ESSENTIALS IN OPHTHALMOLOGY

G. K. Krieglstein · R. N. Weinreb
Series Editors

Glaucoma

Cataract and Refractive Surgery

Uveitis and Immunological Disorders

Vitreo-retinal Surgery

Medical Retina

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Paediatric Ophthalmology, Neuro-ophthalmology, Genetics

Cornea and External Eye Disease

Editors Uwe Pleyer
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Uveitis and Immunological Disorders

With 73 Figures, Mostly in Colour,
and 26 Tables

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Foreword

Essentials in Ophthalmology is a new review series covering all of ophthalmology categorized in eight subspecialties. It will be published quarterly; thus each subspecialty will be reviewed biannually.

Given the multiplicity of medical publications already available, why is a new series needed? Consider that the half-life of medical knowledge is estimated to be around 5 years. Moreover, it can be as long as 8 years between the description of a medical innovation in a peer-reviewed scientific journal and publication in a medical textbook. A series that narrows this time span between journal and textbook would provide a more rapid and efficient transfer of medical knowledge into clinical practice, and enhance care of our patients.

For the series, each subspecialty volume comprises 10–20 chapters selected by two distinguished editors and written by internationally renowned specialists. The selection of these contributions is based more on recent and noteworthy advances in the subspecialty than on systematic completeness. Each article is structured in a standardized format and length, with citations for additional reading and an appropriate number of illustrations to enhance important points. Since every subspecialty volume is issued in a recurring sequence during the 2-year cycle, the reader has the opportunity to focus on the progress in a particular subspecialty or to be updated on the whole field. The clinical relevance of all material presented will be well established, so application to clinical practice can be made with confidence.

This new series will earn space on the bookshelves of those ophthalmologists who seek to maintain the timeliness and relevance of their clinical practice.

G. K. Krieglstein
R. N. Weinreb
Series Editors

Preface

Essentials in Ophthalmology is a publication series that includes eight volumes covering the following topics: Glaucoma, Cataract and Refractive Surgery, Uveitis and Immunological Disorders, Vitreo-Retinal Surgery, Medical Retina, Ocular Plastics and Orbit, Neuro-Ophthalmology, Paediatric Ophthalmology, and Cornea and External Eye Disease. A volume on each topic will be published every 2 years with one being published each quarter. Every 2 years, each volume will be repeated in a new and updated version with different topics.

Each volume is designed to bridge the gap between primary research literature and daily practice. Although current and practical information is stressed in each volume, the latest in research information and directions is also highlighted. The series is designed to be user friendly with numerous tables and illustrations. The target audience includes ophthalmologists and optometrists in practice and training as well as libraries.

The third volume in this series is entitled *Uveitis and Immunological Disorders.* Our knowledge and understanding of these diseases has increased exponentially over the past decade, especially in the areas of immunopathogenesis and immunogenetics. This volume will provide the practitioner with practical information on how to diagnose and treat these difficult and, in some cases, blinding disorders. In addition, there are important discussions of mechanisms underlying these conditions that incorporate the most recent, up-to-date research material available. A "Summary for the Clinician" as well as "Core Messages" enhance the value of the chapters. These aids will help the reader to focus on the important messages in each chapter.

The scope of chapters ranges from diseases that are relatively common and usually require only topical therapy such as ocular allergy and dry eye to diseases that may result in blindness without systemic therapy such as ocular cicatricial pemphigoid, scleritis and some forms of uveitis. Two of the chapters, "Immune Mechanisms in Uveitis" and "Uveitis and Genetics", provide the reader with the latest laboratory research in these areas.

This volume has information of interest to a wide range of ophthalmic subspecialists. For example, the anterior segment subspecialist will find an interest in subjects such as ocular allergy, dry eye, cicatricial pemphigoid, corneal transplantation, scleritis and herpes viruses. Retina and uveitis specialists will have a special interest in the chapters dealing with uveitis and its mechanisms and therapy, including medical and surgical. Lastly, the chapter covering uveitis in children provides important and useful information for paediatric ophthalmologists.

Uwe Pleyer
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Targets in Ocular Allergy

1

Alessandra Micera, Sergio Bonini, Alessandro Lambiase, Roberto Sgrulletta, Stefano Bonini

Core Messages

- Allergic conjunctivitis is inflammation of the ocular surface with IgE, cells, cytokines and mediators
- Several chemokines/adhesion molecules, cytokines and neuropeptides are detectable in situ in allergic conjunctival tissues
- Growth factors, and mainly nerve growth factor (NGF) and transforming growth factor β1 (TGF-β1), are also found to be increased in the blood, tears and tissues of patients with allergic conjunctivitis
- A significant correlation is observed between plasma levels of NGF and the increased number of mast cells (MCs) and eosinophils (EOSs) in the tarsal and bulbar vernal keratoconjunctivitis (VKC) conjunctiva
- Fibrosis can be observed in the conjunctiva of VKC (giant papillae formations) as a consequent manifestation of allergy
- Overproduction and deposition of collagens and tissue remodelling in VKC may be due to imbalance of matrix metalloproteinases and their physiological inhibitors
- Various growth factors (NGF, TGF-β1) and cytokines, produced by both inflammatory and stromal cells, have been proposed to cause tissue repair
- VKC and atopic keratoconjunctivitis (AKC) diseases can compromise the cornea, with ulcers and scarring ultimately leading to visual loss

1.1 Introduction

Allergy or type I hypersensitivity is an immunoglobulin E (IgE) driven reaction that can take place in different organs. The effector phases of IgE-associated immune responses occur in three temporal patterns: (1) an acute reaction, which develops within seconds or minutes after allergen exposure; (2) a late phase reaction, which develops within a few hours after allergen exposure; and (3) a chronic inflammatory response, which can persist for days, months or years [1]. Allergic inflammatory disorders may result in ocular surface allergy, and in most cases ocular allergy is associated with other systemic allergies such as rhinoconjunctivitis [1]. The immunopathogenic mechanisms in allergic disorders involve a combination of IgE-mediated and T-cell-mediated responses. Type-2 T-helper lymphocytes are predominant in inflamed allergic conjunctival tissues [2]. They are thought to play a prominent role in the development of allergic disorders by producing regulatory and inflammatory cytokines such as interleukin (IL)-3 (local differentiation of mast cells and eosinophils), IL-4 (development of mast cells, IgE synthesis and vascular cell adhesion molecules) and IL-13 and IL-5 (development of eosinophils, eosinophil chemotaxic, survival and degranulation) [1]. The IgE-mediated conjunctival allergic reaction can be reproduced easily by specific conjunctival provocation, which induces an early reaction followed by a predominant infiltration of eosinophils (EOSs) and mast cells (MCs), which are thought to play a pivotal role in disease pathogenesis [3]. EOSs and their proteins (ECP) are increased

and activated in tears and tissues of all allergic eye diseases even though the mechanisms of EOS recruitment to the ocular surface are not completely understood [4]. Tear fluid has also been found to contain a small quantity of histamine and tryptase [5], well-known products of MCs [1]. Interestingly, the conjunctival epithelium has also been considered to play a key role in ocular allergic disorders [6].

1.2 Chronic Allergic Eye Diseases

Chronic allergic ocular disease encompasses several disorders, such as seasonal atopic conjunctivitis, perennial atopic conjunctivitis, atopic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC) [7]. *Seasonal atopic conjunctivitis (SAC)* is a time-limited disease and in most cases conjunctivitis is only one manifestation of additional allergic reactions (rhinitis, hay fever or a hay fever like symptomatology, and in severe cases conjunctivitis is associated with different forms of pulmonary affection). *Atopic keratoconjunctivitis* is a severe, bilateral, ocular allergic disease affecting adults. A familial history for atopy and an association with systemic atopic dermatitis are common. Symptoms commonly include itching, burning, and tearing. Signs include involvement of mainly upper conjunctiva in the form of a papillary conjunctivitis. The corneal epithelium reveals mild to moderate inflammatory changes that can result in scarring and neovascularization leading to blindness. Many patients may develop associated staphylococcal blepharitis. Ocular complications include blepharoconjunctivitis, cataract, ocular herpes simplex and keratoconus. *Vernal keratoconjunctivitis* is essentially a chronic recurrent seasonal childhood disease (worldwide spread, especially in countries around the Mediterranean basin), which in approximately half the children's population is associated with other allergic manifestations [8]. Unlike severe AKC, VKC tends to resolve spontaneously after several years. Genetic studies have not been performed that confirm a relationship of VKC with a particular genotype. The disease is mostly seasonal, lasting from the beginning of spring (marked exacerbation of clinical symptoms) until autumn/winter (milder symptoms), even though for children the disease remains variably active the year around. Nevertheless, perennial cases that are persistent throughout the year are not rare, especially in patients living in warm subtropical or desert climates. Its predominance during the high pollen season highly strengthens the widely accepted hypothesis that VKC is an immunologically mediated hypersensitivity reaction to environmental antigens. The characteristic features of the two clinical forms of VKC, the giant papillae on the upper tarsal conjunctiva (tarsal VKC) or the gelatinous limbal infiltrates (limbal VKC), leave no doubt as to the diagnosis of vernal disease. All VKC forms are characterized by intense itching, tearing, mucous secretions and a severe photophobia that often forces children to live virtually in the dark. The commonly noted foreign body sensation is caused by conjunctival surface irregularity and copious mucous secretions, while the presence of pain is indicative of a compromised cornea, which can be present in the form of a superficial punctate keratitis, epithelial macroerosions or ulcers and plaque. According to the other allergic conjunctivitis, pronounced infiltration of lymphocytes, mainly of the Th2 subtype, EOSs and MCs has been reported for VKC [4, 6, 9]. In particular, Th2-derived IL-4 and IL-13, essential to IgE antibody production in tissue eosinophilia and asthma airway remodelling [1], have been reported in tears of both VKC and AKC as well as in patients with SAC, VKC and AKC, respectively [10].

1.3 Effector Cells and Cytokine Release

Allergic conjunctivitis is due to direct exposure of the ocular mucosal surfaces to environmental allergens. The pathogenesis of allergic conjunctival disorders is multifactorial and not fully understood. Ragweed represents the primary responsible allergen (75% of cases of rhinoconjunctivitis). Conjunctival symptoms include itching, tearing, and perhaps burning. Clinical examination may reveal various forms of im-

munologic reaction, all of these presenting as various forms of "red eye". Photophobia and blurred vision may also occur. Clinical signs include milky or pale pink conjunctivae with vascular congestion, which may progress to conjunctival swelling (chemosis). The immunology hypersensitivity reactions include mast cell degranulation, eosinophils, and other inflammatory cells and modulators. The hallmark and effector cells of allergic phenomena are mast cells and eosinophils.

Mast cells (MCs) are bone-marrow-derived tissue cells containing prominent cytoplasmic granules. They have a clear and pivotal role in allergy, but also take part in wound healing and native immunity [11]. In type I hypersensitivity reactions, MCs elicit the early stages of the allergic inflammatory response. In fact the cross-linking of IgE antibodies bound to high-affinity IgE receptors by the antigen and the release of various preformed (histamine) and newly synthesized mediators causes immediate MC degranulation. In addition, the later release of chemiotactic factors, cytokines and chemokines, from activated MCs also induces the onset of the late-phase reaction, characterized by tissue infiltration and activation of various inflammatory cells and notably the eosinophils. The cytokines/growth factors detected in situ in human MCs include interleukin (IL)-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), granulocyte/monocyte-colony forming factor (GM-CSF), stem cell factor (SCF), nerve growth factor (NGF), fibroblast growth factor (β-FGF) and MIP-1α [12]. In particular, by releasing IL-5, GM-CSF, TNF-α, and tryptase, MCs promote eosinophil recruitment, activation and survival into the tissue [12].

Eosinophils (EOSs) are blood granulocytes produced in the bone marrow under the influence of IL-3, IL-5 and GM-CSF that infiltrate the tissues in various pathological situations, such as parasitic infections, allergic inflammation and neoplastic diseases [13]. EOSs possess cytoplasmic granules that, among non-EOS-specific mediators, contain characteristically preformed basic proteins, namely EOS peroxidase (EPO), EOS-derived neurotoxin (EDN), major basic protein (MBP), and EOS cationic protein (ECP). Recently, it has been reported that EOSs maintain their ability to release ECP even after repeated stimulation, implying that mature EOSs may not require a significant ECP resynthesis [14]. In addition, their secretory granules contain several preformed cytokines such as GM-CSF, IL-2, IL-6, IL-4, IL-5, transforming growth factor β (TGF-β), NGF and TNF-α. The presence of EOSs in inflammation and allergy has been recognized as the critical element inducing the inflammatory process and causing tissue damage, even though more recently EOS presence has also been associated with tissue repair. Another recent observation is that an MC-EOS cross-talk exists that may amplify the local allergic inflammatory response [12]. MCs enhance EOS survival and functional activity by TNF-α and tryptase and EOSs cause histamine, GM-CSF and PGD_2 release from MC by MBP and SCF. Moreover, other mediators such as GM-CSF, IL-3, γ-IFN and NGF also take part in this cellular cross-talk [12]. Early studies have shown a role for different growth factors in the growth and survival of both MCs and EOSs [12].

1.4 Chemokines and Adhesion Molecules

Chemokines and adhesion molecules have been found to strengthen associated with the selective infiltrate entity observed in these ocular disorders, controlling the specific cell recruitment in the conjunctiva [15, 16]. Chemokines have been shown specifically to attract and activate EOSs [17], acting through chemokine receptor-3 (CCR3) expressed on EOSs [17]. Increased eotaxin-1 and eotaxin-2 (a recently discovered chemokine that is functionally very similar to eotaxin-1) preferentially recruit circulating EOSs, attracting them to the epithelium and subepithelial stroma and inducing EOS degranulation with release of epithelium-damaging proteins [17]. A recent study showed that cultured conjunctival FBs produce eotaxin-1 in response to IL-4, IL-13 and TNF-α, strongly supporting the hypothesis of a real contribution of FBs in allergic status [18]. These EOS-derived

compounds have been shown to be highly concentrated in tears of all allergic patients, likewise representing a diagnostic marker, mainly localized in corneal ulcers and highly toxic to corneal epithelial cells. In normal conjunctiva, the adhesion molecule ICAM-1 is downregulated and confined to the vascular endothelium. Otherwise, ICAM-1/-3 expression is highly increased in active VKC biopsies and not confined to the lower levels, but expressed by vascular, epithelial, stromal and inflammatory cells [19]. ICAM-1 is necessary to monocyte, lymphocyte and neutrophil adhesion to activated endothelium. Several proinflammatory cytokines contribute ICAM-1 induction [19].

1.5 Neuropeptide and Growth Factor Involvement

Allergic inflammation might also follow the release of neuropeptides, mainly substance P, as observed in VKC [20], which cause characteristic features of allergic inflammation, including vasodilatation, increased vascular permeability and a contribution to further release of histamine from MCs. Specifically, tryptase- and histamine-releasing factors might greatly contribute to SP release and to amplifying the chronic allergic reaction, triggering nerves to release neuropeptides by binding to protease-activated receptors.

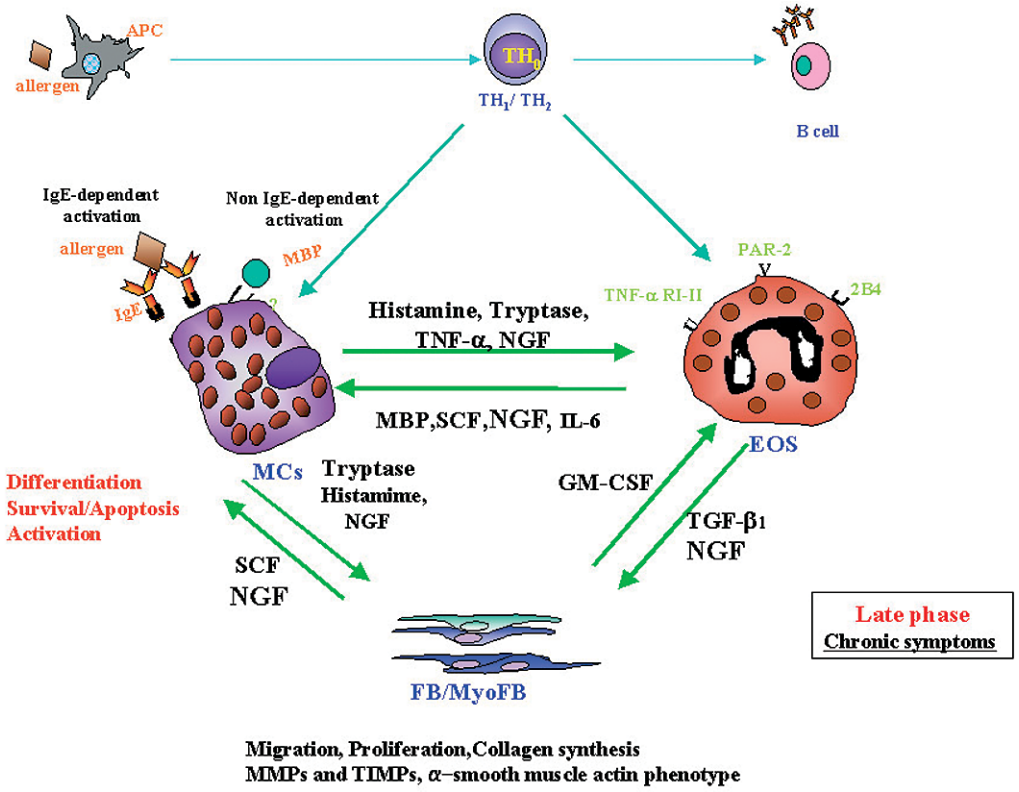

Fig. 1.1. A possible cross-talk between mast cells (*MCs*), eosinophils (*EOS*) and fibroblasts (*FBs*) during the early and late phase of allergic reactions. MCs and EOS effects exerted on FBs seem to be related to the release of either fibrogenic or fibrolytic mediators influencing tissue repair/fibrosis. Furthermore, FBs influenced MCs and EOS by increasing their survival and functional activity and contributing to the local inflammation

Growth factors, and mainly NGF and TGF-β1 [21], are also found increased in the blood, tears and tissues of patients with allergic conjunctivitis. NGF involvement in allergic disorders was postulated after the discovery of an increased NGF plasma level in patients with VKC, which correlated with increased total IgE levels and ECP [22]. A significant correlation was also observed between plasma levels of NGF and the increased number of MCs and EOSs in the tarsal and bulbar VKC conjunctiva [22]. The discovery of the increased NGF plasma levels in VKC prompted the investigation of the presence of NGF in other allergic rhinoconjunctivitis [23]. The fact that NGF levels correlated with total IgE antibody titre strengthens support for the hypothesis of a link between NGF and an allergic response in the visual system [24]. Additional evidence of an involvement of NGF and NGF receptors in allergic ocular disorders has come from a newly described ocular allergic condition, named inflamed juvenile conjunctival nevus (IJCN [25]). In the conjunctiva of IJCN patients increased EOSs and MCs have been found to synthesize and release NGF as well as express its specific receptor [25]. The increase in the local NGF expression has been correlated to an incremented number of MCs and EOSs [25]. TGF-β1 has been reported in several active VKC patients, mainly as a source of EOSs, without significant differences between the tarsal and the limbal forms [26]. Increased levels of TGF-β1, together with IL-1 and IL-6, in active VKC tissues and tears indicate a local production of these cytokines, mainly of EOS derivation [26]. Moreover, these increased levels of TGF-β1, IL-1 and IL-6 might be related to collagen hyperproduction [26]. Taken together, all these data indicate that growth factors are synthesized, released and utilized by MCs, EOSs and FBs, and that NGF and TGF-β1 might be viewed as an additional marker of ocular allergic conditions as a result of their relevant association with allergic ocular disorders (Fig. 1.1).

1.6 Tissue Remodelling and the Contribution of Fibroblasts

Tissue remodelling and formation of giant papillae are some of the consequences of chronic allergic disorders, including the eye [1, 21]. Remodelling involves both production and deposition of extracellular matrix (ECM) components, as well as degradation and clearance of newly synthesized products [21]. Any inflammatory reaction can induce tissue damage and a resulting healing process, which is a very complex event involving interactions of both inflammatory and structural cells [1]. Three main overlapping phases have been identified in tissue response to injury, which can continue for months to years: inflammation, granulation tissue formation – including fibroblast (FB) proliferation, migration, differentiation and remodelling. This process can be physiological and well balanced or can result in an exaggerated pathological process, which includes remodelling and/or fibrosis [21]. FBs represent the main target and effector cells of these processes due to their ability to migrate to the injured area, proliferate, produce ECM, differentiate into myofibroblasts (myoFBs) and finally to contract the wound [21]. Previously viewed as simple structural cells providing the cellular and ECM scaffold to tissues, FBs are now recognized as regulatory cells exerting active roles in tissue homeostasis, by producing cytokines and several growth factors with autocrine/paracrine activities [12]. Several in vitro studies suggest that myoFBs may play a role in fibrosis, remodelling, and repair processes associated with IgE-mediated hypersensitivity, at least in other organs where chronic allergic conditions might develop [21]. MyoFBs are specialized cells thought to rise locally from FBs and displaying the contractile protein α-smooth muscle actin (α-SMA). MyoFBs represent the main transient cellular phenotype responsible for wound contraction and their deletion, via apoptosis, or as recently hypothesized via their phenotypical reversion to FBs, is necessary to terminate successfully the repair process. Although somewhat controversial, prolonged survival of myoFBs

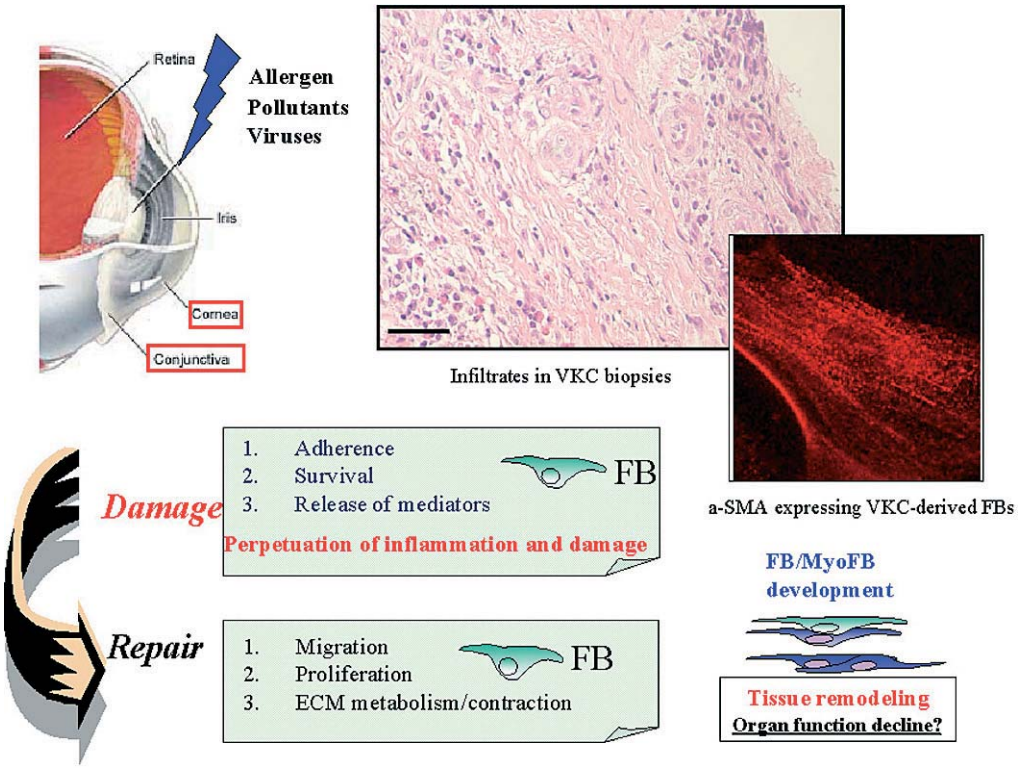

Fig. 1.2. Repeated/prolonged exposure of the ocular surface to allergens can lead to a perpetuation of local inflammation and damage (mediators from both mast cells and eosinophils), resulting in a repair process due to migration/proliferation/active metabolism of fibroblasts/myofibroblasts. Remodelling of the entire structure might sometimes induce function decline. In the frame is shown a picture from a VKC biopsy stained for haematoxylin and eosin

might contribute to the abnormal ECM deposition and fibrosis.

Fibrosis can be observed in the conjunctiva of VKC, an interesting example of a chronic inflammatory response result in tissue remodelling (Fig. 1.2). In VKC, fibrosis can be a consequent and sometimes a concomitant manifestation of allergy [27], and only recently have the role of FBs and myoFBs in VKC fibrosis been taken into consideration. Structural changes in the conjunctiva, such as giant papillae formation, epithelial in growth, and subepithelial fibrosis associated with ECM deposition, are present and may contribute to the chronicity of the disease. No data have been reported on the presence of myoFBs in VKC. Normal cultured conjunctival FBs are induced to express α-SMA protein upon growth factor stimulation, and VKC derived FBs directly express this protein (Micera et al., unpublished data). FBs from VKC biopsies express in vitro α-SMA protein and appear to have a cytokine profile typical of activated myoFBs (Micera et al., unpublished data).

1.7 Metabolism of Extracellular Matrix

The analysis of ECM in VKC demonstrated a reduction in proteoglycans and a substantial increase in total collagen with an altered ratio between collagen type I and III, due to a consistent increase in collagen type III [27]. Moreover, a high amount of procollagens type I and III in the tears during active tarsal VKC has also been reported. This increased deposition of collagen

types I, III and IV in the giant papillae might be viewed as a result of increased expression of cytokines and growth factors from EOS, MCs or other inflammatory cells which are known to stimulate resident FBs to overproduce ECM. Thus, in vitro VKC-derived FBs spontaneously produce collagen types I and III (Micera et al., unpublished data). The Th2-type IL-4 and -13 seem to control collagens I and III on normal FBs, whereas Th1-type derived IFN-γ may have an inhibitory effect on FBs, according to other tissues [1]. Overproduction and deposition of collagens and tissue remodelling may be due to an imbalance between matrix metalloproteinases (MMPs) and their physiological inhibitors, tissue inhibitors of MMPs (TIMPs). Thus, MMP-1 and MMP-9 were found altered in the tear in VKC [28]. MMPs are a family of zinc- and calcium-dependent enzymes involved in many physiological and pathologic processes, including progression of tumours, metastasis, inflammatory diseases, and wound healing, by several cytokines. Some of the 17 known members of the human MMP family, collagenase I (MMP-1), gelatinase A and B (MMP-2 and -9), stromelysin (MMP-3), and matrilysin (MMP-7), are those most involved in anterior segment disease and wound healing. These enzymes are produced and secreted by a variety of cells, including epithelial cells, inflammatory cells, and conjunctival FBs. MMP-1 can cleave the triple helix of interstitial collagen types I, II, and III. Stromelysin and matrilysin are involved in degrading a variety of ECM components such as proteoglycans, fibronectin, and laminin. Gelatinase A and B are involved in cleaving collagen types IV (the main component of basal membrane), V, VII, and X; fibronectin; laminin; elastin; and collagen degradation products; and TIMP-1 is the natural inhibitor of both collagenase I and gelatinase.

Various growth factors and cytokines, produced by both inflammatory and stromal cells, have been proposed to cause tissue repair. However, the main pro-fibrogenic factor remains TGF-β [21] , which is responsible for the stimulation of ECM formation, the inhibition of its degradation and the chemoattraction of FBs. There are three different isoforms of TGF-β, namely TGF-β1 (mainly profibrogenic), TGF-β2 (mainly immunoregulatory) and TGF-β3. TGF-β1 is the main isoform involved in the fibrotic process [21]. TGF-β1 is not the only growth factor found increased in ocular allergic disorders showing tissue remodelling [21]. NGF recently has been postulated to be another growth factor important in the stromal-epithelial interaction during the wound-healing processes occurring in different organs [29]. The healing effect of topical long-term treatment of NGF of human corneal neurotrophic ulcers has indicated that this factor might influence FBs, myoFBs and epithelial cells during the repair process occurring in the eye [29], showing the potential of NGF as a pharmacological tool for disorders characterized by impaired healing.

Finally, VKC and AKC diseases can compromise the cornea severely, with ulcers and scarring ultimately leading to visual loss. In particular, VKC affected boys and AKC patients might develop keratoconus [30]. Whether these children develop keratoconus as a result of a susceptible genetic background or as a secondary manifestation remains an open question.

Summary for the Clinician

- **Ocular allergic manifestations range from mild to severe diseases**
- **Itching is a cardinal symptom: no itching, no allergy!**
- **Transient or persistent corneal damage represents signs of the severity of the disease and is the expression of severe inflammation**
- **In the mildest form a common treatment may be suggested (multiple action drugs)**
- **Topical steroids or topically available new immunosuppressive agents such as cyclosporine represent alternative treatment for severe diseases**

1.8 Conclusions

The topical therapy for chronic allergic ocular disorders implies the use of steroids to quieten the disease and of MC stabilizers or leukotriene inhibitors, mainly in association with steroid treatment. MCs and EOSs have been shown to participate in the development of allergic in-

flammatory disorders and tissue repair/fibrosis by modulating the activities of immunocompetent cells and of FBs and ECM homeostasis. MC and EOS effects exerted on FBs seem to be related to the production of different mediators and in turn FBs can promote local inflammation by producing factors responsible for recruitment, adhesion or cell activation. This would imply that novel immunotherapeutic tools directed to different targets, including antagonists of some cytokines or growth factors, should be studied to develop new strategies for controlling especially MC activation, EO recruitment, and Th2 activity.

References

1. Holgate ST, Church MK, Lichtenstein LM (2001) Allergy, 2nd edn. Mosby International, London
2. Metz DP, Hingorani M, Calder VL, et al. (1997) T-cell cytokines in chronic allergic eye disease. J Allergy Clin Immunol 100:817–824
3. Abelson MB, Chambers WA, Smith LM (1990) Conjunctival allergen challenge. A clinical approach to studying allergic conjunctivitis. Arch Ophthalmol 108:84–88
4. Bonini S, Bonini S, Vecchione A, et al. (1988) Inflammatory changes in conjunctival scrapings after allergen provocation in humans. J Allergy Clin Immunol 82:462–469
5. Bonini S, Lambiase A, Sgrulletta R, Bonini S (2003) Allergic chronic inflammation of the ocular surface in vernal keratoconjunctivitis. Curr Opin Allergy Clin Immunol 3:381–387
6. Bonini S, Lambiase A, Sacchetti M, Bonini S (2003) Cytokines in ocular allergy. Int Ophthalmol Clin 2003 43:27–32
7. Trocme SD, Sra KK (2002) Spectrum of ocular allergy. Curr Opin Allergy Clin Immunol 2:423–427
8. Leonardi A (2002) Vernal keratoconjunctivitis: pathogenesis and treatment. Progr Retin Eye Res 21:319–339
9. Leonardi A, DeFranchis G, Zancanaro F, et al. (1999) Identification of local Th2 and Tho lymphocytes in vernal conjunctivitis by cytokine flow cytometry. Invest Ophthalmol Vis Sci 40: 3036–3040
10. Uchio E, Ono SY, Ikezawa Z, Ohno S (2000) Tear levels of interferon-g, interleukin (IL)-2, IL-4 and IL-5 in patients with vernal keratoconjunctivitis, atopic keratoconjunctivitis and allergic conjunctivitis. Clin Exp Allergy 30:103–109
11. Galli SJ, Williams CMM (2000) The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. J Allergy Clin Immunol 105:847–859
12. Smith S, Levi-Schaffer F (2000) Cross-talk between eosinophils, mast cells and fibroblasts. Chem Immunol 78:81–92
13. Trocme SD, Aldave AJ (1994) The eye and the eosinophil. Surv Ophthalmol 39:241–52.
14. Simon HU, Weber M, Becker E, Zilberman Y, Blaser K, Levi-Schaffer F (2000) Eosinophils maintain their capacity to signal and release eosinophil cationic protein upon repetitive stimulation with the same agonist. J Immunol 165: 4069–4075
15. Abu El-Asra AM, Geboes K, Al-Kharashi S, Tabbara KF, Missotten L, Desmet V (1997) Adhesion molecules in vernal keratoconjunctivitis. Br J Ophthalmol 81:1099–1106
16. Abu El-Asrar AM, Struyf S, Al-Kharashi SA, et al. (2000) Chemokines in the limbal form of vernal keratoconjunctivitis. Br J Ophthalmol 84:1360–1366
17. Forssman U, Uguccioni M, Loetscher P, et al. (1997) Eotaxin-2, a novel CC chemokine that is selective for the chemokine receptor CCR3, and acts like eotaxin on human eosinophil and basophil leukocytes. J Exp Med 185:2171–2176
18. Solomon A, Puxeddu I, Levi-Schaffer F (2003) Fibrosis in ocular allergic inflammation: recent concepts in the pathogenesis of ocular allergy. Curr Opin Allergic Clin Immunol 3:389–393
19. Leonardi A, Jose PJ, Zhan H, Calder VL (2003) Tear and mucus eotaxin-1 and eotaxin-2 in allergic keratoconjunctivitis. Ophthalmology 110:487–492
20. Lambiase A, Bonini S, Micera A, Tirassa P, Magrini L, Bonini S, Aloe A (1997) Increased plasma levels of substance P in vernal keratoconjunctivitis. Invest Ophthalmol Vis Sci 38:2161–2164
21. Micera A, Puxeddu I, Aloe L, Levi-Schaffer F (2003) New insights on the involvement of Nerve Growth Factor in allergic inflammation and fibrosis. Cytokine Growth Factor Rev 14:369–374
22. Lambiase A, Bonini St, Bonini Se, Micera A, Magrini L, Bracci-Laudiero L, Aloe L (1995) Increased plasma levels of nerve growth factor in vernal keratoconjunctivitis and relationship to conjunctival mast cells. Invest Ophthalmol Vis Sci 36:2127–2132
23. Bonini S, Lambiase A, Bonini S, Angelucci F, Magrini L, Manni L, Aloe L (1996) Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. Proc Natl Acad Sci USA 93:10955–10960

24. Bonini S, Aloe L, Bonini S, Rama P, Lamagna A, Lambiase A (2002) Nerve growth factor (NGF): an important molecule for trophism and healing of the ocular surface. Adv Exp Med Biol 506: 531–537
25. Levi-Schaffer F, Micera A, Zamir E, Mechoulam H, Puxeddu I, Piliponsky AM, Aloe L, Pe'er J (2002) Nerve growth factor and eosinophils in inflamed juvenile conjunctival nevus: a link with allergy. Invest Ophthalmol Vis Sci 43:1850–1856
26. Hingorani M, Calder V, Jolly G, Buckley RJ, Lightman SL (1998) Eosinophil surface antigen expression and cytokine production vary in different ocular allergic diseases. J Allergy Clin Immunol 102:821–830
27. Leonardi A, Borghesan F, DePaoli M, Plebani M, Secchi AG (1998) Procollagens and inflammatory cytokine concentrations in tarsal and limbal vernal keratoconjunctivitis. Exp Eye Res 67:105–112
28. Leonardi A, Brun P, Abatangelo G, Plebani M, Secchi AG (2003) Tear levels and activity of matrix metalloproteinase (MMP)-1 and MMP-9 in vernal keratoconjunctivitis. Invest Ophthalmol Vis Sci 44:3052–3058
29. Lambiase A, Rama P, Bonini S, Caprioglio G, Aloe L (1999) Topical treatment with nerve growth factor for corneal neurotrophic ulcers. N Engl J Med 338:1174–1180
30. Bawazeer AM, Hodge WG, Lorimer B (2000) Atopy and keratoconus: a multivariate analysis. Br J Ophthalmol 84:834–836

Dry Eye: Inflammation of the Lacrimal Functional Unit

Stephen C. Pflugfelder, Michael E. Stern

Core Messages

- Dry eye (LKC, lacrimal keratoconjunctivitis) can best be defined as a disease resulting in altered tear film composition
- Regardless of the aetiology, systemic autoimmune disease such as Sjögren's syndrome or local autoimmune diseases such as keratoconjunctivitis sicca, patients will have an immune based inflammation of the ocular surface and lacrimal glands
- Tears are secreted by the "lacrimal functional unit" composed of the ocular surface, the main and accessory lacrimal glands and the interconnecting innervation
- Current concepts of the tear film define it as a mucin/aqueous gel covered by a layer of lipid
- A decrease in systemic androgens associated with aging or various pathologies has been found to be one of the predisposing factors behind the initiation of LKC
- Effects on the ocular surface in patients with LKC include an increase in apoptosis (programmed cell death)

2.1 Introduction

2.1.1 Basics

Dry eye disease has traditionally been defined as a tear deficiency. Research over the past decade, however, has led to an appreciation of the central role that ocular surface inflammation plays in dry eye disease. Dry eye may now be viewed as a syndrome in which an unstable tear film of altered composition fails to support ocular epithelial health and instead promotes ocular surface inflammation.

Recent research has also led to recognition that the ocular surface and tear secreting glands act in concert as a functional unit [33, 41]. The lacrimal functional unit maintains ocular surface health by a homeostatic mechanism: sensory neural input from the ocular surface is integrated and directed to the secretory apparatus in order to manipulate the volume and composition of the tear film (Fig. 2.1).

Dry eye disease can arise in a number of different ways. For example, Sjögren's syndrome, a systemic autoimmune disease, probably initiates dry eye by autoimmune-mediated inflammation of the lacrimal glands, causing altered tear volume and composition (including proinflammatory cytokines), in turn leading to inflammation of ocular surface tissues. In another example, disease of the meibomian glands, which secrete the lipid layer protecting the tear film, may initially impact tear composition by allowing excess evaporation, later causing ocular surface inflammation, which can impact neural control of the lacrimal glands. Interconnectedness within the lacrimal functional unit means that the varied causes of dry eye disease all eventually manifest an unstable, proinflammatory tear film, which leads to inflammation of the ocular surface (Fig. 2.2), including apoptosis of epithelial cells within the main and accessory lacrimal glands and the conjunctiva, and chronic firing of ocular surface sensory nerves, experienced as ocular pain. In the past, the ocular surface disease associated with dry eye has been called keratoconjunctivitis sicca;

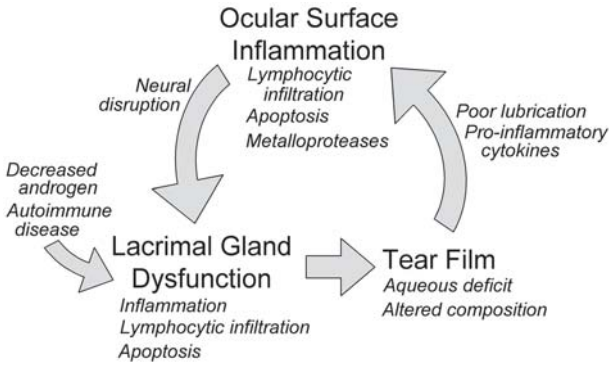

Fig. 2.1. The lacrimal functional unit maintains the health of ocular surface tissues by a homeostatic mechanism. A properly refreshed tear film provides protection, lubrication and a trophic environment for the ocular surface epithelia. Tear film components are secreted by the lacrimal and meibomian glands, and from conjunctival goblet cells, under neural control derived from afferent innervation of the ocular surface. Glandular function is also influenced by hormonal regulation

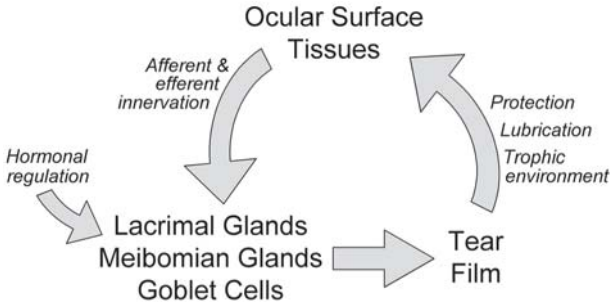

Fig. 2.2. Lacrimal keratoconjunctivitis results from dysfunction of the lacrimal functional unit. Ocular surface inflammation is promoted by a proinflammatory tear film, and by increased osmolarity due to a deficit of aqueous production. Lacrimal gland inflammation, accompanied by lymphocytic infiltration and apoptosis, is responsible for the unrefreshed tear film and its altered composition. Inflammatory effects on neural control of gland function, hormonal imbalance, and autoimmune disease can all contribute to lacrimal gland dysfunction

however, the term lacrimal keratoconjunctivitis (LKC) seems a more accurate description, in that dryness is not initially evident, and will be used in this article.

Estimates of the prevalence of dry eye vary widely, probably because of different criteria used in epidemiological studies. Prevalence estimates range from 0.7% of subjects 65 years and older that had irritation symptoms together with two clinical signs of LKC, to 15% who self-reported ocular dryness symptoms [26, 38]. Prevalence of dry eye disease increases with age, is greater in women, and may increase over time due to the advent of refractive surgeries such as LASIK (laser-assisted intrastromal keratomileusis). The disease pathophysiology can be initiated by the interruption of corneal sensory nerves that occurs during LASIK, causing dysfunction of the lacrimal functional unit [7]. Costs to society, such as time lost from work and the expense of medical care, must be substantial as well. As a suggestion of how substantial, worldwide sales of artificial tears exceeded $500 million in 2002.

2.1.2 Lacrimal Functional Unit

Tear flow is reflexively regulated by the lacrimal functional unit (Fig. 2.1). The lacrimal functional unit comprises the ocular surface, including the cornea, conjunctiva, and meibomian glands, the main and accessory lacrimal glands, and the neural network that connects them [33, 41]. Its overall purpose is to maintain corneal clarity and the quality of the image projected onto the retina. Corneal clarity depends, in turn, on the integrity of the tear film and the health of the ocular surface.

The lacrimal functional unit operates by a homeostatic mechanism. The status of the ocu-

lar surface is monitored by sensory nerves carrying information to the lacrimal centre in the brain stem. Autonomic secretomotor nerves direct secretory tissues and glands, including the main and accessory lacrimal glands, the meibomian glands, and the conjunctival goblet cells. The major variables which can be adjusted to influence the system's status (and thereby maintain or return to stasis) are the volume and composition of the tear film [33, 41].

The tear film serves four important functions: it provides a smooth optical surface for normal vision, it maintains ocular surface comfort, it protects ocular surface tissues from environmental and infectious insults, and it contain factors important for maintenance of epithelial cell health. The tear film and the anterior surface of the cornea combine to provide approximately 80 % of the refractive power for the eye's focusing mechanism. Small changes in tear film stability and volume can result in tear film break-up, causing optical aberrations that can significantly degrade the quality of vision, primarily by decrease in contrast sensitivity. Tear film break-up likely contributes to the visual fatigue and photophobia experienced by many LKC patients [4]. Ocular surface comfort depends on the tear film's lubricating properties, which decrease the shear forces exerted by the superior lid margin during a normal blink cycle [6]. The mucin layer of the tear film is critical for this lubrication. The tear film protects the ocular surface, the most environmentally exposed mucosal surface of the body, from extremes of temperature and humidity, allergens, irritants and infectious agents. The surface lipid layer, secreted by the meibomian glands, prevents evaporation of the aqueous component and consequent increases in osmolarity of the tear film in adverse environments. Some of the proteins present in the tear film, such as immunoglobulin A, lactoferrin, lysozyme, and peroxidase, help resist bacterial or viral infections. Because the corneal epithelium lacks vasculature, it is dependent on tear film electrolytes and oxygen for tissue health, and on tear film growth factors to stimulate the constant regeneration of the corneal epithelium and for wound healing. Antioxidants in the tear film help maintain a reducing environment and scavenge free radicals.

Traditionally the tear film was envisioned as three distinct components: a mucin layer coating the surface epithelium, an aqueous layer making up the majority of the tear film, and a thin lipid layer sitting on top to slow evaporation. That view has evolved to the currently proposed structure of a mucin/aqueous gel containing electrolytes, proteins, and regulatory factors that decreases in density toward the lipid layer (Table 2.1) [6, 33]. The mucin component functions as a surfactant for the ocular surface, allowing the tear film to spread evenly over the hydrophobic epithelium. It includes the glycocalyx, composed of transmembrane mucins anchored to the epithelial cell surface [11], and soluble mucins, shed by epithelial cells and secreted by conjunctival goblet cells and the lacrimal glands [16]. Soluble mucins interact with the glycocalyx and the aqueous component to form a water-trapping gel. The mucin component may also help prevent adherence of inflammatory cells, bacteria, and debris to the ocular surface [11]. The aqueous component solubilizes oxygen, electrolytes, and numerous proteins and regulatory factors. Normal tear osmolarity, about 300 mOsm/l, is important to maintain normal epithelial cell volume, for maintenance of correct nerve membrane potential, and for cellular homeostasis and secretory function. The main and accessory lacrimal glands secrete the aqueous component of tears, although their relative contributions to tear volume are unresolved. The main lacrimal gland is responsible for reflex tearing, which can flush infective or irritating particles from the ocular surface. The composition of the lipid layer is complex, with polar lipids found mostly at the lipid-aqueous interface, and non-polar lipids found at the lipid-air interface. The very diverse array of lipids found in the tear film are secreted by the meibomian glands, whose ducts exit just anterior to the mucocutaneous junction of the lids. Blinking helps to spread the lipid layer uniformly over the tear film surface, a process assisted by the low surface tension of the lipid-air interface.

The lacrimal functional unit is provided information about the status of the ocular surface by afferent innervation via the first (ophthalmic) division of the trigeminal ganglion (or the second division for the lower lid). The

Table 2.1. Components of the tear film

Component	Secreted by:	Functions
Lipid	Meibomian glands	Minimize evaporation
Aqueous	Main and accessory lacrimal glands	Solubilize mucins, electrolytes, proteins Flush irritants (reflex tears)
Mucin	Goblet cells, epithelia, lacrimal glands	Lubrication; surfactant between hydrophobic epithelium and aqueous component

cornea, the most densely innervated epithelial surface in the body, and the rest of the ocular surface epithelia are populated by sensory neural receptors of a morphologically unspecified type, called free nerve endings. Sensations evoked are painful in nature, but aside from relatively infrequent traumatic events such as debris on the corneal surface, they are usually subconscious, and an individual is unaware of sensory input from the ocular surface. Reflex tearing and eyelid closure are the obvious responses to stimulation of corneal nerves.

Nerves from the parasympathetic sphenopalatine (pterygopalatine) ganglion are associated with the secretory glands of the lacrimal functional unit. Parasympathetic cholinergic nerves are primarily responsible for signalling reflex tear secretion, and acetylcholine (M_3) receptors are present on secretory epithelia of lacrimal glands and on mucin-producing goblet cells in the conjunctiva [5]. Other parasympathetic neurotransmitters have also been detected near the lacrimal epithelium and meibomian glands [13]. Evidence for nerves of sympathetic origin has been found for the main and accessory lacrimal glands, the meibomian glands, and conjunctival goblet cells. Maintenance of the lacrimal gland secretory environment is also regulated by serum-derived factors, including androgen, oestrogen, progesterone, cortisol, insulin, thyroxin, and growth factors [43].

Lacrimal glands are composed of numerous lobules with secretory acini and ducts that converge into excretory ducts. The acini appear as rosettes of polarized columnar secretory epithelial cells in cross section whose apical surfaces terminate in the central lumen. The mid and apical regions of acinar cells contain numerous secretory granules of protein products to be released, whereas the basal regions contain the nucleus surrounded by a prominent endoplasmic reticulum and Golgi apparatus. When a neurotransmitter molecule binds a cognate receptor on the exterior of the acinar cell's basolateral membrane, it activates heterotrimeric G proteins on the cytoplasmic side [13, 25]. Their G_α subunits dissociate, exchange GDP for GTP, and initiate a cascade of intracellular regulatory events leading to Ca^{2+} influx and elevated cAMP. These mediators cause preformed transport vesicles (derived from the Golgi apparatus), containing proteins destined for secretion, to fuse with the apical cell membrane of the acinar cell, releasing the vesicle contents [44]. Secretion of water by lacrimal epithelial cells depends mostly on osmotic pressure generated by secretion of electrolytes, although secretion of proteins and mucins may contribute. The same receptor binding event that triggers protein secretion activates at least seven ion transporters that function together to secrete Na^+, K^+, and Cl^- ions, resulting in secretion of water into the lacrimal ducts [45].

2.2 Specific Pathologies of the Lacrimal Functional Unit

2.2.1 Dysfunction of the Afferent System

Decreased afferent sensory input from the ocular surface results in decreased lacrimal function and epithelial mucin production, which in turn can lead to LKC. For example, patients with familial dysautonomia (Riley-Day syndrome), a

hereditary sensory and autonomic neuropathy that causes corneal anaesthesia, produce a reduced amount of tears when crying and suffer from severe LKC. Their reflex lacrimation response to irritants such as onion odour is absent; however, affected children produce abundant tears after parenteral administration of a cholinergic agonist, indicating that lacrimal gland function is retained.

Surgical damage or amputation of trigeminal afferent nerves is a common cause of reduced corneal sensation. It has long been recognized that surgical sectioning of the trigeminal ganglion (to relieve trigeminal neuralgia) leads to LKC. Experimental trigeminal ablation in animal studies decreased conjunctival goblet cell density and corneal epithelial glycogen, and resulted in morphological alterations of ocular surface epithelia similar to those characteristic of LKC. Ocular surgical procedures that decrease corneal sensation include penetrating keratoplasty, photorefractive keratectomy, and LASIK.

Several ocular and systemic diseases cause trigeminal dysfunction and decreased tear production. Herpes zoster ophthalmicus can reduce corneal sensation in the distribution of the first division of the trigeminal nerve, and herpes simplex keratitis can result in sectoral or diffuse reduction of corneal sensation; both conditions can decrease tear production [12]. Diabetes mellitus can cause a polyneuropathy that reduces corneal sensation and causes secondary tear deficit and LKC. Reduced corneal sensation and aqueous tear deficiency are considered risk factors for diabetic keratoepitheliopathy.

2.2.2 Dysfunction of the Efferent System

Dysfunction of the efferent component of the lacrimal functional unit can affect autonomic nerves that stimulate tear secretion (secretory fibres), or those that regulate eyelid blinking and the tear drainage pump, or both. A common cause of efferent dysfunction is use of systemic anticholinergic medications, such as antihistamines, antispasmotics, antiemetics, and antidepressants [26]. Interestingly, a mechanism of secretory dysfunction found in Sjögren's syndrome may mimic the effects of anticholinergics: circulating autoreactive antibodies interact with M_3 acetylcholine receptors on lacrimal gland secretory cells [2, 15]. Age-related generalized dystrophy of the parasympathetic nervous system can also decrease secretory drive.

2.2.3 Glandular Dysfunction

Dysfunction of lacrimal and other secretory glands of the lacrimal functional units can result from a number of conditions (Table 2.2).

Sjögren's syndrome is a major cause of dry eye. It is a systemic autoimmune disease, primarily of females, characterized by progressive lymphocytic infiltration of lacrimal and salivary glands, which results in aqueous tear deficiency, LKC, and dry mouth. It can develop secondarily to connective tissue disease, most commonly rheumatoid arthritis. Specific autoantibodies are usually detectable. Lacrimal dysfunction and LKC are typically more severe in Sjögren's syndrome patients than in non-Sjögren's patients [31]. Several mechanisms contribute to lacrimal dysfunction in Sjögren's syndrome. The lymphocytic infiltrate, predominately B and T helper ($CD4^+$) cells, along with a lesser proportion of cytotoxic T cells ($CD8^+$), is accompanied by disruption of normal lacrimal gland architecture, with loss or dysfunction of secretory acini and proliferation of ductal epithelia [30]. Inflammatory cytokines released by infiltrating lymphocytes and by diseased epithelial cells can inhibit stimulation of lacrimal secretion [interleukin (IL)-1], or may promote apoptosis of lacrimal secretory epithelia [tumour necrosis factor (TNF)-α, interferon [INF]-γ, IL-12 and IL-18] [19, 49]. Among the autoantibodies developed by Sjögren's patients are antibodies which bind the M_3 acetylcholine receptor on secretory epithelial cells in lacrimal and salivary glands, and are proposed to inhibit their neural stimulation [2, 15]. Aqueous tear deficiency and altered tear composition, including hyperosmolarity and elevated concentrations of proinflammatory cytokines in tears,

promote inflammation and additional pathological changes of other ocular surface tissues, contributing to LKC [41].

Decreased androgen levels and increased oestrogen levels are associated with Sjögren's syndrome (and with other autoimmune diseases as well), consistent with the fact that 90–95% of Sjögren's patients are female [43]. The exact mechanisms are not yet elucidated, but may involve the pervasive influences of these hormones on innate and adaptive immunity.

Dry eye is a complication of acute and chronic graft-versus-host disease. In this condition, lacrimal production may be obstructed by accumulation of normal-appearing granules, together with amorphous material and cellular debris, in the acinar and ductule lumens. Tear deficiency in patients suffering graft-versus host disease may be accompanied by severe LKC, conjunctival scarring, and corneal epithelial defects [29].

Age-related degeneration, including pathological changes such as atrophy, and lobular, diffuse, and periductal fibrosis, may be the most common cause of decreased lacrimal function [8]. Decreased androgen levels [43] and aging effects on corneal sensory nerves may contribute to age-related lacrimal gland dysfunction. Immunological mechanisms may be involved as well, although circulating autoantibodies are not evident in non-Sjögren's LKC.

2.3 Lacrimal Keratoconjunctivitis Inflammation

Dysfunction of the lacrimal functional unit leads to ocular surface epithelial disease, which has been traditionally called keratoconjunctivitis sicca. We feel that the newly introduced term, lacrimal keratoconjunctivitis (LKC), more completely describes the array of pathological features associated with this syndrome (Table 2.3). Although our view of ocular surface pathology resulting from secretory dysfunction is incomplete, changes in tear fluid composition are clearly central in its development. As the lacrimal functional unit fails, tear concentrations of growth factors [for example, epidermal growth factor EGF)] and anti-inflammatory factors decrease [28], and concentrations of proinflammatory cytokines, that can originate from diseased lacrimal glands, meibomian glands, or ocular surface epithelia, increase [32, 40]. Tear concentrations of the soluble mucin, MUC5AC, are decreased in Sjögren's patients. Increased levels of proteases, including plasmin and matrix metalloproteinases (MMPs), profoundly impact tear composition and corneal epithelial integrity [39, 40]. Inflammation and neural sensitization result, together with ocular surface epithelial disease, the most clinically recognizable manifestation of LKC.

Table 2.3. Pathophysiology of the ocular surface in lacrimal keratoconjunctivitis

Tear film
Tear film break-up, poor lubrication
Increased osmolarity and proinflammatory cytokines
Cornea
Loss of epithelial barrier function
Conjunctiva
Squamous metaplasia
Apoptosis of epithelial cells (especially goblet cells)
T-lymphocyte infiltration

Table 2.2. Conditions associated with lacrimal dysfunction

Efferent dysfunction
Anticholinergics, anti-M_3 autoantibody
Lacrimal inflammation
Lymphocytic infiltration
Proinflammatory cytokines
Apoptosis
Hormonal imbalance
Decreased androgen/increased estrogen
Cicatricial obstruction of lacrimal ducts
Due to autoimmune and inflammatory diseases

2.3.1 Corneal Epithelial Disease

The decreased tear production and altered tear composition associated with dysfunction of the lacrimal functional unit contribute to poor lubrication of the ocular surface and a destabilized tear film. This is evident clinically as rapid tear break-up (see Sect. 1.4), with visible discontinuities in the tear film [24, 34]. The unstable tear film is associated with corneal epithelial surface irregularities that are detectable by computerized videokeratoscopy [4]. The blurred, fluctuating vision, photosensitivity, and reduced contrast sensitivity reported by LKC patients likely result from effects of corneal epithelial disease.

The normal corneal epithelium is much less permeable than the conjunctival epithelium because of its barrier function, which is critical for corneal smoothness and clarity. Disruption of the barrier function, assessed clinically by increased permeability to fluorescein dye, is a well-recognized feature of LKC: corneal epithelial permeability in untreated dry eye patients is 2.7–3 times greater than in patients with normal tear function. Studies of the rabbit cornea showed that it is impermeable to molecules larger than 3 kDa in molecular weight, and that the barrier to permeability lies entirely in the epithelium. The permeability of reepithelialized corneal tissue (after wounding) correlated with the degree of surface vascularization: conjunctival epithelium transdifferentiated into a corneal phenotype with minimal vascularization was relatively impermeable, whereas vascularized epithelium retaining a conjunctival phenotype displayed increased permeability characteristic of conjunctival epithelium [14]. Disruption of the mucin layer associated with cell membranes of apical corneal epithelial cells may compromise their barrier function [9]. Death, loss, or dysfunction of well differentiated apical corneal epithelial cells, as can occur in LKC, exposes the relatively permeable subapical layer of less well differentiated cells, obviating the corneal epithelial barrier function.

The corneal epithelial barrier function also depends on the integrity of tight junctional complexes between adjacent cells of the apical epithelium. Exposure of cultured corneal epithelial cells to low concentrations of surfactants or to a proinflammatory stimulus disrupted tight junctions [47]. Altered expression of tight junction complex proteins, such as ZO-1, ZO-2, and occludin, or their proteolytic degradation, appeared to be responsible for the disruption. Hyperosmolar stress and proinflammatory cytokines, conditions found on the ocular surface in LKC, activate transcription factors NF-KB and AP-1, which regulate tight junction development, and such conditions also increase expression of matrix metalloproteinase-9 (MMP-9) by the corneal epithelium [1, 39]. MMP-9 is increased in the tear film of LKC patients, and it is known to cleave tight junction proteins such as occludin, suggesting it plays a role in disruption of the barrier function. Although much remains to be learned, the findings so far suggest an explanation of how the proinflammatory ocular surface environment of LKC disrupts corneal epithelial barrier function.

Neurogenic inflammation of the ocular surface can occur by activation of or damage to the numerous unmyelinated axons that innervate the cornea. Released substance P and calcitonin gene-related peptide (CGRP) can act on anterior segment vascular elements, leading to inflammation and migration of immune cells from the vascular space to the ocular surface. Neurogenic inflammation may contribute to the ocular irritation symptoms of LKC.

2.3.2 Conjunctival Epithelial Disease

Squamous metaplasia, a condition of hyperproliferation and abnormal differentiation of the conjunctival epithelium, occurs in a variety of ocular surface inflammatory diseases, including LKC [31].

An important feature of squamous metaplasia is significantly decreased numbers of mucin-producing goblet cells in both Sjögren's and non-Sjögren's LKC patients. Consistent with this, tear concentrations of the goblet cell-specific soluble mucin, MUC5A, were reduced in

these patient populations [48]. Immunological analysis showed an altered distribution or glycosylation of membrane-bound mucins on apical conjunctival epithelial cells of patients with dry eye symptoms compared with normal patients, which correlated with rose bengal staining, another sign of dry eye disease [3]. Reduced membrane-bound and soluble mucin levels probably impair the spreading capability of the tear film, contributing to tear film break-up.

Hyperproliferation implies an elevated mitotic rate, which was indicated in the bulbar conjunctival epithelium of Sjögren's syndrome patients by increased epithelial stratification, DNA synthesis, and cell proliferation [17]. An elevated mitotic rate was also documented by increased immunostaining for the cell cycle associated protein KI-67 in bulbar conjunctival epithelial biopsies of non-Sjögren's LKC patients compared with those of normal patients [20]. Together with accelerated mitotic rates, increased expression of genes for transglutaminase 1, involucrin, filagrin, and the cytokeratin pair 1/10 in conjunctival tissue from Stevens-Johnson syndrome and ocular cicatricial pemphigoid patients with severe squamous metaplasia [27] may suggest alterations in the differentiation program of conjunctival epithelial cells.

LKC patients exhibit accelerated apoptosis of conjunctival epithelial cells, which is most severe in the goblet cell-rich areas of the bulbar conjunctiva. This phenomenon has been investigated experimentally in dry eye dogs that spontaneously develop LKC and in a mouse model where dry eye is induced by systemically administered anticholinergic agents coupled with a desiccating environment. Apoptosis is readily detected in both systems by indirect staining for chromosomal DNA released as a consequence of cell death [10, 46]. Interestingly, high level expression of the apoptotic indicator proteins fas, fasL, and p53 in conjunctival tissue of dry eye dogs was reversed by treatment with the immunomodulatory drug, cyclosporin A [10]. Similarly, experimentally induced apoptosis was observed in murine conjunctival epithelium (as compared with uninduced control animals), and the induced apoptosis could be reversed by administration of cyclosporin A [46].

Retinoid deficiency can inhibit proper differentiation of ocular surface epithelial cells, resulting in squamous metaplasia and a reduced concentration of mucin-secreting conjunctival goblet cells [36]. Alkaline or acidic chemical insult, in addition to possible damage to the epithelium, can destroy conjunctival goblet cells, and damage lacrimal and meibomian gland ducts. Reduced soluble mucin concentration may destabilize the tear film, and can lead to LKC.

2.3.3 Inflammation

Numerous studies, together with the therapeutic response of LKC to anti-inflammatory drugs, underscore the importance of inflammation in its pathogenesis. Cellular and soluble mediators act in a number of ways in a series of complex interactions to promote and modulate ocular surface inflammation. Some mediators act as chemokines, stimulating chemotaxis of migrating inflammatory cells to sites of inflammation on the ocular surface. Other mediators stimulate expression of adhesion molecules such as ICAM-1 on conjunctival vasculature and epithelial cells. These act by binding proteins called integrins on the surfaces of chemoattracted inflammatory cells to help retain them at sites of inflammation. Certain cytokines and other mediators activate inflammatory cells once they arrive to begin a proinflammatory program of gene expression, secretion of more mediators, and other functions. Other activities of inflammatory mediators include alteration of epithelial proliferation and differentiation, stimulation of protease production and activation (see Sect. 1.3.2), promotion of apoptosis (see Sect. 1.3.2), and sensitization of ocular surface pain receptors.

T-lymphocyte infiltration clearly indicates a well-developed inflammatory process. T-cell infiltration of the conjunctival epithelium and substantia propria is found in both Sjögren's and non-Sjögren's LKC [31, 42]. Not only are T-cell numbers elevated, but the population is shifted from predominately cytotoxic T_{Killer} cells (CD8 marked) to T_H cells (T-helper cells;

CD4 marked), which also display increased levels of CD11a (an α-integrin subunit) and CD23 (an IgE receptor) on their surfaces, indicators of an activated state [21]. Treatment of LKC patients with the immunomodulatory drug cyclosporin A decreases the numbers of T cells in the conjunctiva.

Increased levels of a number of proinflammatory cytokines, including IL-1α and β, IL-6, TGFβ1, and TNF-α, have been detected in the conjunctival epithelium of LKC patients, as well as in tear fluid (IL-1α and β, IL-6) [18, 32, 40]. Evidence suggests that at least some of these cytokines are synthesized by activated conjunctival epithelial cells. Correlating with this proinflammatory cytokine spectrum, increased IL-1β and a decreased ratio of IL-1α to IL-1 receptor antagonist (IL-1Ra) were observed in conjunctival epithelium [40]. Elevated levels of the cell surface immune activation markers ICAM-1, HLA-DR, and CD40/CD40 ligand (CD40L) are also present in the conjunctival epithelium of both Sjögren's and non-Sjögren's LKC patients [18, 46].

Exactly how ocular surface inflammation in LKC arises is not completely understood. Clearly, systemic autoimmune disease, such as Sjögren's syndrome, or androgen deficiency predispose some classes of patients, and desiccating environmental stress appears to be an important trigger. Consistent with this, human corneal epithelial cells respond to a hyperosmolar environment (as would result from desiccation of the tear film) by activating a cascade of stress associated protein kinases, which in turn activate transcriptional regulators of inflammatory cytokine and MMP production. Inflammatory mediators produced by ocular surface epithelial cells could initiate or contribute to an inflammatory cascade leading to dysfunction of other components of the lacrimal functional unit, for example, tear-secreting glands. Cytokines from ocular surface epithelial cells could also affect proliferation, differentiation, or apoptosis of other epithelial cells. Finally, the proinflammatory cytokines IL-1α, TNF-α, and TGF-β1 are potent stimulators of MMP production (including gelatinases, collegenases, and stromelysins) by cultured human corneal epithelial cells [22, 23]. In addition to their degradation of tight junction proteins (see Sect. 1.3.1), MMPs can proteolytically activate latent proinflammatory cytokines such as IL-1β, TNF-α, and TGF-β, and neural peptides such as substance P. Although only parts of the overall picture are visible at present, evidence is accumulating that epithelial cells, in addition to T lymphocytes, are important and direct participants in the ocular surface inflammation characteristic of LKC.

2.4 Diagnosis

Diagnosis of LKC or dry eye disease is complicated by symptoms that are common to other ocular surface disorders as well. However, two sorts of complaints are suggestive of dry eye: exacerbation of ocular irritation by environmental stress, such as the low humidity of airplanes, smoky environments, or drafts from air conditioners, and exacerbation by activities that require prolonged visual attention, such as reading or viewing a video display terminal. A history consistent with Sjögren's syndrome, or with other autoimmune disorders, should also raise the possibility of dry eye. To diagnose LKC or dry eye with confidence, objective tests are necessary, and many clinicians prefer to use multiple tests.

An unstable tear film, resulting from inflammatory compositional alterations of the aqueous, mucin and lipid components, is the hallmark of dry eye [41]; therefore measurement of the tear film break-up time (TBUT) is probably the most important clinically used objective test for LKC or dry eye because it assesses tear film stability in a nearly direct manner. The test is conducted by introducing fluorescein into the lower conjunctival sac by micropipette, insertion of a fluorescein strip wetted with saline, or, perhaps better, a commercial pre-wetted fluorescein strip that minimizes the volume change of the tear film. The patient is asked to blink, and the time interval between a complete blink and the appearance of the first dry spot or discontinuity in the precorneal tear film (viewed through a yellow filter) is recorded. Topical anaesthesia and lid holding are discouraged be-

cause they reduce tear break-up time. Three repetitions are recommended, and although there is no consensus, an average value of less than 10 s is considered abnormal by many. Dry spots or discontinuities are thought to occur where the tear film thins to the extent that the lipid layer "sinks" into the mucin layer because of their proximity and similar hydrophobicity, and the aqueous layer retracts locally from the hydrophobic spot; however, this explanation is not universally accepted. Anomalous results may be caused by discontinuities of the corneal surface that cause persistent tear break-up in a single location. Tears deficient in either aqueous, mucin, or lipid components may exhibit tear film instability; therefore the test does not distinguish lacrimal dysfunction from meibomian gland dysfunction.

Non-invasive alternative methods of measuring tear film break-up substitute a regular pattern reflected from the tear film for instillation of fluorescein. These methods avoid artefacts that are possible with the fluorescein method, but require specialized instrumentation, for example, computerized videokeratoscopy.

The Schirmer test measures tear volume and/or production. The test is performed by insertion of a standardized strip of filter paper over the lid margin at the junction of the medial and lateral third of the lower lid. Tear production is measured by the number of millimetres of the paper strip wetted in 5 min. A Schirmer I (without anaesthesia) value of 5 mm/5 min or less indicates dry eye; however, because of variability in the test, this cut-off value may not be especially sensitive for diagnosis. Several variations of the Schirmer test have attempted to improve its reproducibility. Instillation of topical anaesthesia prior to sampling tear production (sometimes called the Schirmer II test) minimizes reflex tear production. Use of a phenol red-impregnated cotton thread instead of a strip of filter paper is less irritating; wetting is readily visualized by colour change due to the slightly alkaline pH of tears.

Meibomian gland disease usually does not show a decrease in aqueous tear production. It can be evaluated by biomicroscopic examination of the glands, looking for ductal orifice metaplasia, reduced expressibility of secretions, increased turbidity and viscosity of expressed secretions, and dropout of glandular acini.

Diagnostic dye staining is a simple and practical method to evaluate the severity of ocular surface damage in LKC. Fluorescein dye stains the epithelium and underlying tissue in areas where barrier function has been disrupted due to death or desquamation of the apical epithelium. It also stains the stroma at sites of other epithelial defects. The barrier function of healthy corneal epithelium prevents such penetration. Fluorescein is introduced from a strip as for the tear break-up test, and viewed through a yellow filter. A cornea with LKC shows a number of stained dots and perhaps some confluent staining areas or some more linear staining patterns characteristic of filamentary keratitis. Several grading schemes have been proposed to quantify the severity of corneal staining, which correlates well with other measures of LKC [4].

Rose bengal, applied from a strip or as a solution, stains the conjunctiva more effectively than the cornea. It stains devitalized epithelial cells and also healthy epithelial cells that are not protected by a normal mucin layer; therefore, it evaluates the protective status of the preocular tear film [35]. The classic rose bengal staining pattern for dry eye is two triangles (nasal and temporal) in the interpalpebral conjunctiva – the region that is usually exposed when the eyes are open. Rose bengal staining also correlates well with other objective measures of LKC, such as the Schirmer test.

Impression cytology uses cellulose acetate filter strips applied with gentle pressure to different areas of the conjunctiva to obtain superficial cells for analysis. Topical anaesthesia is recommended for patient comfort. Goblet cell density and epithelial morphology can be readily assessed from the filters. For example, the extent and severity of squamous metaplasia can be graded based on loss of goblet cells, enlargement and increased ratio of cytoplasm to nucleus of superficial epithelial cells, and increased keratinization relative to normal samples. Squamous metaplasia of the bulbar conjunctiva is especially prevalent in Sjögren's syndrome LKC, although it can occur in a variety of other dry eye conditions [31].

Impression cytology is a highly sensitive means to detect pathologic changes on the conjunctival surface, and thereby confirm a clinical diagnosis. Impression cytology samples can also be immunostained to detect cell surface mucins as well as specific markers for particular types of infiltrating inflammatory cells. Routine use of impression cytology in clinical settings is probably limited by lack of facilities to stain and microscopically examine the filters.

Summary for the Clinician

- **Irritation from desiccating environments or from prolonged visual concentration suggests dry eye**
- **Autoimmune disease is an important predisposing factor**
- **Diagnosis is complicated by varying symptoms – multiple objective tests for dry eye are recommended**
- **Tear break-up time and the Schirmer test evaluate tear film stability and tear volume/production, respectively**
- **Fluorescein staining of the cornea and rose bengal staining of the conjunctiva reveal the severity of damage to ocular surface epithelia**

2.5 Therapies

Traditional therapies for dry eye disease or LKC are mostly aimed at augmenting the depleted tear film. Recently, an anti-inflammatory therapy that treats the underlying disease process has become commercially available.

The first goal of therapy is to minimize factors that exacerbate dry eye or LKC. Use of systemic anticholinergic medications (antihistamine and antidepressants), which may decrease tear production, should be minimized or eliminated. Exposure to desiccating environments should be avoided, where possible. Lowering computer displays to decrease the interpalpebral aperture, and periodic breaks from reading or computer work to close one's eyes may help.

Artificial tear solutions contain polymers, electrolytes, and buffering agents to mimic normal tear viscosity, osmolarity and pH. Aqueous preparations may be used as required for temporary improvement of symptoms; preservative-free preparations (lacking benzalkonium chloride) are recommended for patients who use tears more than 4 times per day. Newer emulsion tears, which contain a lipid component, are also effective, but the number of doses may be limited to about four per day because excessive use may cause blurred vision. Artificial tears may temporarily relieve symptoms and discomfort, but they do not reverse conjunctival squamous metaplasia, indicating that they do not treat the underlying pathology of dry eye.

Punctal occlusion is a useful and practical therapy for conserving tears. Occlusion by semipermanent plugs of silicone or thermolabile polymers inserted into the punctal orifice has the advantage of being readily reversible. Punctal plugs can improve symptoms and ocular surface dye staining. Permanent punctal occlusion by thermocautery or radio frequency needle can be performed using a topical anesthetic. If punctal occlusion is performed, it is still important to resolve inflammation within the lacrimal functional unit in order to avoid retaining proinflammatory tears on the ocular surface.

Anti-inflammatory therapy should be considered for moderate or severe dry eye (e.g. any patient with corneal epithelial disease), or for patients with symptoms that persist despite artificial tear use and improvements in their environment. A recently FDA-approved therapy for ocular inflammation associated with LKC contains cyclosporin A 0.05% in an emulsion vehicle (Restasis, Allergan, Inc.). Cyclosporin A is an anti-inflammatory compound that acts by binding an intracellular protein (cyclophilin) involved in a regulatory cascade that ultimately controls transcription factors required for T-cell activation and cytokine production. Cyclophilin binding of cyclosporin A also inhibits an early event in mitochondrially mediated apoptosis. Clinical trials of cyclosporin A 0.05% instilled BID showed significantly greater improvement in two objective measures of dry eye disease or LKC, corneal fluorescein staining and anaesthetized Schirmer test values, than was observed for the vehicle [37]. Clinical improve-

ment was accompanied by decreased expression of immune activation markers and apoptosis markers, and decreased numbers of T lymphocytes in the conjunctiva of cyclosporin A-treated patients, indicating a reversal of the inflammatory disease underlying LKC [21]. It should be noted that the emulsion vehicle itself showed provided significant short-term improvements in symptoms, but failed to improve the indicators of inflammation in conjunctival tissue. The excellent safety profile of cyclosporin A permits prolonged BID dosing.

Topically administered corticosteroids have been reported to improve dry eye signs and symptoms in several studies. However, because of increased risks of elevated intraocular pressure, cataract formation, and infection, they should be used in short pulses or minimal doses.

Whether or not punctal occlusion or anti-inflammatory therapy is employed, the therapeutic regimen should attempt to eliminate or minimize environmental factors that may contribute to dry eye or LKC and should include artificial tears as a palliative treatment.

Summary for the Clinician

- **Minimize environmental factors and medications that can exacerbate dry eye**
- **Artificial tears are useful for temporary relief of symptoms**
- **Punctal occlusion can help conserve tears, but inflammation must also be addressed, to avoid retention of inflammatory tears on the ocular surface**
- **Anti-inflammatory therapy that treats the underlying pathology should be considered for moderate to severe dry eye**

References

1. Afonso A, Sobrin L, Monroy DC, et al. (1999) Tear fluid gelatinase B activity correlates with IL-1α concentration and fluorescein tear clearance. Invest Ophthalmol Vis Sci 40:2506–2512
2. Bacman S, Berra A, Sterin-Borda L, et al. (2001) Muscarinic acetylcholine receptor antibodies as a new marker of dry eye Sjogren syndrome. Invest Ophthalmol Vis Sci 42:321–327
3. Danjo Y, Watanabe H, Tisdale AS, et al. (1998) Alteration of mucin in human conjunctival epithelia in dry eye. Invest Ophthalmol Vis Sci 39: 2602–2609
4. De Paiva CS, Lindsey JL, Pflugfelder SP (2003) Assessing the severity of keratitis sicca with videokeratoscopic indices. Ophthalmology 110:1102–1109
5. Diebold Y, Rios JD, Hodges RR, et al. (2001) Presence of nerves and their receptors in mouse and human conjunctival goblet cells. Invest Ophthalmol Vis Sci 42:2270–2282
6. Dilly PN (1994) Structure and function of the tear film. Adv Exp Med Biol 350:239–247
7. Donnenfeld ED, Solomon K, Perry HD, et al. (2003) The effect of hinge position on corneal sensation and dry eye after LASIK. Ophthalmology 110:1023–1029
8. Draper CE, Adeghate EA, Singh J, et al. (1999) Evidence to suggest morphological and physiological alterations of lacrimal gland acini with ageing. Exp Eye Res 68:265–276
9. Dursun D, Monroy D, Knighton R, et al. (2000) The effects of experimental tear film removal on corneal surface regularity and barrier function. Ophthalmology 107:1754–1760
10. Gao J, Schwalb TA, Addeo JV, et al. (1998) The role of apoptosis in the pathogenesis of canine keratoconjunctivitis sicca: the effect of topical cyclosporin A therapy. Cornea 17:654–663
11. Gipson IK, Inatomi T (1998) Cellular origin of mucins of the ocular surface tear film. Adv Exp Med Biol 438:221–227
12. Heigle TJ, Pflugfelder SC (1996) Aqueous tear production in patients with neurotrophic keratitis. Cornea 15:135–138
13. Hodges RR, Zoukhri D, Sergheraert C, et al. (1997) Identification of vasoactive intestinal peptide receptor subtypes in the lacrimal gland and their signal-transducing components. Invest Ophthalmol Vis Sci 38:610–619
14. Huang AJ, Tseng SC, Kenyon KR (1990) Alteration of epithelial paracellular permeability during corneal epithelial wound healing. Invest Ophthalmol Vis Sci 31:429–435
15. Humphreys-Beher MG, Brayer J, Yamachika S, et al. (1999) An alternative perspective to the immune response in autoimmune exocrinopathy: induction of functional quiescence rather than destructive autoaggression. Scand J Immunol 49:7–10
16. Inatomi T, Spurr-Michaud S, Tisdale AS, et al. (1996) Expression of secretory mucin genes by human conjunctival epithelia. Invest Ophthalmol Vis Sci 37:1684–1692

17. Jones DT, Ji A, Monroy D, Pflugfelder SC (1998) Evaluation of ocular surface cytokine, mucin, and cytokeratin expression in Sjögren's syndrome. Adv Exp Med Biol 438:533–536
18. Jones DT, Yen M, Monroy D, et al. (1994) Evaluation of cytokine expression in the conjunctival epithelia of Sjogren's syndrome patients. Invest Ophthalmol Vis Sci 35:3493–3504
19. Kimura-Shimmyo A, Kashiwamura S, Ueda H, et al. (2002) Cytokine-induced injury of the lacrimal and salivary glands. J Immunother 25(Suppl 1):S42–51
20. Kunert KS, Tisdale AS, Gipson IK (2002) Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. Arch Ophthalmol 120:330–337
21. Kunert KS, Tisdale AS, Stern ME, et al. (2000) Analysis of topical cyclosporine treatment of patients with dry eye syndrome: effect on conjunctival lymphocytes. Arch Ophthalmol 118:1489–1496
22. Li DQ, Lokeshwar BL, Solomon A, et al. (2001) Regulation of MMP-9 production by human corneal epithelial cells. Exp Eye Res 73:449–459
23. Li D-Q, Tie Yan Shang TY, Kim H-S, et al. (2003) Regulated expression of collagenases (MMP-1, -8, -13) and stromelysins (MMP-3, -10, -11) by human corneal epithelial cells. Invest Ophthalmol Vis Sci 44:2928–2936
24. Liu Z, Pflugfelder SC (1999) Corneal surface regularity and the effect of artificial tears in aqueous tear deficiency. Ophthalmology 106:939–943
25. Meneray MA, Fields TY, Bennett DJ (1997) G_s and $G_{q/11}$ couple vasoactive intestinal peptide and cholinergic stimulation to lacrimal secretion. Invest Ophthalmol Vis Sci 38:1261–1270
26. Moss SE, Klein R, Klein BE (2000) Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol 118:1264–1268
27. Nakamura T, Nishida K, Dota A, et al. (2001) Elevated expression of transglutaminase 1 and keratinization-related proteins in conjunctiva in severe ocular surface disease. Invest Ophthalmol Vis Sci 42:549–556
28. Nava A, Barton K, Monroy DC, et al. (1997) The effects of age, gender, and fluid dynamics on the concentration of tear film epidermal growth factor. Cornea 16:430–438
29. Ogawa Y, Okamoto S, Wakui M, et al. (1999) Dry eye after haematopoietic stem cell transplantation. Br J Ophthalmol 83:1125–1130
30. Pepose JS, Akata RF, Pflugfelder SC, et al. (1990) Mononuclear cell phenotypes and immunoglobulin gene rearrangements in lacrimal gland biopsies from patients with Sjögren's syndrome. Ophthalmology 97:1599–1605
31. Pflugfelder SC, Huang AJ, Feuer W, et al. (1990) Conjunctival cytologic features of primary Sjogren's syndrome. Ophthalmology 97:985–991
32. Pflugfelder SC, Jones D, Ji Z, et al. (1999) Altered cytokine balance in the tear fluid and conjunctiva of patients with Sjogren's syndrome keratoconjunctivitis sicca. Curr Eye Res 19:201–211
33. Pflugfelder SC, Solomon A, Stern ME (2000) The diagnosis and management of dry eye: a twenty-five-year review. Cornea 19:644–649
34. Pflugfelder SC, Tseng SCG, Sanabria O, et al. (1998) Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. Cornea 17:38–56
35. Pflugfelder SC, Tseng SCG, Yoshino K, et al. (1997) Correlation of goblet cell density and mucosal epithelial mucin expression with rose bengal staining in patients with ocular irritation. Ophthalmology 104:223–235
36. Rao V, Friend J, Thoft RA, et al. (1987) Conjunctival goblet cells and mitotic rate in children with retinol deficiency and measles. Arch Ophthalmol 105:378–380
37. Sall K, Stevenson OD, Mundorf TK, et al. (2000) Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. Ophthalmology 107:631–639
38. Schein OD, Munoz B, Tielsch JM, et al. (1997) Prevalence of dry eye among the elderly. Am J Ophthalmol 124:723–728
39. Sobrin L, Liu Z, Monroy DC, et al. (2000) Regulation of MMP-9 activity in human tear fluid and corneal epithelial culture supernatant. Invest Ophthalmol Vis Sci 41:1703–1709
40. Solomon A, Dursun D, Liu Z, et al. (2001) Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci 42:2283–2292
41. Stern ME, Beuerman RW, Fox RI, et al. (1998) The pathology of dry eye: the interaction between the ocular surface and lacrimal glands. Cornea 17:584–589
42. Stern ME, Gao J, Schwalb TA, et al. (2002) Conjunctival T-cell subpopulations in Sjogren's and non-Sjogren's patients with dry eye. Invest Ophthalmol Vis Sci 43:2609–2614
43. Sullivan DA, Wickham LA, Rocha EM, et al. (1998) Influence of gender, sex steroid hormones and the hypothalamic-pituitary axis in the structure and function of the lacrimal gland. Adv Exp Med Biol 438:11–42
44. Sundermeier T, Matthews G, Brink PR, et al. (2002) Calcium dependence of exocytosis in lacrimal gland acinar cells. Am J Physiol 282: C360–C365

45. Walcott B, Moore L, Birzgalis A, et al. (2002) A model of fluid secretion by the acinar cells of the mouse lacrimal gland. Adv Exp Med Biol 506: 191–197
46. Yeh S, Song XJ, Farley W, et al. (2003) Apoptosis of ocular surface cells in experimentally induced dry eye. Invest Ophthalmol Vis Sci 44:124–129
47. Yi X, Wang Y, Yu FS (2000) Corneal epithelial tight junctions and their response to lipopolysaccharide challenge. Invest Ophthalmol Vis Sci 41: 4093–4100
48. Zhao H, Jumblatt JE, Wood TO, et al. (2001) Quantification of MUC5AC protein in human tears. Cornea 20:873–877
49. Zhu Z, Stevenson D, Ritter T, et al. (2002) Expression of IL-10 and TNF-inhibitor genes in lacrimal gland epithelial cells suppresses their ability to activate lymphocytes. Cornea 21:210–214

Ocular Cicatricial Pemphigoid

3

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Core Messages

- Mucous membrane pemphigoid (MMP) is a group of chronic systemic autoimmune disorders characterized primarily by T-cell and B-cell dysfunction. Ocular cicatricial pemphigoid (OCP) is the MMP subset affecting the eye
- A key defect in the immune system response causes production of autoantibodies (IgG and IgA) directed against the β_4-subunit of $\alpha_6\beta_4$-integrin, and epiligrin (laminin type 5) in the conjunctival epithelium (hemidesmosome-epithelial membrane complex)
- HLA-DR2, HLA-DR4 [HLA-DR*0401], HLA-DQw7 [DQB1*0301], HLA A2, HLA-B*8, HLA-B*35 and HLA-B*49 genotypes may confer an increased genetic susceptibility to OCP
- A conformational change in the β4 peptide may lead to antigenic stimulation of certain B-cell clones that elaborate antibodies against the conjunctival basement membrane zone (BMZ) glycoproteins
- Proinflammatory cytokine mediated fibroblast activation leads to collagen production and subsequent cicatrization of the conjunctiva
- Conjunctival fibrosis produces an environment that leads to progressive corneal damage and loss of vision
- Extraocular manifestations of OCP must be sought in all patients to rule out potentially lethal systemic involvement (ex. esophagus or trachea)
- Staging using the Modified Foster Staging System early as a part of initial evaluation helps in management and prognosis
- Demonstration of linear deposition of immunoreactants [(IgG, IgA, IgM) or complement (C3)] at the basement membrane zone (BMZ) of biopsied inflamed conjunctiva is necessary for definitive diagnosis
- An algorithmic approach of immunomodulation is recommended for treating the disorder

3.1 Introduction

Ocular cicatricial pemphigoid (OCP) belongs to a family of diseases known as mucous membrane pemphigoid (MMP). It is a group of chronic systemic autoimmune disorders characterized primarily by T-cell and B-cell dysfunction, with production of autoantibodies targeted to adhesion molecules in the conjunctival basement membrane zone. Cicatricial pemphigoid can affect not only the conjunctiva but also mucous membranes in the mouth, trachea, pharynx, larynx, esophagus, vagina, anus and urethra.

3.2 Pathophysiology

The pathophysiologic mechanisms of OCP and its associated conjunctival fibrosis have not been completely elucidated. It is thought that a critical defect in immune system regulation causes production of autoantibodies (IgG and IgA) [9, 24] directed against key subunits of integrin, epiligrin and laminin in the conjunctival epithelium (hemidesmosome-epithelial membrane complex) [16, 31].

Many potential "triggers" have been proposed. These include genetic predisposition and exposure to topical or systemic medication. It is thought that human leukocyte antigen DR2 (HLA-DR2), human leukocyte antigen DR4 (HLA-DR4) and human leukocyte antigen DQw7 (HLA-DQw7) genotypes confer an increased genetic susceptibility to development of OCP [1, 2]. This may occur in a "double-hit" manner where an environmental insult in a genetically susceptible individual leads to disease.

Some patients may develop drug-induced pemphigoid in response to systemic or topical medication. Drugs implicated include systemic practolol, topical pilocarpine, epinephrine, phospholine iodide, timolol, and idoxyuridine [14, 27].

Autoantibodies isolated from sera of patients with OCP recognize and bind to the β_4-subunit of the $\alpha_6\beta_4$-integrin [10]. Autoantibodies to epiligrin (laminin 5) [13], β_4-integrin [29], and α_6-integrin [15] have been identified in some patients. The antibodies bind to the target antigen and begin a sequence of events that results in chronic inflammation and, eventually, in fibrosis. The fibrosis progresses to involve the lacrimal ductules and meibomian ducts, leading to tear film abnormalities (decreased mucin and aqueous component of tears), disorientation of lash follicles, with trichiasis, conjunctival shrinkage, and keratinization. These complications conspire to produce corneal damage and loss of vision.

3.3 Epidemiology

OCP is relatively rare, with an estimated 20,000 cases in the United States [10]. The disease appears to be distributed worldwide, with no specific geographical predilection. It is most often recognized in the elderly. In a study of 130 patients referred to us, the average age was 64 years, with a range of 20–87 years [9]. Determination of the exact age spectrum for OCP, however, is difficult, because most patients in early stages remain undiagnosed. The disease affects more women than men, with a ratio of 1.6 to 1 [21].

3.4 Clinical History

OCP patients typically present with symptoms of chronic conjunctivitis. Complaints include, but are not limited to: redness, dry eyes, blepharospasm, itching, burning sensation, light sensitivity, foreign body sensation, swelling and heaviness of eyelids, decreased vision and diplopia. Often, non-ocular areas such as skin, mouth, pharynx, larynx, trachea, esophagus, vagina, urethra and anus may also be affected.

3.5 Clinical Signs

The external examination discloses conjunctival hyperemia, with subepithelial fibrosis to one degree or other, along with (variably) eyelid and corneal abnormalities (Table 3.1).

Table 3.1. Clinical signs on slit lamp examination

Eyelids	Conjunctiva	Cornea
Trichiasis	Papillae/follicles	Superficial punctate keratitis
Distichiasis	Keratinization	Epithelial defect
Blepharitis	Subepithelial fibrosis[a]	Stromal ulcer
Meibomian gland dysfunction	Conjunctival shrinkage	Neovascularization
	Foreshortening/obliteration of fornices[b]	Keratinization
	Symblepharon	Limbitis
	Ankyloblepharon	Perforation

[a] Fine white striae surrounding superficial vessels of substantia propria.
[b] Inferior fornix usually involved initially.

3.6 Staging

We proposed a combined staging system that incorporates the strengths of the original Foster [12] and Mondino [21] systems of classification. We find it useful to describe four stages that help in management and prognosis of patients with cicatricial pemphigoid. And although OCP is typically bilateral, it may be asymmetrical. Therefore, it is important to stage each eye separately.

Stage 1 findings include conjunctival inflammation, mucoid discharge, small patches of rose bengal-staining conjunctival epithelium, and conjunctival subepithelial fibrosis. The fibrosis at this stage is typically subtle and best seen with slit lamp biomicroscopy. It appears as fine white striae most easily seen surrounding the superficial blood vessels in the substantia propria (Fig. 3.1). These connective tissue striae contract and lead to conjunctival shrinkage, producing foreshortening of the inferior fornix and blunting of the angle of reflection of the conjunctiva from the eyelid and fornix onto the globe, the characteristic features of stage 2 disease (Fig. 3.2). Stage 2 can be subclassified into subcategories *a* through *d* to describe the degree of fornix foreshortening:

a. 0–25 %
b. 25–50 %
c. 50–75 %
d. 75–100 %

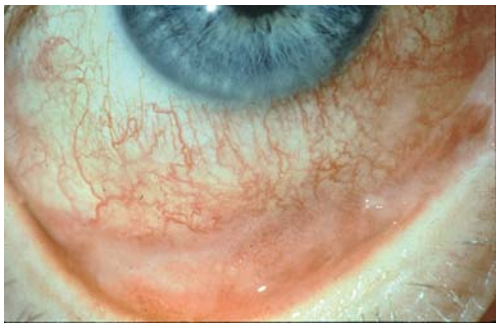

Fig. 3.1. Subepithelial fibrosis, tarsal conjunctiva. Note the striae of fibrotic material under the epithelium

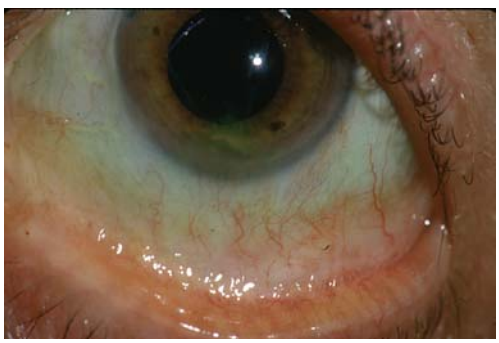

Fig. 3.2. Subepithelial fibrosis, with fornix foreshortening

Symblepharon formation, resulting from the progressive contraction of subepithelial bands of connective tissue, marks stage 3 disease (Fig. 3.3). The percentage of horizontal involve-

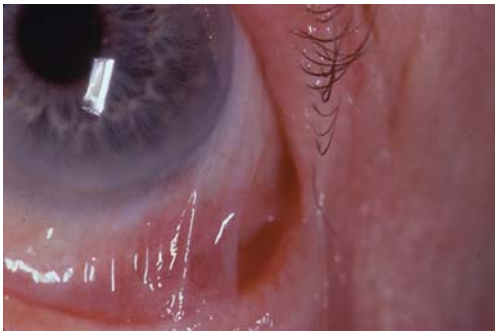

Fig. 3.3. Subepithelial fibrosis with fornix foreshortening and symblepharon formation

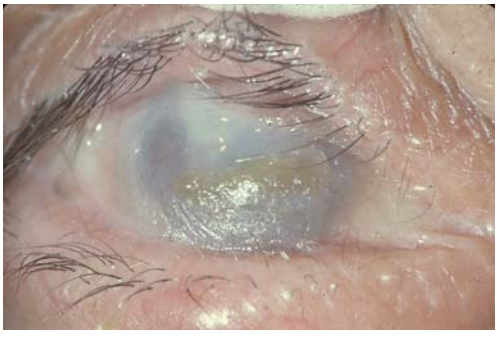

Fig. 3.4. End-stage pemphigoid with total keratinization of the ocular surface

ment by symblephra can also be subclassified from *a* through *d*:

a. 0–25 %
b. 25–50 %
c. 50–75 %
d. Greater than 75 % involvement by symblephra

End stage cicatricial pemphigoid or Stage 4 is defined by severe sicca syndrome, ocular surface keratinization and ankyloblepharon (Fig. 3.4).

3.7 Diagnosis

The diagnosis of OCP is based on the clinical presentation and immunofluorescent studies of the conjunctiva.

3.7.1 Laboratory Tests

Currently no standard laboratory tests are available to either diagnose the disease or to monitor its activity. Such assays are in the process of development. Some studies have correlated decreased levels of interleukins, while other studies have demonstrated increased levels of tumor necrosis factor alpha in patients with active disease [17]. Patients on immunosuppressive therapy are routinely monitored with blood studies in order to avoid serious side effects.

3.7.2 Conjunctival Biopsy

Demonstration of linear deposition of immunoreactants [immunoglobulin (IgG, IgA, IgM) or complement (C3)] at the conjunctival epithelial basement membrane zone (BMZ) of biopsied inflamed conjunctiva is the gold standard for diagnosis of OCP (Fig. 3.5). The biopsy, processing, and staining is best performed by ophthalmologists and technicians experienced in handling the delicate specimens. A negative or inconclusive biopsy does not exclude the diagnosis; such a result may occur as a consequence of poor handling of tissue or improper technique, and "true" false negatives may also occur [11].

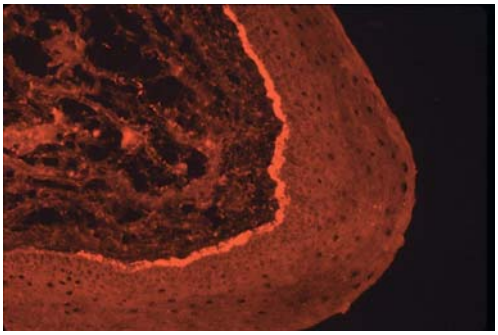

Fig. 3.5. Immunofluoresence probing of biopsied conjunctiva of a patient suspected of having cicatricial pemphigoid. The antibody is goat anti-human IgG. Note the striking positivity of the epithelial basement membrane zone. The negative control, directed against human albumin, was negative, thereby confirming the clinical suspicion of cicatricial pemphigoid

3.7.3 Histology

Hematoxylin and eosin staining shows infiltration of the inflamed conjunctiva with plasma cells, neutrophils, eosinophils, Langerhans cells and macrophages. The PAS positive goblet cell population is variably decreased (dependent on severity and chronicity of inflammation).

Giemsa stain results show that the total mast cell number and ratio of connective tissue mast cells to mucosal mast cells are significantly higher than in normal conjunctiva.

3.8 Treatment

3.8.1 Medical

Ocular cicatricial pemphigoid is a chronic, systemic, autoimmune disease with evidence of immune system dysregulation. Unfortunately, most patients are diagnosed in advanced stages, when the most aggressive measures must be implemented to save sight.

3.8.1.1 Topical

No topical agent has been found effective in treating OCP. Artificial tears form part of a "cocktail" that together helps maintain a hospitable physiological ocular environment.

3.8.1.2 Injections

Some patients may require subconjunctival steroid injections or subconjunctival injections of mitomycin C to slow disease progression while systemic therapy takes effect. Such regional therapy is usually transient and of limited benefit for long-term use [6].

3.8.1.3 Systemic

Systemic corticosteroids are effective in controlling active OCP [23]. The high doses required for such control, and the inevitable recurrence of inflammation upon cessation of steroids, restricts their use and essentially makes them less efficacious than immunomodulatory agents [20, 22]. Long-term steroid therapy is associated with inescapable side effects (e.g. avascular necrosis of femoral head, osteoporosis, hypertension, glucose intolerance with development of steroid-induced diabetes mellitus). The high potential for complications mandates that corticosteroids should be reserved for short-term therapy in conjunction with long-term immunomodulatory therapy (IMT) in severely inflamed eyes. If corticosteroids are integrated into the treatment program, they should be used for no longer than 3 months, inclusive of tapering.

3.8.1.4 Immunomodulation

When used correctly by properly trained doctors, this mode of therapy is not only effective, but also quite safe [9, 12, 22, 25]. In fact, such medications can be safer to use than long-term systemic corticosteroids [12].

We recommend a stepwise approach to treating OCP. The first step involves assessment of a number of factors including: patients' health and medical conditions, severity of disease, and the rate of progression of disease. Patients with end-stage disease often have irreversible ocular damage and typically do not benefit from systemic immunomodulation. Treatment should be tailored to the patient, with the goal of abolition of all inflammation and prevention of further scarring.

Before any treatment recommendation can be made, the patient undergoes a formal medical evaluation. This includes a complete blood count and liver and renal function tests. Patient compliance is, of course, critical to successful therapy; the patient must be willing to return for regular follow-up visits with monitoring of blood parameters. The patient is advised to contact us and/or to promptly seek medical care if he/she develops symptoms of systemic disease (chills and fever).

For patients with mild to moderately active OCP, we generally begin therapy with diaminodiphenylsulfone (Dapsone), provided there are no contraindications (sulfa allergy, glucose-6-phosphate deyhydrogenase deficiency). Dapsone is a synthetic sulfone with both anti-inflammatory and antimicrobial activity. The drug interferes with neutrophil chemotaxis and prevents lysosomal enzyme release and phagocytosis. While Dapsone is highly effective when used short term, Fern and associates [8] have demonstrated this benefit to be temporary, with disease recurrence within weeks to months while on therapy. Patients who demonstrate a positive response can be maintained on a reduced dose.

This drug should not be used in G6PD-deficient patients and not in those with sulfa allergies. We typically begin with 25 mg twice daily, and advance to no higher than 50 mg three times daily. Sulfapyridine is a possible alternative [7].

Physicians should monitor patients with a complete blood count, net hemoglobin levels, reticulocyte count and liver enzymes, and at every visit exclude subclinical potential side-effects. These potential side-effects include hemolytic anemia and leukopenia that can occur especially 8–10 weeks after initiation of therapy. Dapsone should be stopped in such cases, and an alternative, such as azathioprine or methotrexate or mycophenolate mofetil, instituted. Patients whose OCP is incompletely controlled with Dapsone may require azathioprine or methotrexate as supplemental therapy. If the OCP remains active, azathioprine or mycophenolate mofetil may be substituted for Dapsone.

Azathioprine is a purine analog that is metabolized to produce 6-mercaptopurine, which inhibits both DNA and RNA synthesis. Dantzig first proposed the use of azathioprine for the treatment of mucus membrane pemphigoid, including that affecting conjunctiva [3].

Patients can be started on 2.0 mg/kg per day of azathioprine, and the dose can be increased to a maximum of 3.0 mg/kg per day. Monitoring is performed every 6 weeks with liver function tests and complete blood count. Potential treatment side-effects include chemical hepatitis, leukopenia, and arthralgias.

Methotrexate is a folic acid antagonist with an exceptional safety and efficacy record. It has both anti-inflammatory and immunosuppressive actions; we employ methotrexate at an initial dose of 7.5 mg once a week, with a maximum of 35 mg once a week. The weekly dosing reduces the likelihood of potential adverse effects. Uncommon but possible adverse effects include bone marrow suppression, hepatic toxicity, pneumonitis, and oral ulcerations. Clinical studies have shown that the concurrent administration of folic acid or folinic acid with methotrexate does not reduce the efficacy of the drug and alleviates some of its potential toxicities. The concurrent use of folic acid, 1 mg/day, regular follow-up care, and the appropriate monitoring of hematologic parameters and hepatic enzyme levels minimize the risk of the potential adverse effects.

Other potential side-effects include nausea, fatigue and thinning of hair (often noticeable only to the patient). Patients intolerant to the gastrointestinal effects can often be successfully maintained on methotrexate by conversion to subcutaneous injection.

Patients are initially monitored 3 weeks into therapy with a complete blood count and liver function tests, and then every 6 weeks thereafter.

Mycophenolate mofetil (CellCept) has been used with good success in the care of patients with active cicatricial pemphigoid in our clinic at the Massachusetts Eye and Ear Infirmary. We typically begin at 1 g once daily, observing for tolerance to the medication for 1 week, before proceeding to our preferred dose of 1 g twice daily. Liver enzyme and CBC determinations are performed monthly, and the dosage can be increased in increments of 500 mg, but we have never exceeded 3 g/day.

Cyclophosphamide is the preferred initial mode of treatment for patients with severe inflammation. It has proven to be highly successful in treating active OCP [12]. Patients are initially started on 2 mg/kg/day (all taken in the morning). An increase in dosage is based on a combination of factors, including presence of any side-effects, and bone marrow tolerance. Patients are monitored with a complete blood count every 4 weeks; leukopenia is an expected side-effect when therapeutic levels have been reached. The dosage is adjusted to keep the peripheral white cell count at 4,500 cells/mm^3, with at least 1,500 neutrophils/mm^3 and 70,000 platelets/mm^3. While the likelihood of potential side-effects is low, the physician must promptly address thrombocytopenia, gastrointestinal symptoms, infection, and hemorrhagic cystitis. Urinalysis and renal function tests are routinely done every month to detect early manifestations of renal toxicity.

Resistance to chemotherapy, intolerance to its side-effects or failure to respond to a long-term immunomodulatory regimen are indications for prescribing intravenous immunoglobulin (IVIg) therapy. IVIg blocks the F_c receptor of B cells (thus inhibiting them), induces suppressor T (T_s) cell activity, decreases production of IL-10, modulates complement uptake on target cells and influences T-cell function. IVIg can stop OCP progression by decreasing the levels of anti-β-4 antibodies [18]. We give IVIg at a dose of 2–3g/kg per cycle, split into thirds, given over 5 h on each of three consecutive days. The rationale for spreading the dose over 3 days is to prevent the potential complications of volume overload and vaso-occlusion that are associated with high doses of IVIg delivered over a short interval. The total number of cycles is dependent on individual patient response but ranges from 15–29 cycles [18]. Commitment of both the patient and physician to a structured program of infusion is critical. We monitor patients with an antibody titer prior to staring treatment and then monthly until the end of the treatment program. There are two major contraindications to therapy: selective IgA deficiency and serious reaction to intravenous or intramuscular human immunoglobulin.

It has long been known that activated lymphocytes expressing the CD25 molecule (IL-2 receptor) play a role in inflammation associated with OCP. Daclizumab is a humanized IgG_1 monoclonal antibody directed against the α-chain of the CD25 molecule. Our experience with daclizumab in patients with OCP has been favorable, especially in patients resistant to conventional agents [26]. Like IVIg, daclizumab has few potential side-effects. The drug is given intravenously at a dose of 1 mg/kg per cycle. Cycles are repeated every 2 weeks for the first 12 weeks (total of seven doses). Then treatment is given every 3 weeks until the 24th week of treatment (total of four doses). Subsequently, the patients return every month until week 52 for an eye exam and IV infusions. In this last phase, the patients receive a total of seven doses [26]. Additional sessions may be required, depending on individual response to therapy. A small decrease in white cell count may occur. Treatment is discontinued if the count drops below 3,500 cells/mm^3, and then restarted once the counts return to normal. Patients should be monitored every 6 weeks with complete blood count, liver function tests, and renal function tests.

3.8.2 Surgery

Any attempt at surgery in patients with ocular cicatricial pemphigoid must be preceded by complete control of inflammation. Surgery is often employed to address the confounders that create or maintain a hostile ocular environment: trichiasis, posterior lid margin keratinization, sicca syndrome, corneal exposure, and entropion.

3.8.2.1 Epilation

Extraction of aberrant eye lashes is essential to prevent continued damage to the cornea and to allow an objective evaluation of the efficacy of a treatment program. Among the options available, permanent electro-epilation offers the best hope of eliminating the misdirected eyelashes; recurrences can be similarly retreated.

3.8.2.2 Dry Eyes

Dry eye syndrome often occurs in conjunction with meibomian gland dysfunction. Dry eyes can be treated with a vigorous regimen of ocular lubricants and punctual occlusion (punctual plugs or cautery). The latter often provides lasting relief. Meibomian gland dysfunction (MGD) is treated with warm compress, followed by lid massage to milk the glands and restore flow of meibum. Systemic tetracyclines are also prescribed.

3.8.2.3 Amniotic Membrane Transplantation

Amniotic membrane transplants (AMT) were first used in ophthalmology by De Rotth in 1940, who reported partial success in the treatment of conjunctival epithelial defects after symblepharon [4]. Human amniotic membrane is derived from placenta, which consists of the inner amniotic membrane made of a single layer of amnion cells fixed to collagen-rich mesenchyme six to eight cells thick loosely attached to chorion. It is composed of three layers: a single epithelial layer, thick basement membrane, and avascular stroma. Human amniotic membrane is nonimmunogenic and has a unique combination of anti-inflammatory proteins and growth factors that promote wound healing.

We use amniotic membrane grafting to promote healing of persistent epithelial defects *after* we have corrected problems that promote development of such defects in the first place, such as nocturnal lagophthalmos, trichiasis, and keratinization of the posterior lid margin. We use an "onlay" technique, in which the amniotic membrane is placed over the entire anterior surface of the globe, tacked down to conjunctiva, with 8–0 nylon interrupted sutures. A bandage soft contact lens is applied over this, and the amnion "melts" over the ensuing 4 weeks, typically revealing a total healing of the previously persistent epithelial defect.

Fornix reconstruction can be performed employing amniotic membrane, particularly if the degree of fornix reconstruction that is required is minimal; extensive dissections and reformation of totally obliterated fornices must be accomplished with mucous membrane grafting, and we typically use oral mucosa from inside the lower lip.

3.8.2.4 Limbal Stem Cell Transplantation

In autoimmune conditions such as ocular cicatricial pemphigoid (OCP) the amount of stem cell damage depends on severity and duration of disease activity. Chronic, severe disease can eliminate most, if not all, of the limbal stem cell population. In such cases, the corneal surface is replaced by conjunctival epithelium, with resultant corneal neovascularization and an irregular, semi-opaque surface that blurs vision and causes photosensitivity. Provided all OCP inflammation has been abolished, and provided anatomic abnormalities have been sufficiently corrected (sicca, trichiasis, exposure), limbal stem cell transplantation (LSCT) may be performed in an attempt to rehabilitate the ocular surface. But it is abundantly clear that patients with autoimmune processes, such as OCP, have a worse outcome after LSCT than patients with noninflammatory disorders.

3.8.2.5 Lamellar Keratoplasty/ Penetrating Keratoplasty

The most common indications for penetrating keratoplasty (PK) and lamellar keratoplasty (LK) are corneal perforation and extensive corneal scarring [29]. However, the outcome of even successful PK is poor in eyes with end-stage chronic cicatrizing conjunctival diseases such as OCP. This is primarily due to the im-

munologically driven conjunctival inflammation, trichiasis/distichiasis, severe dry eye and extensive corneal neovascularization. Graft failure occurs due to many factors either acting independently or in conjunction with one another; these include: immunologically driven inflammation, trichiasis/distichiasis, persistent epithelial defects, stromal ulceration, dry eye, meibomian gland disease, posterior lid margin keratinization, hypesthesia, exposure, corneal perforation, limbal stem cell deficiency, and graft rejection. In spite of the overall poor outcome, some success has been reported in patients adequately controlled with immunosuppressive therapy [29].

3.8.2.6 Keratoprosthesis

In patients with severe damage secondary to chronic cicatrizing disease, keratoprosthesis (KPro) may offer the only feasible alternative for recovery of functional vision. Suitable candidates are often those patients for whom penetrating keratoplasty has either been unsuccessful or not feasible. We again emphasize that before any surgical procedure, including KPro, is undertaken the underlying inflammatory process should be adequately controlled to ensure a successful outcome. Investigators have shown favorable long-term visual outcome in patients with ocular cicatricial pemphigoid [5]. The likelihood of complications remains high, with necrosis of tissue flanking the prosthesis leading the list. Other potential complications include extrusion of prosthesis, retinal detachment, and glaucoma.

3.9 Collaborative Care

An integral component of managing patients with ocular cicatricial pemphigoid is a close collaboration between the ophthalmologist and other health care professionals. While it is ideal for the primary ophthalmologist to be both skilled in giving chemotherapy and in managing any potential complications, this is not always possible. We often find it useful to collaborate with an ophthalmologist and a hematologist (or rheumatologist) more local to the patient's residence. The ophthalmologist monitors OCP activity and can titrate the therapy after discussion with the hematologist about blood parameters. This triad of care, in our experience, increases compliance and increases the likelihood of a favorable visual outcome.

3.10 Prognosis

Ocular cicatricial pemphigoid is a potentially blinding systemic disease. Treatment is challenging since most patients often present in late stages. We have previously shown that after withdrawal of immunosuppressive therapy for OCP, one-third of the patients remained in remission for an average of 4 years; of the 22% who relapsed, all regained control readily upon reinstitution of therapy [25]. Relapses are usually slow to develop; thus the disease can be controlled with reinstitution of therapy. Patients with both active and inactive disease must be periodically monitored for possible relapses later in life.

Summary for the Clinician

- **Ocular cicatricial pemphigoid is a chronic autoimmune disease with both systemic and ocular manifestations**
- **Ocular involvement can lead to progressive cicatrization with profound loss of vision**
- **Linear deposition of immunoglobulins at the basement membrane zone (BMZ) of conjunctival biopsy specimens of OCP patients is diagnostic**
- **The goal of therapy is to suppress inflammation, promote healing, and prevent cicatrization**
- **Steroid-sparing agents have fewer, preventable and reversible side effects in comparison to steroids for treatment of OCP**

References

1. Ahmed AR, Foster S, Zaltas M, et al. (1991) Association of DQw7 (DQB1*0301) with ocular cicatricial pemphigoid. Proc Natl Acad Sci U S A 88:11579–11582
2. Ahmed AR, Konqui A, Park MS, Tiwari JL, Terasaki PI (1984) DR antigens in bullous pemphigoid. Arch Dermatol 120:795
3. Dantzig PI (1974) Immunosuppressive and cytotoxic drugs in dermatology. Arch Dermatol 110:393–406
4. De Rotth A (1940) Plastic repair of conjunctival defects with fetal membranes. Arch Ophthalmol 23:522–525
5. Dohlman CH, Terada H (1998) Keratoprosthesis in pemphigoid and Stevens-Johnson syndrome. Adv Exp Med Biol 438:1021–1025
6. Donnenfeld ED, Perry HD, Wallerstein A, et al. (1999) Subconjunctival mitomycin C for the treatment of ocular cicatricial pemphigoid. Ophthalmology 106:72–78
7. Elder MJ, Leonard J, Dart JK (1996) Sulphapyridine – a new agent for the treatment of ocular cicatricial pemphigoid. Br J Ophthalmol 80:549–552
8. Fern AI, Jay JL, Young H, MacKie R (1992) Dapsone therapy for the acute inflammatory phase of ocular pemphigoid. Br J Ophthalmol 76:332–335
9. Foster CS (1986) Cicatricial pemphigoid. Trans Am Ophthalmol Soc 84:527–663
10. Foster CS (1993) Immunologic disorders of the conjunctiva, cornea, and sclera. In: Albert DM, Jakobiec FA (eds) Principles and practice of ophthalmology, vol 1. Philadelphia, Saunders, pp 196–199
11. Foster CS, Dutt JE, Rice BA, Kupferman AE, Lane L (1994) Conjunctival epithelial basement membrane zone immunohistology: normal and inflamed conjunctiva. Int Ophthalmol Clin 34:209–214
12. Foster CS, Wilson LA, Ekins MB (1982) Immunosuppressive therapy for progressive ocular cicatricial pemphigoid. Ophthalmology 89:340–353
13. Fujimoto W, Toi Y, Okazaki F, et al. (1999) Anti-epiligrin cicatricial pemphigoid with IgG autoantibodies to the beta and gamma subunits of laminin 5. J Am Acad Dermatol 40:637–639
14. Hirst LW, Werblin T, Novak M et al. (1982) Drug-induced cicatrizing conjunctivitis simulating ocular pemphigoid. Cornea 1:121
15. Kanitakis J, Zambruno G, Vassileva S, Giannetti A, Thivolet J (1992) Alpha-6 (CD 49f) integrin expression in genetic and acquired bullous skin diseases. A comparison of its distribution with bullous pemphigoid antigen. J Cutan Pathol 19:376–384
16. Kirtschig G, Marinkovich MP, Burgeson RE, Yancey KB (1995) Anti-basement membrane autoantibodies in patients with anti-epiligrin cicatricial pemphigoid bind the alpha subunit of laminin 5. J Invest Dermatol 105:543–548
17. Lee SJ, Li Z, Sherman B, Foster CS (1993) Serum levels of tumor necrosis factor-alpha and interleukin-6 in ocular cicatricial pemphigoid. Invest Ophthalmol Vis Sci 34:3522–3525
18. Letko E, Bhol K, Foster SC, Ahmed RA (2000) Influence of intravenous immunoglobulin therapy on serum levels of anti-beta 4 antibodies in ocular cicatricial pemphigoid. A correlation with disease activity. A preliminary study. Curr Eye Res 21:646–654
19. Miserocchi E, Baltatzis S, Roque MR, Ahmed AR, Foster CS (2002) The effect of treatment and its related side effects in patients with severe ocular cicatricial pemphigoid. Ophthalmology 109:111–118
20. Mondino BJ, Brown SI (1981) Ocular cicatricial pemphigoid. Ophthalmology 88:95–100
21. Mondino BJ, Brown SI (1983) Immunosuppressive therapy in ocular cicatricial pemphigoid. Am J Ophthalmol 96:453–459
22. Mondino BJ, Brown SI, Lempert S, Jenkins MS (1979) The acute manifestations of ocular cicatricial pemphigoid: diagnosis and treatment. Ophthalmology 86:543–555
23. Mondino BJ, Ross AN, Rabin BS, Brown SI (1977) Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 83:443–450
24. Neumann R, Tauber J, Foster CS (1991) Remission and recurrence after withdrawal of therapy for ocular cicatricial pemphigoid. Ophthalmology 98:858–862
25. Papaliodis GN, Chu D, Foster CS (2003) Treatment of ocular inflammatory disorders with daclizumab. Ophthalmology 110:786–789
26. Pouliquen Y, Patey A, Foster CS, Goichot L, Savoldelli M (1986) Drug-induced cicatricial pemphigoid affecting the conjunctiva. Light and electron microscopic features. Ophthalmology 93:775–783
27. Tugal-Tutkun I, Akova YA, Foster CS (1995) Penetrating keratoplasty in cicatrizing conjunctival diseases. Ophthalmology 102:576–585
28. Yancey KB, Kirtschig G, Yee C, Lazarova Z (1995) Studies of patients with anti-epiligrin cicatricial pemphigoid. J Dermatol 22:829–835

Immunomodulation for Corneal Transplantation

4

Douglas J. Coster, Keryn A. Williams

Core Messages

- Despite the immune-privileged nature of the eye, irreversible rejection is the major cause of human corneal allograft failure
- Allograft rejection is best described as a delayed-type hypersensitivity reaction controlled by CD4-positive T cells
- Inflammation erodes corneal privilege, and thus meticulous surgery and rapid postoperative attention to any inflammatory episode are important factors in reducing the incidence of corneal allograft rejection
- Topical glucocorticosteroids are the mainstay of immunosuppression for corneal transplantation, but the most appropriate regimen of treatment is uncertain
- The balance of evidence suggests a clinical benefit for histocompatibility matching in corneal transplantation, but logistic difficulties in matching remain
- The possible benefits of systemic immunosuppression with corticosteroids, anti-proliferative agents and calcineurin inhibitors in corneal transplantation need to be weighed against the substantial side effects associated with these agents
- Future approaches to immunosuppression for corneal transplantation include the use of monoclonal antibodies, and gene transfer to the donor cornea to reduce rejection

4.1 Introduction

Corneal transplantation is the most frequently performed clinical allograft. Despite being widely practiced the usefulness of the procedure is limited by allograft rejection, the most common cause of graft failure.

Developments in immunomodulation, largely related to developments in immunosuppression, have resulted in remarkable improvements in organ transplantation. Unfortunately the same improvement has not been seen in corneal transplantation. Documented outcomes for renal transplantation are better than for corneal transplants. The overall renal graft survival reported from registries is greater than 90 % [1, 2]. The corresponding figure for corneal transplantation is closer to 50 % [3]. Most corneal graft failures are due to allograft rejection and there is need for more effective modulation of the immune response in patients receiving corneal transplants.

Not that all patients receiving corneal grafts need immunosuppression. Patients with keratoconus or stromal dystrophies seldom reject their grafts. This is considered to be the "low risk" group of patients in whom the cornea is an immunologically privileged site. Unfortunately these patients are in the minority. In Australia and other Western countries only 30 % of corneal grafts are done for keratoconus and only 1 % for stromal dystrophies. The remainder has acquired disease and almost all have had corneal inflammation at some stage. For this group of patients the immunological privilege of the cornea has been eroded to some degree and modulation of the immune response is re-

quired to minimize the impact of corneal allograft rejection.

The problem with immunomodulation for corneal transplantation is related to the clinical context in which it is practised. Solid organ transplantation is done for patients who are seriously ill, their life threatened by organ failure. For this group of patients the risks of immunosuppression are justified. Corneal transplantation on the other hand is done for patients who are disabled but not at risk of death from their disorder. For most of these patients the risks of systemic immunosuppression cannot be justified. In a small minority the prospect of recovering vision justifies the risks systemic immunosuppression exposes them to. What is required for corneal transplantation is the holy grail of transplantation biology – specific immunosuppression which avoids the significant clinical problems associated with current practice.

4.2 Why Has Corneal Transplantation Fallen Behind Solid Organ Grafting?

At least three factors have contributed to the improvement in outcome for renal transplantation that has occurred over the last 40 years.

1. Histocompatibility matching
2. Better systemic immunosuppression
3. Use of antibodies for graft rescue

None of these approaches is widely applicable to clinical corneal transplantation.

Matching for Class I and Class II histocompatibility antigens has not found a routine place in corneal transplantation. The benefit of this approach is contentious and for those who accept that there are benefits, the logistics are complicated, more so in some places than others. Nor has systemic immunosuppression, so effective in solid organ transplantation, been adopted for corneal transplantation. Again the benefits are arguable. In addition the clinical context is limiting. Patients with corneal blindness are not faced with death should their graft fail as is so often the case for patients receiving solid organ grafts. The systemic administration of antibodies to treat allograft rejection, another strategy successfully employed in solid organ transplantation, has similar limitations when considered for corneal transplantation. Neither can the limitations of systemic administration of conventional immunosuppressive agents be avoided by delivering them locally to the eye as drops or ointment. Steroids are incompletely effective when delivered as drops but other agents such as the calcineurin blockers and antiproliferative agents are ineffective if delivered locally to the eye, and antibodies are too large to cross the cornea and enter the eye, precluding topical use.

4.3 Immunomodulation for Corneal Transplantation

For some patients requiring corneal transplantation immunomodulation is warranted. There is a group of patients who are severely disabled by corneal disease and in whom a functioning corneal graft would restore vision and substantially improve their quality of life. The evaluation of patients from this point of view requires knowledge of the risk of failure, the actuarial data, as predicted by acknowledged clinical prognostic indicators. It also requires examination of the clinical context. This entails an examination of the benefits of the acquisition of a successful corneal graft on a patient's lifestyle and the consequences of graft failure for the individual should this occur. It also requires an assessment of their attitude to success and failure, a highly variable matter, and some broader quality of life issues. Finally the surgeon must evaluate the risks entailed in the measures required to give the patient the best chance of graft survival. For example, the risks of systemic immunosuppression may include serious illness or even death. HLA matching does not offer such risk directly to the patient, but may result in substantial delays in surgery that can be an important consideration for some, particularly the elderly.

4.4 Actuarial Survival Data on Corneal Transplantation

Long-term data on corneal graft survival and the factors affecting survival are available from registries. Overall survival is around 50% at 10 years (Fig. 4.1) and the clinical indicators best predicting graft outcome are set out in Table 4.1 [3]. With these data it is relatively easy to predict the probability of success or failure for a particular patient. What is more arbitrary is the definition of high-risk patients. There is no universal agreement about this in the literature, which makes interpretation of published outcomes difficult. Any definition of a high risk group is arbitrary. Our criteria for grouping patients into high-, medium- and low-risk patients are set out in Table 4.2.

Table 4.1. Prognostic indicators for corneal transplantation. Summary of the multivariate analysis reported in the 1999 report of the Australian Corneal Graft Register

Indication for graft
Number of previous ipsilateral grafts
Eye inflamed at time of graft
Graft size
Lens status after graft
Neovascularization of the graft
Early removal of graft sutures
Postoperative rise in intraocular pressure

Clinical Context. The probability of a complicated outcome is only part of the concept of risk. The consequences of an adverse outcome must also be considered. Outcome data should be applied to patients in their particular clinical context. For patients undergoing corneal transplantation the consequences of success or failure can be quite different from patient to patient. For patients with corneal disease in one eye and a normal contralateral eye very little is to be gained by the patient in a functional sense from a successful outcome. Visual ability is determined by how good the vision is in the better eye rather than the level of vision in the poorer eye. Grafts in anything but the favourable circumstances of keratoconus or stromal dystrophy are best avoided when vision is normal in the contralateral eye. Other patients have much to gain from achieving a functioning graft in high risk situations. Those with most to gain are those with poor vision due to corneal disease in an only eye. These are important considerations because conventional approaches to modulating the immune system are prone to complications which can be serious and life threatening.

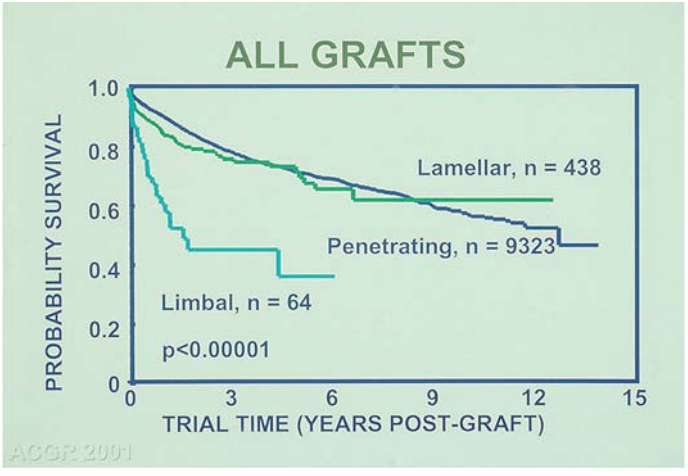

Fig. 4.1. Australian Corneal Graft Registry: corneal graft survival with time. (From the Australian Corneal Graft Registry Report 1999)

Table 4.2. Arbitrary assignment of risk of rejection of corneal grafts. Only one of the clinical features of the high-risk group is required for the patient to be considered to be at high risk of rejection

High risk	Vascularization in three to four segments
	Previous graft failure
	Inflammation at the time of surgery
Moderate risk	Vascularization in one to two segments
	Previous inflammation
	Previous raised intraocular pressure
	Child (<12 years)
	Atopic
	Pseudophakic or aphakic
Low risk	Uncomplicated keratoconus
	Uncomplicated stromal dystrophy

4.5 Options for Immunomodulation

A number of theoretical options exist for reducing the clinical impact of the corneal allograft response. They are based on a need to interfere with two processes, the erosion of the immunological privilege that results from corneal inflammation, and the corneal allograft response itself. Some approaches are more practical than others. A knowledge of the mechanisms involved in immunological privilege and the way it is eroded and the corneal allograft response is necessary to understand existing and potential strategies for immunomodulation.

4.6 Corneal Privilege

A number of related factors contribute to the immunological privilege of the cornea.

1. The blood-eye barrier. The normal cornea is avascular. Only the peripheral cornea depends directly on the vasculature. The central cornea depends on the tear film and the aqueous humor for metabolic requirements. The aqueous humor is secreted by the ciliary body and there is normally no leakage of cells or proteins across the vessels of the tissues lining the anterior chamber such as those in the iris. The separation of the intravascular space and the ocular tissues is referred to as the blood-eye barrier [4–7].
2. Absence of blood vessels and lymphatics. The cornea is normally avascular and devoid of lymphatics. This is an impediment to both the afferent and efferent limbs of the allograft response [8–10].
3. Modest expression of HLA. The epithelium, endothelium, and stromal keratocytes express HLA Class I antigens but only modestly [11]. Similarly Class II expression, which is confined to the Langerhans cells in the peripheral epithelium and interstitial dendritic cells in the peripheral stroma, is only modest. Epithelial cells express ABO antigens [12, 13]. Laboratory experiments in rats indicated the minor transplantation antigens are important in corneal allograft rejection [14].
4. Relative absence of mature antigen-presenting cells. Antigen-presenting cells are necessary to initiate the corneal allograft response. Cells with antigen-presentation capability are virtually absent from the normal cornea. There are a few interstitial dendritic cells in the peripheral corneal stroma [15, 16] and Langerhans cells in the peripheral epithelium [17, 18]. These are outside the operative field for most corneal grafts. Recently a population of CD45+ cells resident in the central cornea of mice has been described [19, 20]. The lineage of these cells, whether they are related to dendritic cells or monocytes or macrophages, is in dispute.

They have been shown to have some allostimulatory capacity after maturation.

5. Constitutive expression of Fas ligand (CD95L). In common with other privileged sites such as testes and brain, the cornea expresses Fas ligand. This entity promotes apoptosis in cells bearing Fas such as lymphocytes [21–23].
6. Immunosuppressive cytokines in aqueous humor. Transforming growth factor (TGFβ) [24, 25], α-melanocyte-stimulating hormone (MSH) [26] and vasoactive intestinal peptide (VIP) [27] are present in normal aqueous fluid.
7. Anterior chamber-associated immune deviation. Antigen introduced into the aqueous humor results in antigen-specific suppression of delayed hypersensitivity [28].

Inflammation Erodes Corneal Privilege. Most of the processes contributing to immune privilege are affected by inflammation. Leakage of cells and proteins from vessels is a fundamental element of acute inflammation [7, 29]. Chronic inflammation results in neovascularization and new vessels are inherently leaky. Lymphatics also develop under these conditions [10]. New blood vessels are easily seen clinically and new vessels in the recipient bed or developing later in the graft are associated with a high risk of graft failure [30]. Lymphatics are not seen clinically in the cornea but can be demonstrated histologically in corneas that have been inflamed [31, 32] and may contribute to the erosion of graft privilege.

Under inflammatory conditions there is upregulation of HLA expression in constitutive corneal cells [33, 34]. In addition inflammation brings about recruitment of bone-marrow-derived cells with antigen-presenting capability into the cornea. These persist for many years. Once a cornea has been inflamed it never returns to normal in this regard. The number of bone-marrow-derived cells in the cornea correlates with the subsequent occurrence of allograft rejection [35].

There is little a clinician can do to protect privilege other than to minimize inflammation. There is an opportunity and a need to do this when treating any patient with keratitis. A cornea that has been inflamed is never again the same. At the time of surgery, trauma should be minimized by meticulous microsurgical technique and the use of biocompatible surgical materials. In the postoperative period inflammation should be suppressed with topical corticosteroids and any episodes of intercurrent inflammation dealt with promptly and effectively. These simple measures are important. The differences in outcomes from one centre to the next are considerable and are likely to be due to attention to these simple details [3].

4.7 Mechanisms of Corneal Allograft Rejection

Allograft rejection is the most common cause of corneal graft failure. The corneal allograft response shares much with other allograft responses; however, there are some important differences which are worthy of attention because they have a bearing on how corneal allograft rejection might best be abrogated.

4.8 The Afferent Limb: Sensitization to Corneal Alloantigens

The foreign antigens introduced into the eye with the graft are on the cell membranes of the epithelial cells, stromal keratocytes and any other cells present in the stroma, and the corneal endothelium. Only the donor endothelial cells persist for the life of the graft. The epithelial cells are replaced within weeks or months by host epithelium. The stromal cells suffer a similar fate but the process may be somewhat slower. Early in the postoperative phase donor antigens could be shed from any donor corneal cells, but if sensitization occurs later the only persisting donor cells may be in the endothelium.

Clinical corneal allograft rejection occurs later compared to what is seen with other organs. The acute rejections seen with solid organ transplantation do not occur with corneal grafts. Even the high risk grafts have a high survival rate at 1 year. The attrition is, however,

steady and prolonged. Corneal allograft rejection is often seen to complicate an episode of intercurrent inflammation such as an episode of herpetic keratitis, conjunctivitis, or other such episode of minor inflammation. The explanation of the relatively late occurrence of corneal allograft rejection and its association with inflammation is bound up with the underlying mechanisms. Inflammation upregulates histocompatibility antigen expression, and recruits immunocompetent cells into the cornea.

Host antigen-presenting cells enter the cornea with wound healing and inflammation. The propensity to rejection correlates with the number of Class II positive cells in the host cornea at the time of surgery [35]. It is as if they are waiting in the residual host cornea, in close proximity to the graft, and enter during wound healing and inflammation. Presumably when these cells present antigen they can do so locally. The conjunctiva is rich in lymphoid tissue [36] and antigen processing can occur there [37]. Furthermore blockade of co-stimulatory molecules in the conjunctiva has been shown to prevent experimental CD4-positive T-cell-mediated herpetic keratitis [38] and expression of recombinant IL-10 by corneal endothelial cells can prevent experimental corneal allograft rejection, supporting the concept of antigen presentation [39] in or around the eye.

There are other cells in close proximity to the graft that can present antigen to the immune system. The iris and trabecular meshwork contain Class II positive interstitial dendritic cells [40]. They are in a position to process antigen or antigen fragments released from the endothelium. Antigen injected into the anterior chamber fluid can be tracked to the spleen [41], and antigen injected into the posterior chamber of the eye can be tracked to the local nodes, at least in the mouse [42].

Another group of antigen-presenting cells is located in the peripheral cornea and adjacent conjunctiva. Unlike the dendritic cells in the uvea these cells interact with antigen in an environment low in TGFβ, and are more likely to direct the immune response along the Th1 response seen in the corneal allograft response [43]. At least some antigen released from the cornea appears in the cervical draining nodes in mice [44]. The balance of evidence suggests that the superficial draining lymph nodes drain the rodent anterior segment and are the main site for the presentation of corneal antigens to T cells. Whatever the equivalent is in humans is not exactly clear, but based on the observation of clinical disorders involving the eye and lymphadenopathy, such as viral keratoconjunctivitis, the draining nodes are in the face and the cervical region. That antigen presentation, and as a consequence T-cell activation and clonal expansion, can occur remote from the eye has therapeutic implications. Agents which affect these events such as the calcineurin inhibitors that interfere with IL-2 driven clonal expansion, are unlikely to be effective if given topically to the eye – their point of action is elsewhere in the draining nodes.

4.9 The Efferent Limb: Histological Correlates

The corneal allograft response is a typical DTH response [45–48], and resembles the allograft response in other organs. The integrity of the blood-eye barrier is breached, resulting in an influx of white cells and proteins into the anterior chamber and cornea. Fibrin accumulates in the anterior chamber and white cells can be seen circulating in the convection currents in the aqueous humor. The cornea becomes infiltrated with macrophages, monocytes, neutrophils, CD4-positive and CD8-positive T cells [47, 49–52]. It is clear from experimental systems that CD4-positive cells have a crucial role in the corneal allograft response [53]. Therapeutic strategies directed at this cell as a means of suppressing the corneal allograft response are attractive. Humoral responses do not seem to be important. Patients who have rejected corneal grafts do develop donor-specific antibodies [54, 55], but corneal grafts are rejected in antibody-deficient hosts [56]. The allograft response can be directed at all of the cellular components of the eye, but it is damage to the corneal endothelium which is critical. This layer of cells has minimal replicative capacity [57]. Unlike the other corneal cells of donor origin, endothelial cells persist in the graft but in decreased num-

bers and with a slow attrition rate. Because endothelium is the only persisting donor element in corneal grafts and because it has a vital function, keeping the cornea transparent, and little capacity for coping with damage, endothelial rejection is the most damaging aspect of the corneal allograft response.

4.10 Current Approaches to Immunomodulation for Corneal Transplantation

4.10.1 Anti-inflammatory Measures

Inflammation is a major threat to corneal graft survival. Inflammation prior to surgery, even many years prior to surgery, erodes corneal privilege and decreases the chances of subsequent corneal transplantation. Inflammation resulting from surgery, or occurring in the postoperative period, even years later, threatens graft survival. Anti-inflammatory measures are the cornerstone of routine care of patients having corneal transplants. There are two planks to this approach.

4.10.2 Atraumatic Microsurgical Technique

The employment of meticulous microsurgical technique and non-reactive surgical materials such as fine monofilament nylon have reduced the degree of postoperative inflammation in patients having corneal transplants. The anecdotal reports of improvements in surgical outcomes reported in the 1950s are attributable to developments in microsurgery and the introduction of topical corticosteroids.

4.10.3 Topical Corticosteroids

All patients receiving corneal transplants are given topical corticosteroids in the postoperative period. The topical administration of 11β-hydroxyl compounds such as prednisolone salts or dexamethasone is the mainstay of immunosuppression for clinical corneal transplantation. There is argument about the appropriate dosage and the desirable period of administration. The unexpectedly good results in the Collaborative Corneal Transplantation Trial (CCTS) in both arms of the trial were attributed to the high dose of topical corticosteroids used, which was considered to be higher than what is generally used in clinical practice [37].

Corticosteroids alone will not overcome the threat of allograft rejection in high-risk cases. In this group of patients the allograft rejection and graft failure rate remain high despite the use of maximal doses of topical corticosteroids.

4.10.4 HLA Matching for Class I and Class II Antigens

Tissue matching in corneal transplantation is controversial. There is widespread acceptance in Europe that matching at the Class I locus affords some benefit. This is not generally accepted in the US and the CCTS demonstrated no beneficial effect for either Class I or Class II matching but an unexpected and surprising benefit from ABO matching [58]. The validity of the study is open to criticism because of the high error rates in the matching [59]. The CTTS findings notwithstanding, there is a body of evidence supporting a modest beneficial effect from Class I matching in patients with high-risk corneal grafts [60–64]. The situation with Class II matching is less clear-cut. There are reports of benefits, others of no benefit, and some of an adverse effect [65–69].

The balance of evidence supports a clinical benefit for HLA-A, -B, and -DR matching. This approach can only be part of an approach to immunomodulation. High level matches are not only difficult to achieve, they do not afford complete protection from allograft rejection for high-risk patients, because of the role of minor transplantation antigens. Ideally matching should be used routinely for patients having corneal grafts and who are at high risk of rejection. It is the only way of improving the outlook for these patients which is free of side effects.

The problem is logistics. A matching program is clearly achievable in Europe with a large, essentially homogeneous population, and sophisticated resources shared across national borders. In more sparsely and heterogeneously populated regions this is more difficult to achieve. In such places, including Australia, it will be necessary to conduct a cost-benefit analysis to review the cost of the reshaping of existing eye banking procedures to bring about more widespread sharing of corneas, monitoring of typing procedures, and outcome surveillance. It is an analysis which should be undertaken because the cost of retransplanting failed grafts is considerable, both to the individual and society.

4.10.5 Systemic Immunosuppression

Systemic immunosuppression is used for all patients receiving grafts of vascularized organs. Improvement in this aspect of immunomodulation has been an important factor in the remarkable achievements in such cases [70]. This approach has been applied less frequently to corneal transplantation and there are relatively few reports of the effect on clinical outcomes. Three groups of agents have a role to play in this field, corticosteroids, calcineurin inhibitors, and antiproliferative agents.

4.10.6 Systemic Corticosteroids

Corticosteroids are potent modulators of the immune system but have low specificity and serious side effects. Their well-known and serious side effects, including osteopaenia, weight gain, hyperglycaemia, hypercholesterolaemia, hypertension, and skin fragility, can occur with modest doses administered over relatively short periods. Despite this they are widely used in solid organ transplantation, where their combination with other immunosuppressive agents enables the use of lower doses of the individual agents with a corresponding reduction in the side effects. However, even when used in combination, the side effects from systemic administration of corticosteroids are considerable and they are generally avoided as maintenance therapy for patients receiving corneal transplants, even for high-risk patients.

They are used for graft rescue – to save corneal grafts that are undergoing allograft rejection – and for graft maintenance when the use of other agents is precluded by side effects, such as cyclosporin toxicity. The level of evidence for their efficacy is low. This is related to the long history of usage. Introduced into clinical practice in the 1960s they have assumed a place in the treatment of corneal graft rejection but have not been subjected to formal assessment as happens with new therapeutic agents today. Their role is so accepted that it would not be possible to arrange such a trial now. Furthermore there are theoretical grounds for using systemic corticosteroids to treat allograft rejection. Clonal expansion, the process that primes the immune system towards subsequent rejection, occurs in the facial and cervical lymph nodes beyond the reach of topical steroids [71]. In addition high levels of circulating corticosteroids are capable of redirecting the immune system [72]. The mechanisms are believed to be attributable to the removal of circulating T cells [73, 74], the activated CD4-positive cells which are primed in the lymph nodes to trigger the next rejection episode being particularly susceptible [75], and pronounced suppression of inflammation.

There are data on the relative merits of oral and intravenous bolus steroids in the treatment of corneal allograft rejection, although the message is mixed. Some studies show a benefit from the use of intravenous methyl prednisolone [76–78], and others show no benefit at all [79]. Perhaps the appropriate approach is to continue to use oral prednisolone for the treatment of corneal allograft rejection because the balance of evidence suggests that there is no advantage from using intravenous therapy. Both routes of administration can result in complications, with the intravenous route having some additional and serious acute problems [58]. When using oral corticosteroids in this situation it is advisable to avoid prolonged therapy. In our practice we restrict the course of oral prednisolone to 3 weeks. If there has not been an improvement in the clinical picture in 3 weeks, it is assumed

the rejection is steroid resistant and oral administration ceased.

4.10.7 Antiproliferative Agents

Azathioprine has been used for many years as an immunosuppressive agent; it was the first immunosuppressive used after corticosteroids and continues to be widely used in the management of patients with organ transplants.

Azathioprine is an imidazole derivative of 6-mercaptopurine (6-MP). In vivo it is rapidly broken down into 6-MP, which readily crosses cell membranes and is converted into a number of purine analogues. These interfere with cell division and growth although the precise mechanisms are not known. The immunosuppressive effect is related to the prevention of proliferation of the cells involved in the immune response. Azathioprine was first reported as an effective treatment of high-risk corneal transplants in 1967 [81].

Mycophenolate, a more recently introduced immunosuppressive drug, works in a similar manner, suppressing the proliferation of cells involved in the immune response. It is thought to do this more specifically in that it has less effect on non-lymphoid cells. Mycophenolate is a potent, selective noncompetitive and reversible inhibitor of monophosphate dehydrogenase which inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because lymphocytes are dependent for their proliferation on de novo synthesis of purines, whereas other cells can utilize salvage pathways, mycophenolate has a more potent effect on the growth and development of lymphocytes than other rapidly dividing cells. It has been shown to be as effective as cyclosporin in the treatment of high-risk corneal transplants [82].

4.10.8 Calcineurin Blockers

The introduction of cyclosporin and later FK506 resulted in spectacular improvements in the outcome of all forms of organ transplantation [72]. Registry studies would suggest that it has had no effect on the outcome of corneal transplantation because it has found little place in this context. This is because side effects are common when it is used in immunosuppressive doses and some of the complications are life threatening. Furthermore graft rejection still commonly occurs in high-risk patients even when the drug is used optimally.

Cyclosporin is a cyclic endecapeptide produced as a metabolite of a fungus. In vivo experiments indicate that it interferes with the development of cell-mediated responses such as allograft immunity, delayed cutaneous hypersensitivity, graft versus host disease and T-cell-dependent antibody production. Cyclosporin appears to block resting lymphocytes in Go or G1 phase of the cell cycle and inhibits antigen-triggered release of lymphokines including IL-2. Cyclosporin seems to affect only lymphocytes, and in particular does not suppress haemopoiesis and has no effect on phagocytic cells, making infection a less common complication than is seen with antiproliferative agents.

FK506 is a macrolide lactone with potent immunosuppressive activity. At a cellular level it inhibits the formation of cytotoxic lymphocytes that are crucial contributors to the allograft response. It also suppresses T-cell activation and T-helper-cell-dependent B-cell proliferation, as well as lymphokine production, including IL-2, and the IL-2 receptor. At a molecular level the effects of the drug are related to binding to a cytosolic protein (FKPB) and a combination of this drug-protein complex, calcium, calmodulin and calcineurin, which inhibits the phosphatase activity of calcineurin [83].

Despite the specificity of these drugs for the lymphoproliferative system the side effects are considerable when used systemically in immunosuppressive doses – and the topical preparations of the drug have not been conclusively proved to be effective.

Systemic administration of cyclosporin has been advocated for the prevention of allograft rejection in high-risk cases. There is considerable in vivo work demonstrating a beneficial effect in animal models of corneal transplantation [84–86]. There is also evidence of effect

claimed from a number of controlled [87, 88] and uncontrolled clinical studies [89] – and other studies challenging the benefits of this approach [90–92]. What is broadly accepted is that the complications from systemically administered cyclosporin are frequent and often serious [93]. This limits the usefulness of the drug in the clinical context of corneal transplantation.

4.10.9 Combination Therapy

Both azathioprine and mycophenolate are used, in combination with cyclosporin or FK506, in patients receiving corneal transplants in whom systemic immunosuppression can be justified. The benefits of this approach for solid organ transplantation are well documented [70] – the benefits in corneal transplantation less so.

In the absence of data supporting any particular regimen for systemic immunosuppression in our institution we have opted to use the same regimen used for renal transplantation in our institute. This simplifies prescribing and surveillance for side-effects.

Currently we use cyclosporin and azathioprine or mycophenolate for 1 year unless side effects demand a change or cessation of the treatment. This is usually commenced immediately after surgery. There would seem little need to commence immunosuppression prior to surgery because corneal grafts are not subjected to the acute rejection suffered by solid organ grafts in the early postoperative period. The dosage is adjusted on the basis of drug levels. Our approach is set out in Table 4.3. It is very important to monitor these patients for side effects. Immunosuppressive doses of these agents are often complicated by side effects, even with short-term administration.

4.10.10 Side Effects of Systemic Immunosuppression

The side effects of systemic immunosuppression are common and can be serious and life threatening. The immune system is an integral part of our defence against the outside world and its suppression leads to vulnerability. It is difficult to identify the precise relationship between a particular agent and a specific side effect. The drugs are usually used for patients with serious systemic disorders and often in combination with other powerful agents. Some of the side effects encountered are non-specific in that they are a consequence of the immunosuppression. Others are due specifically to a particular agent.

Overwhelming infection, skin and hair and oral changes, hypertension and nephrotoxicity can complicate cyclosporin usage. Antiproliferative drugs such as azathioprine and mycophenolate can cause marrow suppression and gut problems and the therapeutic range is narrow so that side effects are relatively frequent.

Just as there is no supporting evidence for one particular treatment there is no evidence for the length of time over which immunosuppression should be maintained. We arbitrarily chose 1 year as the period of treatment, but long-term maintenance may be desirable as it is with organ grafts. Long-term immunosuppres-

Table 4.3. Systemic immunosuppression. Dual therapy as used by authors

	Initial dose	Desired blood level	Other monitoring
Cyclosporin	75–100 mg bd	80–100/mg/l (trough)	Serum electrolytes monthly. Looking for impaired renal function
Mycophenolate	1 g bd	1.0–3.5/mg/l	Complete blood counts, weekly for the 1st month, twice monthly until 3 months, then monthly. Look for neutropenia

sion can be complicated by neoplasia [93]. This is the reason for us choosing 12 months as the time to cease treatment.

4.11 Novel Approaches to Immunomodulation

The results of corneal transplantation point to a need for more effective immunomodulation suitable for use in this clinical setting. The approaches employed for patients with organ grafts are not effective in achieving the improvements witnessed in other areas of transplantation. An ideal solution to this problem would be highly specific, blocking alloresponses to only the relevant alloantigens, taking into account the aspects of the allograft response peculiar to the cornea, and which could be delivered locally to the eye, avoiding the need for systemic administration and associated side effects. Two approaches are under development, the use of monoclonal antibody fragments and gene therapy.

4.11.1 Monoclonal Antibody Fragments

Antibody-based treatments have been used in transplantation for many years. Initially heterologous antilymphocyte serum or globulin was used and more recently monoclonal antibodies have been employed. OKT3, an anti pan T-lymphocyte monoclonal antibody, has been used for the treatment of organ rejection. This systemic approach has not found a place in corneal transplantation. It can only be used once and the systemic administration can be hazardous. As with other approaches to systemic immunosuppression the risks are readily justified for essential organ grafts but more difficult to justify for corneal transplantation. One group has reported the treatment of corneal allograft rejection by injecting monoclonal antibodies into the anterior chamber of the eye [94, 95]. Despite their optimistic initial report this approach has not led anywhere and no other groups have reported adoption of this approach, although more recently there have been anecdotal reports of systemically administered monoclonal antibodies, CAMPATH-1H (anti-CD52) [96, 97] and anti-CD25 [98], to treat corneal allograft rejection. A large number of antibodies have been used in experimental corneal transplantation. This and the clinical experience were recently reviewed [99].

A problem with conventional antibody-based therapies for eye disease is that the molecules are too large to gain access to the eye by crossing the cornea. They cannot be administered as eye drops but instead must be given systemically. This has prompted the development and application of monoclonal antibody fragments which retain their ability to interact with the appropriate receptor but are small enough to cross the cornea if delivered topically into the precorneal tear film [100]. Antibody fragments have been engineered which interfere specifically with the elements operating in the proximal end of the afferent limb of the allograft response. They have been shown to retain their essential reactivity and to be absorbed across the cornea. Whether they are effective in abrogating the corneal allograft response remains to be determined.

4.11.2 Gene Therapy

Gene therapy is also being investigated as a way of modulating the corneal allograft response to prolong graft survival. Early results are encouraging. Genes for naturally occurring immunosuppressive proteins can be transfected into the corneal endothelium. Transfection of the endothelium of sheep with the gene for IL-10 has resulted in prolongation of corneal graft survival [39]. This cytokine has important functions early in the afferent arm of the corneal allograft response. This approach is promising in that the endothelium is easily accessed in patients having corneal grafts and gene expression is prolonged, which may be related to the non-replication of the corneal endothelium.

Summary for the Clinician

- **Transplant rejection remains the single most important cause of graft failure following penetrating keratoplasty**
- **Local as well as systemic immune processes are involved in transplant rejection and may allow development of more specific preventive and therapeutic options**
- **CD4+ lymphocytes play an essential role in the immune response in corneal graft rejection and are the main target of immunomodulatory therapy**
- **The relative importance of CD4+ subtypes classified as "Th1/Th2" is still a matter of debate but may allow new immunomodulatory strategies, such as gene therapy**

4.12 Conclusion

At present very little can be done to modulate the immune response in patients having corneal transplants and at risk of rejection. Reducing inflammation by minimizing microsurgical trauma and generous use of topical corticosteroids is all that is usually available. However, a strong case can be made for HLA-A, -B and -DR matching, but in many places this would demand a change in eye banking logistics. Conventional systemic immunosuppression, the mainstay of immunomodulation for solid organ grafts, is not readily applicable to corneal transplantation because it is difficult to justify the risks involved. Even where the risk may be justified there is little high level evidence to support the approach or to identify one particular immunosuppressive regimen as more effective than another. Nor will it be easy to assemble this evidence. The 1-year survival of corneal transplants is around 90%, even in the high-risk groups. Failures come later and often. Clinical trials would have to be prolonged and the number of patients in any one centre, or any one region, would be limited. Perhaps a registry approach would be more likely to provide the information required as has been the case with solid organ transplantation. Although we might do a little better by translating some of the approaches successfully employed in other branches of clinical transplantation, it is more likely that developing approaches specifically for corneal transplantation will bring about the improvements in corneal graft outcomes that are required. Interventions need to be developed that can be applied directly to the eye and are highly specific – interfering only with responses to the alloantigens in the graft. The development of relevant antibody fragments for topical administration and gene therapy are two approaches that show promise in this regard.

References

1. http://www.anzdata.org.au/ANZDATA/anzdatawelcome.htm
2. Opelz G (1994) Effect of the maintenance immunosuppressive drug regiment on kidney transplant outcome. Transplantation 58:443–446
3. Williams KA, Muehlberg SM, Bartlett CM, Esterman A, Coster DJ (2000) Report from the Australian Corneal Graft Registry 1999. Snap Printing, Adelaide, pp 1–137
4. Grabner G, Zehetbauer G, Bettelheim H, Honigsmann C, Dorda W (1978) The blood-aqueous barrier and its permeability for proteins of different molecular weight. Graefes Arch Klin Exp Ophthalmol 207:137–148
5. Dernouchamps JP, Heremans JF (1975) Molecular sieve effect of the blood-aqueous barrier. Exp Eye Res 21:289–297
6. Maurice D, Mishima S (1984) Ocular pharmacokinetics. In: Sears M (ed) Pharmacology of the eye. Springer, Berlin Heidelberg New York, pp 19–116
7. Kuchle M, Nguyen NX, Naumann GO (1994) Aqueous flare following penetrating keratoplasty and in corneal graft rejection. Arch Ophthalmol 112:354–358
8. Barker CF, Billingham RE (1973) Immunologically privileged sites and tissues. In: Porter R, Knight J (eds) Corneal graft failure. CIBA Foundation Symposium 15 (new series). Elsevier, Amsterdam; Excerpta Medica, North-Holland, pp 79–104
9. Medawar P (1948) Immunity of homologous grafted skin III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br J Exp Pathol 29:58–69
10. Collin HB (1996) Endothelial cell lined lymphatics in the vascularized rabbit cornea. Invest Ophthalmol 5:337–354

11. Whitsett CF, Stulting RD (1984) The distribution of HLA antigens on human corneal tissue. Invest Ophthalmol Vis Sci 25:519–524
12. Nelken E, Michaelson IC, Nelken D, Gurebitch J (1956) ABO antigens in the human cornea. Nature 177:840
13. Salisbury JD, Gebhardt BM (1981) Blood group antigens on human corneal cells demonstrated by immunoperoxidase staining. Am J Ophthalmol 91:46–50
14. Katami M, Madden PW, White DJ, Watson PG, Kamada N (1989) The extent of immunological privilege of orthotopic corneal grafts in the inbred rat. Transplantation 41:371–376
15. Williams KA, Ash JK, Coster DJ (1985) Histocompatability antigen and passenger cell content of normal and diseased human cornea. Transplantation 39:265–269
16. Catry L, Van den Oord J, Foets B, Missotten L (1991) Morphologic and immunophenotypic heterogeneity of corneal dendritic cells. Graefes Arch Clin Ex Ophthalmol 229:182–185
17. Jager MJ (1992) Corneal Langerhans cells and ocular immunology. Reg Immunol 4:186–195
18. Baudouin C, Brignole F, Pisella PJ, Becquet F, Philip PJ (1997) Immunophenotyping of human dendriform cells from the conjunctival epithelium. Curr Eye Res 16:475–481
19. Liu Y, Hamrah P, Zhang Q, Taylor AW, Dana MR (2002) Draining lymph evidence for major histocompatibility complex (MHC) Class II-positive cells derived from Class II-negative grafts. J Exp Med 195:259–268
20. Hamrah P, Zhang Q, Liu Y, Dana MR (2002) Novel characterization of MHC Class II-negative population of resident corneal Langerhans cell-type dendritic cells. Invest Ophthalmol Vis Sci 43:639–646
21. Wilson SE, Li Q, Weng J, Barry-Lane PA, Jester JV, Liang Q et al. (1996) The Fas-Fas ligand system and other modulators of apoptosis in the cornea. Invest Ophthalmol Vis Sci 37:1582–1592
22. Griffith TS, Yu X, Herndon JM, Green DR, Ferguson TA (1996) CD95-induced apoptosis of lymphocytes in an immune privileged site induces immunological tolerance. Immunity 5:7–16
23. Stuart PM, Griffith TS, Usui N, Pepose J, Yu X, Fergusin TA (1997) CD95 ligand (FasL)-induced apoptosis is necessary for corneal allograft survival. J Clin Invest 99:396–402
24. Wilbanks GA, Mammolenti M, Streilein JW (1992) Studies on the induction of anterior chamber-associated immune deviation (ACAID), III. Induction of ACAID depends upon intraocular transforming growth factor-beta. Eur J Immunol 22:165–173
25. D'Orazio T, Niederkorn JY (1998) A novel role for TGF-beta and IL-10 in the induction of immune privilege. J Immunol 160:2089–2098
26. Taylor AW, Streilein JW, Cousins SW (1994) Alpha-melanocyte-stimulating hormone suppresses antigen-stimulated T cell production of gamma-interferon. Neuroimmunomodulation 1:188–194
27. Taylor AW, Streilein JW, Cousins SW (1994) Immunoreactive vasoactive intestinal peptide contributes to the immunosuppressive activity of normal aqueous humor. J Immunol 153:1080–1086
28. Streilein JW (1996) Peripheral tolerance induction: lessons from immune privileged sites and tissues. Transplant Proc 28:2066–2070
29. Williams KA, Coster DJ (1997) Rethinking immunological privilege: implications for corneal and limbal stem cell transplantation. Mol Med Today 3:495–515
30. Coster DJ (1981) Factors affecting the outcome of corneal transplantation. Ann R Coll Surg Engl 63:91–97
31. Collin HB (1966) Endothelial cell lines lymphatics in the vascularized rabbit cornea. Invest Ophthalmol 5:337–354
32. Cursiefen C, Schlötzer-Schrehardt V, Küchle M et al. (2002) Lymphatic vessels in vascularized human corneas: immunohistochemical investigation using LYVE-1 and podoplanin. Invest Ophthalmol Vis Sci 43:2127–2135
33. Treselet PA, Foulks GN, Sanfilippo F (1984) The expression of HLA antigens by cells in the human cornea. Am J Ophthalmol 98:763–772
34. Pepose JS, Gardner KM, Nestor MS, Foos RY, Pettit Th (1985) Detection of HLA class I and II antigens in rejected human corneal allografts. Ophthalmology 92:1480–1484
35. Williams KA, White MA, Ash JK, Coster DJ (1989) Leukocytes in the graft bed associated with corneal graft failure. Analysis by immunohistology and actuarial graft survival. Ophthalmology 96:38–44
36. Knop N, Knop E (2000) Conjunctiva-associated lymphoid tissue in the human eye. Invest Ophthalmol Vis Sci 4:1270–1279
37. Porgador A, Staats HF, Itoh Y, Kelsall BL (1998) Intranasal immunization with cytotoxic T-lymphocyte epitope peptide and mucosal adjuvant cholera toxin: selective augmentation of peptide-presenting dendritic cells in nasal mucosal-associated lymphoid tissue. Infect Immunol 66:5876–5881
38. Chen H, Hendricks RL (1998) B7 costimulatory requirements of T cells at an inflammatory site. J Immunol 160:5045–5052

39. Klebe S, Sykes P, Coster D, Krishnan R, Williams K (2001) Prolongation of sheep corneal allograft survival by transfer of the gene encoding ovine interleukin 10 to donor corneal endothelium. Transplantation 15:1214–1220
40. McMenamin PG, Crewe J, Morrison S, Holt PG (1994) Immunomorphologic studies of macrophages and MHC Class II-positive dendritic cells in the iris and ciliary body of the rat, mouse, and human eye. Invest Ophthalmol Vis Sci 35:3234–3250
41. Streilein JW (1996) Peripheral tolerance induction: lessons from immune privileged sites and tissues. Transplant Proc 28:2066–2070
42. Egan RM, Yorkey C, Black R, Loh WK, Stevens JL, Woodward JG (1996) Peptide-specific T cell clonal expansion in vivo following immunization in the eye, an immune-privileged site. J Immunol 157:2262–2271
43. Williamson JS, Di Marco S, Streilein JW (1987) Immunobiology of Langerhans cells on the ocular surface. I Langerhans cells within the central cornea interfere with induction of anterior chamber associated immune deviation. Invest Ophthalmol Vis Sci 28:1527–1532
44. Yamagami S, Dana MR (2001) The critical role of lymph nodes in corneal alloimmunization and graft rejection. Invest Ophthalmol Vis Sci 42: 1293–1298
45. Ayliffe W, Alam Y, Bell EB, McLeod D, Hutchinson IV (1992) Prolongation of rat corneal graft survival by treatment with anti-CD4 monoclonal antibody. Br J Ophthalmol 76:602–606
46. Joo CK, Pepose JS, Stuart PM (1995) T-cell mediated responses in a murine model of orthotopic corneal transplantation. Invest Ophthalmol Vis Sci 36:1530–1540
47. Katami M (1995) The mechanisms of corneal graft failure in the rat. Eye 9:197–207
48. Yamada J, Kurimoto I, Streilein JW (1999) Role of CD4+ T cells in immunobiology of orthotopic corneal transplants in mice. Invest Ophthalmol Vis Sci 40:2614–2621
49. Callanan DG, Luckenback MW, Fischer BJ, Peeler JS, Niederkorn JY (1989) Histopathology of rejected orthotopic corenal grafts in the rat. Invest Ophthalmol Vis Sci 30:413–424
50. Larkin DFP, Alexander RA, Cree IA (1997) Infiltrating inflammatory cell phenotypes and apoptosis in rejected human corneal allografts. Eye 11:68–74
51. Kuffova L, Holan V, Lumsden L, Forrester JV, Filipec M (1999) Cell sub-populations in failed human corneal grafts. Br J Ophthalmol 83:1364–1369
52. Matoba AY, Peeler JS, Niederkorn JY (1986) T cell subsets in the immune rejection of murine heterotopic corneal allografts. Invest Ophthalmol Vis Sci 27:1244–1254
53. Hegde S, Niederkorn JY (2000) The role of cytotoxic T lymphocytes in corneal allograft rejection. Invest Ophthalmol Vis Sci 41:3341–3347
54. Jager MJ, Volker-Dieben HJ, Vos A, Broersma L, Kok FG, van der Gaag R (1991) Cellular and humoral anticorneal immune response in corneal transplantation. Arch Ophthalmol 109:972–977
55. Hutchinson IV, Alam Y, Ayliffe WR (1995) The humoral response to an allograft. Eye 9:155–160
56. Goslings WR, Yamada J, Dana MR et al. (1999) Corneal transplantation in antibody-deficient hosts. Invest Ophthalmol Vis Sci 40:250–253
57. Tuft SJ, Coster DJ (1990) The corneal endothelium. Eye 4:389–424
58. The Collaborative Corneal Transplantation Studies Research Group (1992) The collaborative corneal transplantation studies (CCTS). Effectiveness of histocompatibility matching in high-risk corneal transplantation. Arch Ophthalmol 110:1392–1403
59. Hopkins KA, Maguire MG, Fink NE, Bias WB (1992) Reproducibility of HLA-A, -B, and -DR typing using peripheral blood samples; results of retyping in the collaborative corneal transplant studies. Corneal Transplantation Studies Group (corrected). Hum Immunol 33:122–128
60. Batchelor JR, Casey TA, Werb A, et al. (1976) HLA matching and corneal grafting. Lancet 1:551–554
61. Volker-Dieben HJ, Kok-van Alphen CC, Lansbergen Q, Persijn GG (1982) The effect of prospective HLA-A and -B matching and corneal graft survival. Acta Ophthalmol (Copenhagen) 60:203–212
62. Gore SM, Vail A, Bradley VA, Rogers CA, Easty DL, Armitage WJ (1995) HLA-DR matching in corneal transplantation. Systemic review of published evidence. Corneal Transplant Follow-up Study Collaborators. Transplantation 60:1033–1039
63. Foulks GN, Sanfilippo FP, Locascio JA III, MacQueen JM, Dawson DV (1983) Histocompatability testing for keratoplasty in high-risk patients. Ophthalmology 90:230–244
64. Sanfilippo F, MacQueen JM, Vaughn WK, Foulks GN (1986) Reduced graft rejection with good HLA-A and B matching in high-risk corneal transplantation. N Engl J Med 315:29–35
65. Hoffman F, von Keyserlingk HJ, Wiederholt M (1986) Importance of HLA DR matching for corneal transplantation in high-risk cases. Cornea 5:139–143
66. Khaireedin R, Wachtlin J, Hopfenmüller W, Hoffman F (2003) HLA-A, HLA-B, and HLA-DR matching reduces the rate of corneal allograft rejection. Graefes Arch Clin Exp Ophthalmol 241:1020–1028
67. Baggesen K, Lamm LU, Ehlers N (1996) Significant effect of high-resolution HLA-DRB1 matching in high-risk corneal transplantation. Transplantation 62:1273–1277

68. Munkhbat B, Hagihara M, Sato T, et al. (1997) Association between HLA-DPB1 matching and 1-year rejection-free graft survival in high-risk corneal transplantation. Transplantation 63:1011–1016
69. Vail A, Gore SM, Bradley BA, Easty DL, Rogers CA, Armitage WJ (1994) Influence of donor and histocompatibility factors on corneal graft outcome. Transplantation 58:1210–1216
70. Morris PJ (1996) A critical review of immunosuppressive regimens. Transplant Proc 28 (Suppl): 37–40
71. Abrahams C, Gaillard V (1980) Instillation of steroids in the eye: its effect on lymphocytes in regional lymph nodes and in peripheral blood. S Afr Med J 57:993–995
72. Meyer PA, Watson PG, Franks W, Dubord P (1987) 'Pulsed' immunosuppressive therapy in the treatment of immunologically induced corneal and scleral disease. Eye 1:487–495
73. Silverman ED, Myones BL, Miller JJ 3rd (1984) Lymphocyte sub-population alterations induced by intravenous megadose pulse methylprednisolone. J Rheumatol 11:287–290
74. Zweiman B, Atkins PC, Bedard PM, Flaschen SL, Lisak RP (1984) Corticosteroid effects on circulating lymphocyte subset levels in normal humans. J Clin Immunol 4:151–155
75. Berge RJ, Sauerwein HP, Yong SL, Schellekens PT (1984) Administration of prednisolone *in vivo* affects the ratio of OKT4/OKT8 and the LDH-isoenzyme pattern of human T lymphocytes. Clin Immunol Immunopathol 30:91–103
76. Hill JC, Maske R, Watson P (1991) Corticosteroids in corneal graft rejection. Oral versus single pulse therapy. Ophthalmology 98:329–333
77. Hill JC, Ivey A (1994) Corticosteroids in corneal graft rejection; double versus single pulse therapy. Cornea 13:383–388
78. Lam DS, Wong AK, Tham CC, Leung AT (1998) The use of combined intravenous pulse methylprednisolone and oral cyclosporine A in the treatment of corneal graft rejection: a preliminary study. Eye 12:615–618
79. Hudde T, Minassian DC, Larkin DF (1999) Randomized controlled trial of corticosteroid regimens in endothelial corneal graft rejection. Br J Ophthalmol 83:1348–1352
80. Thompson JF, Chalmers DH, Wood RF, Kirkham SR, Morris PJ (1983) Sudden death following high-dose intravenous methylprednisolone. Transplantation 36:594–596
81. Mackay IR, Bignell JL, Smith PH, Crawford BA (1967) Prevention of corneal graft failure with the immunosuppressive drug azothioprine. Lancet 2:479–482
82. Reinhard T, Reis A, Bohringer D, Malinowski M, Voiculescu A, Heering P, Godehardt E, Sunmacher R (2001) Systemic mycophenolate motefil in comparison with systemic cyclosporin A in high-risk keratoplasty patients: 3 years' results of randomized prospective clinical trial. Graefes Arch Clin Exp Ophthalmol 239:367–372
83. Hogan PG, Chen L, Nardone J, Rao A (2003) Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev 17:2205–2232
84. Shepherd WFI, Coster DJ, Chin Foog TC, Rice NSC, Jones BR (1980) Effect of cyclosporin A on the survival of corneal grafts in rabbits. Br J Ophthalmol 64:148–153
85. Zhang EP, Schulte F, Bulfone-Paus S, Hoffman F (2000) The effect of corticosteroid and cyclosporin A on murine corneal allograft rejection. Graefes Arch Clin Exp Ophthalmol 238:525–530
86. Claerhout I, Beele H, Verstraete A, Van den Broecke C, Kestelyn P (2001) The effect of duration and timing of systemic cyclosporine therapy on corneal allograft survival in a rat model. Graefes Arch Clin Exp Ophthalmol 23:152–157
87. Hill JC (1994) Systemic cyclosporin in high-risk keratoplasty. Short-term versus long-term therapy. Ophthalmology 101:12833
88. Hill JC (1995) Systemic cyclosporin in high-risk keratoplasty: long-term results. Eye 9:422–428
89. Sundmacher R, Reinhard T, Heering P (1992) Six years' experience with systemic cyclosporin A prophylaxis in high-risk perforating keratoplasty patients. A retrospective study. Ger J Ophthalmol 1:432–436
90. Poon AC, Forbes JE, Dart JK, Subramaniam S, Bunce C, Madison P, Ficker LA, Tuft SJ, Gartry DS, Buckley RJ (2001) Systemic cyclosporin A in high-risk penetrating keratoplasties: a case-control study. Br J Ophthalmol 85:1465–1469
91. Inoue K, Kimura C, Amano S, Sato T, Fujjita N, Kagayo F, Kaji Y, Tsuru T, Araie M (2001) Long-term outcome of cyclosporine treatment following penetrating keratoplasty. Jpn J Ophthalmol 45:378–382
92. Rumelt S, Berdusky V, Blum-Hareuveni T, Rehany V (2002) Systemic cyclosporin A in high failure risk, repeated corneal transplantation. Br J Ophthalmol 86:988–992
93. Algros MP, Angonin R, Delbose B, Cahn JY, Kantelip B (2002) Danger of systemic cyclosporin for corneal grafts. Cornea 21:613–614
94. Ippoliti G, Fronterre A (1987) Use of locally injected anti-T monoclonal antibodies in the treatment of acute corneal graft rejection. Transplant Prod 19:2579–2580

95. Ippoliti G, Fronterre A (1989) Usefulness of CD3 or CD6 anti-T monoclonal antibodies in the treatment of acute corneal graft rejection. Transplant Proc 21:3133–3134
96. Newman DK, Isaacs JD, Watson PG, Meyer PA, Hale G, Waldmann H (1995) Prevention of immune-mediated corneal graft destruction with the anti-lymphocyte monoclonal antibody, CAMPATH-1H. Eye 9:564–569
97. Dick AD, Meyer P, James T, Forrester JV, Hale G, Waldmann H, et al. (2000) Campath-1H therapy in refractory ocular inflammatory disease. Br J Ophthalmol 84:107–109
98. Schmitz K, Hitzer S, Behrens-Baumann W (2002) Immune suppression by combination therapy with basiliximab and cyclosporin in high risk keratoplasty. A pilot study. Ophthalmology 99: 38–45
99. Thiel MA, Coster DJ, Williams KA (2003) The potential of antibody-based immunosuppressive agents for corneal transplantation. Immunol Cell Biol 81:93–105
100. Thiel MA, Coster DJ, Standfield SD, et al. (2002) Penetration of engineered antibody fragments into the eye. Clin Exp Immunol 128:67–74

Scleritis

Alisa Kim, Sean Dumars, Samir Shah, Bartly Mondino

Core Messages

- Scleritis is highly associated with potentially devastating ocular complications and serious systemic diseases
- Necrotizing scleritis has the highest association with systemic disease and is frequently the first manifestation of a systemic condition
- Scleromalacia perforans is most commonly associated with long-standing rheumatoid arthritis and may present without any symptoms
- Scleritis in predisposed individuals may occur after ocular trauma, including ocular surgery
- Scleritis can develop due to an infectious aetiology and requires early recognition and treatment
- Scleritis should be distinguished from episcleritis, as it represents different clinical entities with very contrasting clinical presentations, disease courses, treatments, and prognoses. Lack of vasoconstriction with phenylephrine is a helpful diagnostic test
- Scleritis is a clinical diagnosis and most commonly presents with severe eye pain
- The clinical presentation of scleritis depends on the anatomic site involved and the extent of inflammation, forming the basis for the classification of scleritis (Table 5.1)
- Ocular complications of scleritis include loss of vision, keratitis, progression of cataract, uveitis, glaucoma, scleral thinning and perforation, staphyloma, retinal detachment, and choroidal detachment
- Evaluation for associated systemic illness must be included in the history, physical exam, and diagnostic testing. Referral to an internist or rheumatologist can be of great assistance
- Ancillary diagnostic testing may be helpful in the diagnosis and management, especially in cases of posterior scleritis
- Scleritis is medically managed with systemic non-steroidal anti-inflammatory agents, corticosteroids, and steroid-sparing immunosuppressants in a stepwise manner
- Five to 10% of cases require surgical management and have the greatest success when the inflammation is suppressed medically
- The classification of scleritis by subtypes offers prognostic information and serves as a guideline for therapy
- Diffuse anterior scleritis is the most benign form, nodular scleritis has an intermediate prognosis, and necrotizing scleritis has the worst prognosis in terms of ocular and systemic morbidity
- Early diagnosis and treatment of scleritis is important in preventing and diminishing ocular and systemic morbidity

Table 5.1. Classification of scleritis

	Prevalence	
	Watson	Foster
I. Anterior scleritis	98%	94%
a) Diffuse	40%	45%
b) Nodular	44%	23%
c) Necrotizing	14%	26%
i) With inflammation	(10%)	(23%)
ii) Without inflammation = scleromalacia perforans	(4%)	(3%)
II. Posterior scleritis	2%	6%

Table 5.2. Scleritis vs. episcleritis

	Episcleritis	Scleritis
Main symptom	Redness	Severe, radiating pain
Redness	Bright red	Bluish red
Maximum	Superficial	Deep
Vascular	Episcleral	Episcleral
Congestion	Vessels	Vessels
Tenderness	Rare	+
Scleral thinning	Rare	+
Vision affected	Rare	+
Intraocular involvement	Rare	+

5.1 Introduction

Scleritis represents a spectrum of relatively rare inflammatory disorders of the sclera. Because of the potentially devastating ocular complications and possible association with serious systemic disease, the diagnosis of scleritis should not be missed. Overall, scleritis most often presents within the 4th–6th decades with a mild predisposition towards women over men (1.6:1) [34, 42, 45]. This entity is rarely seen in children. No specific genetic, racial, or geographic risk factors have been elucidated for scleritis, beyond those seen with associated systemic conditions. One-third to one-half of patients (25–45%) with scleritis present with or progress to bilateral disease; however, disease can be unilateral, simultaneously bilateral, or alternate between each eye [34, 42, 45].

5.1.1 Classification

In 1976, Watson and Hayreh proposed a clinical classification of scleritis (Table 5.1) based upon the anatomic location of the inflammation and the observed alterations in the associated vascular structures [45]. This categorization of disease entities does not infer aetiology, but provides valuable information regarding severity of inflammation, prognosis, management options, and association with systemic diseases and with ocular complications. Few patients (<10%) progress to a different form of scleritis from their initial presentation [42].

Scleritis is defined as anterior or posterior based upon the location of inflammation, relative to the equator of the globe. The majority of scleritis is anterior and can be categorized as non-necrotizing or necrotizing. Diffuse (40–45%) and nodular (23–44%) scleritis are non-necrotizing and represent the most common forms of anterior scleritis (Table 5.2). The necrotizing types of anterior scleritis are less common (14–26%), but represent a more severe disease entity [34, 45]. Necrotizing scleritis is classified as either with inflammation or without inflammation, with the latter being synonymous with scleromalacia perforans.

Posterior scleritis represents a more heterogeneous spectrum of inflammation that is less amenable to classification. The prevalence of 2–12% for posterior scleritis may be underrepresentative, due to its low incidence and underrecognition on clinical examination [3, 34, 45]. Singh and Foster previously subdivided posterior scleritis as either chronic or acute [40]. Ultrasonographic classification categorizes posterior scleritis as diffuse or nodular, based upon

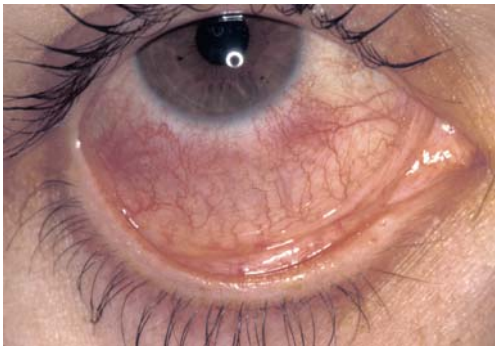

Fig. 5.1. Postsurgical necrotizing scleritis

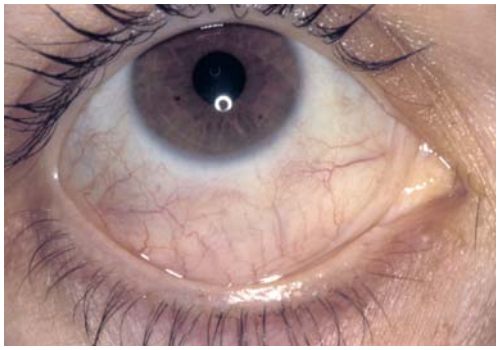

Fig. 5.2. Infectious scleritis

increased eye wall thickness or the finding of scleral nodules, respectively [22].

Although scleritis is most often idiopathic or associated with a systemic disease, scleritis can also be postsurgical or related to an infectious process (Table 5.4). In previous studies, 25–57% of scleritis cases were associated with a known systemic condition [34, 42, 45]. In the Watson and Hayreh series, connective tissue disorders were present in 15% of the patients, of which rheumatoid arthritis constituted 10% [45]. In another series with a higher proportion of necrotizing scleritis cases, half (48%) of the patients had an associated systemic connective tissue or vasculitic disease [34]. Necrotizing scleritis has the highest association (45–95%) with systemic illness and represents the most frequent type of scleritis that is the first manifestation of a systemic condition [34, 45]. Approximately two-thirds of patients with scleromalacia perforans have an associated systemic condition [34], most commonly longstanding rheumatoid arthritis (47%) [45]. Diffuse scleritis appears to be the most benign form with the lowest prevalence of associated systemic illness. Nodular scleritis and posterior scleritis have an intermediate prevalence with associated systemic conditions. Systemic conditions associated with scleritis include Wegener's granulomatosis, relapsing polychrondritis, systemic lupus erythematosus, inflammatory bowel disease, Reiter's syndrome, psoriatic arthritis, polyarteritis nodosa, ankylosing spondylitis, Behçet's disease, giant cell arteritis, and Cogan's syndrome [12, 23, 34, 45]. Vasculitis is a proposed common factor in the pathogenesis of both scleritis and the systemic autoimmune disorders.

Scleritis may occur following ocular trauma, including surgery (Fig. 5.1). The aetiology is unclear, but is hypothesized to be driven by an aberrant, immune-mediated response with a resultant vasculitis. These patients may be predisposed to autoimmune disorders, where trauma initiates the inflammatory cascade that causes scleritis [19, 27, 32, 38]. Surgically induced necrotizing scleritis (SINS) can occur after any type of ocular surgery with scleral manipulation, including cataract surgery, strabismus surgery, filtering blebs, pterygium surgery, and operations for retinal detachments [19, 27, 32, 38]. The onset of progressive scleral inflammation with scleral destruction most frequently occurs within 6 months, but can occur as early as 1 day or as distant as years after the surgery [19, 27, 32, 38]. Inflammation is typically localized to the site or adjacent to the site of surgery, but may progress to involve the entire sclera [19, 32]. Necrotizing scleritis (94%) and posterior scleritis occur more often, compared with other forms of scleritis [27]. The presence of necrotizing scleritis following an uncomplicated ocular surgery may represent the first manifestation of an unrecognized systemic illness. The prevalence of an underlying systemic disease in patients with SINS is approximately 62–90% [19, 27, 32, 37].

Additionally, scleritis may develop due to a primary infectious aetiology (Fig. 5.2) or secondarily as an immune response to a pathogen.

Table 5.3. Systemic diseases associated with scleritis

Rheumatoid arthritis
Wegener's granulomatosis
Inflammatory bowel disease: ulcerative colitis and Crohn's disease
Relapsing polychondritis
Systemic lupus erythematosis
Polyarteritis nodosa
Giant cell arteritis
Behçet's disease
Polymyalgia rheumatica
Reiter's syndrome
Raynaud's disease
IgA nephropathy
Ankylosing spondylitis
Gout
Sarcoidosis
Rosacea
Psoriasis
Lymphoma (Hodgkin's)
Pyoderma gangrenosum
Cogan's syndrome
Necrobiotic xanthogranuloma
Poststreptococcal vasculitis

Table 5.4. Infectious scleritis

Bacterial
Pseudomonas
Proteus mirabilis
Staphylococcus epidermidis
Streptococcus pneumoniae
Viral
Herpes zoster
Herpes simplex
Mumps
Granulomatous
Mycobacterium tuberculosis
Mycobacterium chelonae
Mycobacterium leprae
Syphilis
Fungal
Aspergillus
Pseudallescheria boydii
Sporotrichosis
Parasitic
Acanthamoeba
Toxocariasis
Toxoplasmosis
Onchocerciasis

Various bacterial, viral, fungal, and parasitic infections have been implicated in scleritis [10, 17, 21]. *Pseudomonas* scleritis is the most common cause of infectious scleritis and requires early recognition due to its potential for severe tissue destruction [17]. Infectious scleritis usually results from a traumatic mechanism or from extension of an infection involving other ocular tissues (Table 5.4).

Summary for the Clinician

- **The clinical classification of scleritis based upon anatomic location and alterations in vascular structures (Table 5.1) provides prognostic information regarding severity of inflammation, prognosis, therapy, and association with ocular and systemic morbidity**
- **Scleritis is relatively rare and the majority of cases (94–98 %) are anterior**
- **Twenty-five to 57 % of scleritis cases are associated with a systemic disorder, most commonly rheumatoid arthritis (Table 5.3)**
- **Although most scleritis cases are considered idiopathic or associated with a systemic condition, scleritis can be postsurgical or related to an infectious pathogen (Table 5.4)**

5.1.2 Anatomy

The sclera is the durable, outer connective tissue layer of the eye that protects the intraocular contents and maintains the shape of the globe. The episclera is the most superficial layer, composed of an outer, parietal layer that merges with the overlying conjunctiva and a deeper, visceral layer that connects with the underlying sclera. The scleral stroma is formed of dense collagen bundles with an interlacing array of fibrils, responsible for the opaque appearance and rigidity of the sclera [13].

The sclera is relatively avascular with the exception of perforating vessels. The metabolic requirement of the sclera is low, due to the slow turnover of collagen. The abundant blood supply to the sclera comes from vascular networks in the episclera and choroid. The anterior segment is primarily supplied by the anterior ciliary arteries, which have extensive collateral anastomoses with the posterior ciliary arteries. The anterior ciliary arteries originate from the ophthalmic artery and course anteriorly where the lateral branches merge posterior to the limbus, forming the anterior episcleral arterial circle. The bulbar conjunctival plexus, the superficial episcleral plexus, and the deep episcleral or scleral plexus originate from branches of the anterior episcleral circle. The superficial bulbar conjunctival vessels are radial, freely mobile, and appear bright red when congested. The less superficial episcleral vessels are radial, less mobile, located within the parietal episcleral layer, and appear salmon pink when inflamed. In contrast, the deep episcleral vessels are immobile with a criss-cross configuration, lie within the visceral episcleral layer adjacent to the sclera, and appear a bluish-red hue when congested [13].

5.1.3 Pathogenesis

An associated vasculitis or microangiopathy may play an important role in the pathogenesis of scleritis [11–13, 29, 41]. A proposed component underlying both necrotizing scleritis and systemic autoimmune conditions is an obliterative, immune complex mediated vasculitis. An immune complex mediated or type III reaction may be involved in the pathogenesis of scleritis [12]. Exogenous and endogenous or "self" antigens stimulate the immune system to produce antibodies, leading to immune complex deposition within and outside of vessel walls. The interaction of immune complexes with the complement cascade may play an important role in scleritis. The anterior sclera has higher levels of the recognition component C1 of the complement cascade, compared with the posterior sclera [8, 16]. Inflammatory stimuli, such as interferon-gamma, can induce scleral fibroblasts to increase production of C1, C2, and C4 [16]. Immune complexes interact with C1 to activate the classical complement pathway, resulting in increased vascular permeability and release of chemotactic factors for neutrophils [8]. Tissue damage occurs from membrane attack complex mediated cell lysis and release of lysosomal enzymes, oxygen free radicals, and proinflammatory cytokines by neutrophils. As a result, platelets adhere to damaged endothelium, obstruct blood vessels, and release additional inflammatory mediators, contributing to further inflammatory cell infiltration, fibrinoid necrosis, and vascular occlusion [13]. Histopathologic studies of necrotizing scleritis have confirmed findings of immune-complex deposition, neutrophil infiltration, and fibrinoid necrosis of the vasculature, accompanied by elevated levels of immunoglobulins IgM, IgG, IgA, complement C3, and C4 [12].

Animal models support the role of immune complex deposition in necrotizing scleritis. Intralimbal injections of albumin administered to rabbits pre-sensitized to ovalbumin over 12–18 months can result in an arthus reaction with findings consistent with necrotizing scleritis, secondary to the presence of circulating antibodies to ovalbumin [18].

A T-lymphocyte-mediated delayed hypersensitivity or type IV reaction may also be involved in the pathogenesis of scleritis. T-lymphocyte activation by an unknown antigen can stimulate B-lymphocyte antibody production, leading to immune complex deposition. Conversely, immune complexes can stimulate a cell-mediated immune response. The increased T-lymphocyte helper to suppressor ratio seen in scleritis supports the T-cell-mediated role in the complex interplay of the immune response. Idiopathic scleritis is hypothesized to occur due to a delayed hypersensitivity reaction to an unknown endogenous scleral antigen [13].

Altered immune regulation may be another factor involved in scleritis. Constitutive expression of class II MHC glycoproteins is normally limited to Langerhans cells, macrophages, monocytes, and lymphocytes. However, under inflammatory stimulation by interferon-gamma, scleral fibroblasts can be induced to express class II HLA glycoproteins [16]. The aberrant

capability of fibroblasts to present antigen to T-helper lymphocytes may result in an increased susceptibility to autoimmunity and a loss of self-tolerance. Increased HLA-DR expression in necrotizing scleritis also supports the role of altered immune function in these patients [12, 13, 16].

5.1.4 Histopathology

The histopathology of scleritis is poorly understood given the lack of available tissue in the early stages of inflammation. Previous pathologic studies were based upon tissue obtained from enucleated eyes with advanced disease [15]. Scleral biopsies have rarely been performed given the high rate of associated complications. Although several previous histopathologic examinations have failed to distinguish between the different causes of scleritis [15, 39, 46], recent pathologic studies of necrotizing scleritis may elucidate its pathogenesis [12, 28, 30]. The pathologic findings of scleritis are classified as (1) rheumatoid and rheumatoid-like necrotizing scleritis, (2) idiopathic necrotizing scleritis, (3) postinfectious scleral inflammation, and (4) sarcoidal inflammation [28, 30].

The typical feature of rheumatoid or rheumatoid-like scleritis is central scleral necrosis with a distinct surrounding zone of granulomatous inflammation [12, 28, 30]. Inflammatory cell infiltration with polymorphonuclear leukocytes, histiocytes, and lymphocytes within the episcleral tissue and suprachoroidal area, the presence of an associated necrotizing vasculitis, and scleral fibre necrosis between the pars plana and limbus are other notable findings in rheumatoid scleritis. In the posterior sclera, histologic findings are more suggestive of ischaemia than inflammation. No reactive proliferation of connective tissue or blood vessels is observed. Rheumatoid nodules within the sclera are histologically similar to the skin nodules seen in rheumatoid arthritis.

Although many infectious agents can play a primary or secondary role in scleritis, only a few cases have histologic descriptions. In scleritis following a previous herpes zoster ophthalmicus infection, histologic findings usually include scleral necrosis, an associated vasculitis, and surrounding zonal granulomatous inflammation, primarily in the anterior sclera [28, 30]. The inflammation can be non-granulomatous and focal. Although the scleritis is suspected to be an immune-mediated response to the prior infection, the presence of a reactive proliferation of granulation tissue distinguishes this form from the rheumatoid type. In infectious scleritis, the presence of microabscesses with or without histologic identification of a pathogen can be a distinguishing factor. In scleritis caused by *Pseudomonas,* multiple microabscesses with necrotic neutrophils and gram-negative bacteria can be seen [28, 30].

Idiopathic necrotizing scleritis is characterized by chronic, non-granulomatous inflammation and diffuse lymphocytic infiltration of the anterior sclera, episclera, and uvea [28, 30]. In contrast to rheumatoid-like scleritis, reactive connective tissue and blood vessel proliferation is observed, while findings suggestive of vasculitis are absent. The presence of newly formed vascular channels and focal granulation tissue with fibroblasts, lymphocytes, and histiocytes in idiopathic scleritis may be suggestive of a delayed type of hypersensitivity [29]. Although sarcoidosis rarely has scleral involvement, the histologic findings are distinctive with minimal to no necrosis and well-delineated lesions composed of epithelioid cell collections [30].

Given the higher prevalence of anterior scleritis compared to posterior scleritis, limited histologic information about posterior scleritis is known. Histologic findings of posterior scleritis have been obtained by studying eyes enucleated for the mistaken diagnosis of malignant melanoma. Perivascular lymphocyte accumulation in the episclera and sclera can be seen. Minimal findings suggestive of a primary vasculitis were found [4].

In a case series of seven enucleated eyes treated with systemic steroids, the presence of RPE loss, chronic inflammatory cells, and mast cells was observed in posterior scleritis. All seven cases had findings of fragmented collagen with widespread granulomatous changes suggestive of an associated vasculitis [9].

Summary for the Clinician

- **An immune-complex mediated microangiopathy or vasculitis may play a role in the pathogenesis of scleritis**
- **The higher levels of complement C1 and ability of induced scleral fibroblasts to increase production of complement components may be involved in the inflammatory cascade, resulting in inflammatory cell infiltration, fibrinoid necrosis, and vascular occlusion**
- **The role of a cell mediated delayed hypersensitivity reaction is supported by the increase in T-lymphocyte helper to suppressor ratio in scleritis**
- **Aberrant HLA-DR expression in scleritis may play a role in the altered immune function in these patients**
- **The pathologic findings of scleritis can have characteristic features based upon the aetiology and be classified as rheumatoid, idiopathic, postinfectious, and sarcoidal**

5.2 Clinical Presentation

The diagnosis of scleritis is based upon history and clinical findings seen on examination. Scleritis is almost always associated with overlying inflammation of the episclera or episcleritis. However, the clinical distinction between scleritis and episcleritis is necessary, as they represent different clinical disease entities with very different clinical presentations, diseases courses, treatments, and prognoses (Table 5.2) [45].

5.2.1 History: Ocular Symptoms

The clinical presentation of scleritis depends upon the anatomic site involved and the extent of inflammation. Patients often seek medical attention for severe, deep, boring pain that can be localized to the eye or generalized (66%), radiating along the trigeminal nerve distribution to the brow, temple, sinuses, and/or jaw [43, 45]. Pain is usually only temporarily relieved with analgesics and can be associated with malaise and weight loss, leading to misdiagnoses such as sinusitis, migraines, and brain tumour. Distention of the nerve fibres secondary to scleral edema and necrosis of nerve endings are speculated to be responsible for pain that can be so severe as to awaken the patient. The most severe pain, often out of proportion to the extent of inflammation, is seen with progressive necrotizing scleritis, which can be a stark contrast to the absence of pain in scleromalacia perforans. The eye may feel tender to palpation due to the inflammation [45]. However, tenderness is generally not experienced in necrotizing scleritis without inflammation. In contrast to scleritis, episcleritis is not associated with significant pain or tenderness.

Scleritis typically has a gradual onset of redness with increasing inflammation over several days [45]. In contrast to the brighter redness of episcleritis, scleritis is usually a darker violaceous-red hue due to the depth of the congested vascular plexus. The extent of redness does not correlate with the severity of disease and may be absent in scleromalacia perforans.

Photophobia and lacrimation are non-specific symptoms sometimes associated with anterior scleritis, most often with necrotizing scleritis [45]. Although approximately half of patients report tearing in posterior scleritis, photophobia is atypical. Conjunctival discharge is not a characteristic feature of scleritis and may suggest an infectious process.

Patients may experience an insidious onset of visual impairment, especially if the inflammatory process extends to other ocular tissues [22, 34, 40, 42, 45]. Loss of vision does not occur in cases of episcleritis.

Scleromalacia perforans is most notable for the potential lack of symptoms. Many patients often do not report any subjective symptoms, despite having significant signs of scleritis [45].

In addition to the symptoms described previously, posterior scleritis can present with less common symptoms of proptosis, lid edema, ptosis, and pain worsened with eye movement [3, 22, 43, 45].

5.2.2 Physical Examination: Ocular Signs

Anterior scleritis is a clinical diagnosis that requires careful examination to localize the anatomic location and depth of structures involved in the inflammatory process. In comparison to tungsten, fluorescent, and cobalt blue light, examination of the eye under natural light greatly accentuates visualization of deep discoloration, extent of scleral edema, and areas of increased transparency [45]. With the slit lamp examination, particular attention should be directed towards distinguishing the level of maximum vascular congestion, the most extensive areas of scleral edema, and the presence of episcleral infiltration.

A hallmark finding that distinguishes scleritis from episcleritis is the presence of scleral edema. Edematous sclera can bow forward, displacing the deep episcleral vascular plexus and exacerbating deep vascular congestion. To assess the degree of scleral involvement, blanching the superficial conjunctival and episcleral vasculature with topical 2.5% phenylephrine can improve visualization of the underlying tissue. Further examination using a red-free filter is instrumental in evaluating the vascular architecture, areas of avascularity, and cellular infiltration of the episclera. The anatomic location of the inflammation and typical alterations in the vessels form the basis of the classification of anterior scleritis [45].

Diffuse anterior scleritis is the most benign form of scleritis (Fig. 5.3). The area of involvement can be segmental (60%) or global (40%), with mild to severe inflammation and redness. Distortion of the superficial and deep vascular structures with subsequent loss of the normal radial vascular pattern and replacement with large, tortuous anastomotic channels can be seen [45].

Nodular anterior scleritis can present with a single or multiple scleral nodules (Fig. 5.4). Typically, the nodule is a darker hue of red, separate from the overlying episclera, immobile, and tender to palpation. These features distinguish this form of scleritis from nodular episcleritis. The lack of necrosis within the nodule and the containment of inflammation within the borders of the nodules differentiate this form from necrotizing anterior scleritis with inflammation. All of the vascular layers overlying the nodule are displaced forward [45].

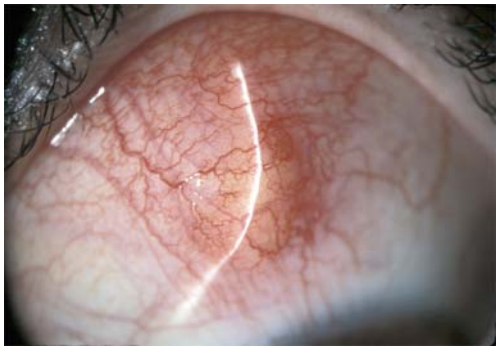

Fig. 5.3. Diffuse anterior scleritis

Necrotizing scleritis with inflammation is the most destructive form of anterior scleritis, attributed to the extensive release of proteolytic enzymes (Fig. 5.5). Commonly, the initial presentation is a localized area of scleral edema with overlying inflammation, greatest at the leading edge. A less common presentation is a focal area of avascular episclera, adjacent to or overlying an area of scleral edema. Thinning of the sclera with increased visualization of the underlying uveal tissue may result in a bluish-grey hue to the sclera. Scleral transparency is attributed to alterations in the collagen and ground substance, allowing increased visualization of the darker uveal tissue. However, transparency does not always indicate thinning of the sclera. A hallmark feature of necrotizing scleritis is findings of scleral ischaemia. Without treatment, the inflammation can progress in both directions circumscribing the globe, until the entire anterior segment is involved. Vascular changes include postcapillary venous congestion, vascular thrombosis, and the development of deep, anastomotic vessels [45].

Minimal to no signs of inflammation are seen with necrotizing scleritis without inflammation or scleromalacia perforans. As the overlying episcleral tissue thins, patches of the sclera may appear yellowish or greyish. The sclera may appear porcelain-like, as the vascularity di-

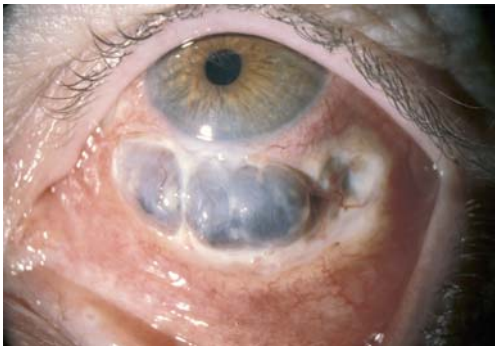

Fig. 5.4. Nodular anterior scleritis

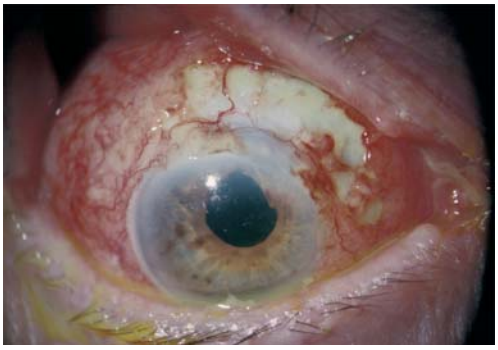

Fig. 5.5. Necrotizing scleritis

minishes. Necrotic sclera can slough or become sequestered. With severe scleral thinning, increased visualization of the dark underlying uvea may occur. Due to decreased sclera vascularity attributed to arteriolar vaso-occlusion, large, abnormal vessels may cross and surround the areas of affected sclera [45].

The findings of posterior scleritis on examination are variable, based upon on the severity, extent, and location of the inflammation. Diffuse scleritis with generalized scleral thickening without nodules or nodular scleritis can develop in the posterior segment [22]. The most common signs of posterior scleritis are posterior extension of anterior scleritis (34%), a serous or exudative retinal detachment (21%), optic disc edema (18%), a circumscribed subretinal mass (13%), choroidal folds, retinal striae, elevated intraocular pressure, and a bullous or annular choroidal detachment [22]. Extension of scleral inflammation to the adjacent choroid can lead to an overlying serous detachment of the neurosensory retina [22, 34, 43, 45], which represents the most common sign of posterior scleritis (21%) [22]. Exudative macular detachments occurred predominantly in young women, with a mean age of 26, and often without any other signs [3]. In posterior scleritis, a circumscribed fundus mass or subretinal granuloma with associated choroidal folds and retinal striae should be differentiated from a choroidal neoplasm. Choroidal folds and retinal striae can occur as independent signs of mild diffuse inflammation [3, 40]. Bullous choroidal detachments and, more commonly, annular cilioretinal effusions can occur as an extension of scleral inflammation [3]. Some patients (9–17%) with posterior scleritis will have no presenting signs [3, 22].

If inflammation extends beyond the globe, proptosis, lid retraction, and restriction of extraocular muscle motility may be seen [3, 45]. Orbital myositis may be associated with posterior scleritis in up to 30% of cases, as an extension of the scleral inflammation [6]. Considerable overlap can be seen between the diagnoses of idiopathic inflammatory pseudotumour of the orbit and posterior scleritis, representing different manifestations within a disease spectrum. However, in idiopathic inflammatory pseudotumour, involvement of the adjacent extraocular orbital structures is the predominant feature [3].

5.2.3 Associated Ocular Manifestations

In the series by Watson and Hayreh, the rate of complications in patients with scleritis was 57%, excluding scleral thinning [45]. Decreased visual acuity, keratitis, cataract, uveitis, and glaucoma are ocular associations indicating the spread of scleral inflammation to adjacent tissues [34, 42, 45]. Complications are more frequent in severe necrotizing scleritis and posterior scleritis [34, 42, 45]. Due to potential ocular complications related to scleritis, early diagnosis and treatment of scleritis and its associated ocular manifestations are critical.

Visual acuity may be compromised in patients with scleritis and must be monitored carefully. Decreased vision occurs most frequently with posterior scleritis (45–84%), necrotizing scleritis (74–82%), nodular scleritis (26%), and least often with diffuse anterior scleritis (9%) [34, 42]. Vision may be limited due to keratitis, anterior uveitis, or cataract in anterior scleritis. In posterior scleritis, vision loss greater than 2 Snellen lines has been reported in 31–45% of patients [22, 34]. Vision loss in posterior scleritis from an axial hyperopic shift in refractive status can occur secondary to scleral edema with anterior displacement of the retina [22, 34, 40]. Decreased vision in posterior scleritis is most commonly related to macular changes followed by optic disc abnormalities, including macular edema, optic disc edema or atrophy, retinal detachment, epiretinal membrane formation, macular cyst or hole, and cataract [22, 34, 40, 42, 45]. In some patients the visual impairment can be severe and permanent, emphasizing the importance of early diagnosis and treatment [22].

A mild to moderate anterior uveitis has been observed in 30–42% of patients with scleritis, most frequently (69%) with necrotizing scleritis [36, 45]. Almost half of the patients with posterior scleritis have an anterior uveitis [34] and 2–100% have a posterior vitritis, depending on the case series [22, 45]. The anterior uveitis often follows a long-standing and intractable course [36, 45], with one-third of cases being bilateral [36]. Approximately half of patients with scleritis-associated uveitis have an associated systemic illness [34, 36], most frequently (19%) rheumatoid arthritis [36]. Anterior uveitis may be an indicator of the activity and extent of scleral inflammation, as the uveitis subsides when the scleral inflammation is adequately treated. Additionally, vision loss (49%), peripheral ulcerative keratitis (22%), and glaucoma (19%) occurred more frequently in patients with uveitis compared with those without uveitis [36]. Due to extension of scleral inflammation to other ocular tissues, scleritis-associated uveitis portends a worse prognosis.

A keratopathy has been associated with 14–29% of patients with scleritis, including peripheral corneal thinning, stromal keratitis, and peripheral ulcerative keratitis [34, 37, 42, 45]. Corneal changes are most frequently seen in patients with necrotizing scleritis (relative risk of 5.3) and least often in patients with diffuse scleritis [37]. An associated systemic illness was found in 87% of patients who had both peripheral keratopathy and scleritis [37]. Patients with scleritis-associated peripheral keratopathy are at increased risk for complications, such as decreased vision (81%), anterior uveitis (62%), and impending corneal perforation (62%) [37]. Peripheral corneal thinning is the most benign form with a well-demarcated peripheral zone of circumferential thinning that precedes corneal vascularization. Indicative of extension of scleral inflammation, stromal keratitis with central or peripheral infiltrates can progress to diffuse corneal clouding or sclerocornea [45]. The most destructive keratopathy is peripheral ulcerative keratitis inferring a worse prognosis [37, 45], with a higher prevalence of necrotizing scleritis (67%), and a greater association with impending corneal perforation (100%) [45].

During any stage of scleral inflammation, the intraocular pressure may be elevated due to several different mechanisms, such as obstruction of the aqueous outflow channels, elevated episcleral pressure, angle closure, or secondary to a steroid response [45]. Although the elevation in intraocular pressure may be transient, glaucoma has been reported in 12–13% of cases of scleritis [34, 45]. Patients with an associated anterior uveitis are at an increased risk for ocular hypertension due to possible obstruction of the trabecular meshwork by inflammatory cells, corticosteroid usage, or secondary angle closure from peripheral anterior synechiae [45]. Intraocular pressure was elevated in 12% of patients with posterior scleritis [22]. In 4–16% of posterior scleritis cases with ocular hypertension, annular ciliochorodial effusions were present, causing secondary angle closure from anterior displacement of the iris-lens diaphragm [3, 22].

Cataracts have been observed in 7–17% of cases of scleritis [34, 45]. Cataract formation may be accelerated by long-standing inflammation or secondary to steroid use. The prevalence of cataract was associated with the type of scleritis, seen most frequently with necrotizing scleritis (41%) and least frequently with diffuse

scleritis (9 %) [34]. Cataract extraction can precipitate scleral inflammation, usually in the form of a necrotizing scleritis [19, 37, 32, 38].

Scleral thinning (22 %) most commonly occurs in necrotizing scleritis and may progress to ectasia [45]. Staphyloma formation occurs only in the presence of increased intraocular pressure, usually greater than 30 mmHg. Scleral defects can develop due to necrosis and sequestration, but rarely result in perforation. If the inflammation is controlled medically, new collagen may form over small defects. Large defects are less likely to become adequately covered with new granulation tissue [45].

5.2.4 Systemic Diseases: Clinical Evaluation

As scleritis can be associated with a systemic condition and may be the initial manifestation of a life-threatening immune-mediated disease, a thorough history and examination are necessary [14, 34, 35, 42, 43, 45]. The evaluation should be directed towards any possible associated rheumatologic, vasculitic, metabolic, pulmonary, renal, cardiac, neurologic, or infectious signs and symptoms. Referral to an internist or rheumatologist can be of great assistance [45].

Summary for the Clinician

- **Scleritis should be distinguished from episcleritis. Vascular congestion that persists after administering phenylephrine 2.5 % is characteristic of scleritis**
- **The most common presenting symptom is severe pain that can radiate along the trigeminal nerve distribution**
- **Examination under natural light accentuates visualization of the anatomic changes with scleritis. Examination with red-free filters facilitates evaluation of vascular structures and regions of avascularity**
- **A hallmark feature that distinguishes scleritis from episcleritis is the presence of scleral edema. Ischemic changes of the scleral are most notable in necrotizing scleritis**
- **Ocular complications of scleritis are most frequent with necrotizing scleritis and posterior scleritis, including scleral thinning, staphyloma, perforation, loss of vision, keratopathy, cataract progression, uveitis, and glaucoma. Extrascleral extension of inflammation portends a worse prognosis**
- **History and physical exam should be comprehensive, including evaluation for any associated systemic conditions**

5.3 Diagnostic Tools

Anterior scleritis is generally a clinical diagnosis, based upon external examination under direct light and slit lamp examination [45]. Fluorescein angiography of the anterior segment may be an adjunctive diagnostic tool in differentiating the type of anterior scleritis, particularly in detecting early necrotizing scleritis [25, 44]. In diffuse anterior scleritis, rapid filling of all vascular networks with decreased transit time, subtle capillary alterations, and abnormal leakage patterns may be seen. The angiographic findings in nodular scleritis are similar, but may be more localized than with diffuse anterior scleritis. In early necrotizing scleritis, failure of episcleral capillary filling, staining of the deep sclera, and limbal conjunctival vascular anomalies may be seen. The most characteristic angiographic finding in necrotizing scleritis is postcapillary venular occlusion with evidence of hypoperfusion and, eventually, non-perfusion. The transit time is much more reduced compared to diffuse and nodular scleritis. Angiographic evidence of new, anastomotic vessels in a granuloma can be appreciated with associated deep scleral staining. In general, fluorescein angiography is not instructive in cases of necrotizing scleritis without inflammation and mainly findings are consistent with areas of vascular closure. The sequential use of fluorescein angiography and indocyanine green angiography may be useful in determining areas of damage that are not clinically visible and in differentiating the subtypes of anterior scleritis [25]. The role of fluorescein angiography (FA) in the diagnosis of posterior scleritis is very limited. Although the inflammatory changes and choroidal vasculature may be not easily as-

sessed, other diseases in the differential diagnoses of serous and exudative detachments, RPE changes, choroidal folds, and retinal striae may be excluded by fluorescein angiography [3, 22].

Indocyanine green angiography (ICG) may play a greater role in the diagnosis and monitoring of progression in posterior scleritis [1]. Focal areas of maximal scleral inflammation and regression of inflammation in response to treatment can be identified with ICG. The principal feature indicative of scleral inflammation is intermediate and late diffuse zonal choroidal hyperfluorescence. In cases with massive subretinal exudation, pinpoint areas of leakage in the underlying choroid can be identified. Regions with delayed choroidal perfusion correspond to focal areas of hypofluorescence that resolve in the late phase, in contrast to similar areas in Voyt Koyanagi Harada syndrome that persist in the late phase. Lastly, enlargement of choroidal veins can be seen and the calibre decreases as a response to treatment. ICG may be useful in assessing therapeutic response [1].

The hallmark features of posterior scleritis seen with both A- and B-mode ultrasonography are helpful in differentiating posterior scleritis from other conditions. B-scan ultrasonography may reveal the characteristic flattening of the posterior aspect of the globe due to retrobulbar edema [3]. Abnormally increased thickening of the posterior ocular coats of the globe >2 mm, optic disc swelling, distension of the optic nerve sheath, retinal detachments, and choroidal detachments can be detected. Fluid can accumulate in the posterior episcleral space and extend around the optic nerve, forming the characteristic "T-sign" on B-scan [3, 22]. A-mode ultrasonography can be helpful in distinguishing scleral nodules with high internal reflectivity from areas of adjacent choroidal thickening with low internal reflectivity. This pattern of reflectivity can also help differentiate scleral nodules from choroidal melanomas with low internal reflectivity and no associated retrobulbar edema. The hallmark feature of retrobulbar edema in posterior scleritis is typically absent in cases of metastatic carcinoma, choroidal haemangiomas, benign lymphoid hyperplasia, and VKH. A limitation of this diagnostic tool is the lack of correlation between serial ultrasound findings and clinical response to treatment [22].

Contrast enhanced orbital CT and MRI scans can show characteristic scleral thickening, confirming the diagnosis of posterior scleritis. Imaging is particularly useful in cases of suspected orbital inflammation, an orbital mass, or in patients with Grave's thyroid disease. Radiological evaluations are not thought to offer a significant advantage over high quality B-scan ultrasonography when orbital extension is absent [22].

Tissue biopsy is generally contraindicated in scleritis, due to poor wound healing and areas of scleral thinning at risk for perforation [45]. If an infectious aetiology is suspected, the appropriate cultures should be obtained and tissue biopsy may be considered.

Patients with scleritis should undergo a targeted evaluation for any possible associated systemic condition [45]. Laboratory tests to consider for immune-mediated connective tissue and vasculitic disorders include erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, antinuclear antibody, antineutrophil cytoplasmic antibody (classic and perinuclear), immune complex testing, complement levels, and complete blood cell count with differential. Serum uric acid and urinalysis can be used to screen for metabolic disorders, such as gout. Latent syphilis can be investigated with serologic tests such as fluorescent treponemal antibody absorption test (FTA-ABS). Investigation with a radiograph of the chest, sacroiliac joint, joints of the extremities, and sinuses should also be considered as part of the systemic evaluation for arthritis, Wegener's granulomatosis, and tuberculosis.

Summary for the Clinician

- **Scleritis is generally a clinical diagnosis. Ancillary testing with FA, ICG, ultrasonography, MRI, and CT can be helpful adjuncts**
- **A classic sign of posterior scleritis is the "T-sign" on B-scan, due to accumulation of fluid in the posterior episcleral space and around the optic nerve. Scleral thickening seen on CT and MRI can be confirmative of posterior scleritis**

- **Tissue biopsy is contraindicated due to the increased risk of perforation**
- **Patients should undergo targeted evaluation with laboratory tests and radiographic studies for any possible associated systemic conditions**

5.4 Medical Management

For non-necrotizing forms of scleritis, such as diffuse and nodular, oral non-steroidal anti-inflammatory medications (NSAIDs) [24, 33, 42, 43, 45], such as indomethacin, are considered first-line treatment with a 92% success rate [33]. When patients fail to respond to oral NSAIDs, systemic corticosteroids may be substituted or more effectively used in combination with NSAIDs [24]. Systemic corticosteroids can be administered as oral prednisone 60 mg daily or intravenously "pulsed" methylprednisolone. Corticosteroids should be discontinued when inflammation is suppressed and remission can be maintained with NSAIDs. If treatment is not successful with the use of corticosteroids, immunosuppressive agents should be added or substituted as a third-line treatment [24, 33, 43]. Patients using NSAIDs need to be followed closely for gastrointestinal symptoms and may require ulcer prophylaxis. Side effects of corticosteroids include peptic ulcer disease, hypertension, secondary diabetes mellitus, osteoporosis, weight gain, adrenal suppression, Cushing's syndrome, myopathy, psychosis, increased intraocular pressure, and progression of cataract formation [24].

For necrotizing scleritis, treatment with NSAIDs is often inadequate. Alone or in combination with NSAIDs, systemic corticosteroids are used as first-line therapy, usually with great success [24, 43, 45]. If inflammation is not adequately suppressed or if the patient is intolerant, treatment with steroid-sparing immunosuppressive medications is indicated [14, 24, 33, 43, 45]. Additionally, immunosuppressive agents can be used as combination therapy to decrease corticosteroid dosage or in place of corticosteroids to decrease the side effects of their long-term use. Immunosuppressive agents that can be used to treat scleritis include oral cyclophosphamide, methotrexate, azathioprine, cyclosporine, and tacrolimus [14, 33].

Often, when the systemic disorder is medically controlled, the scleral inflammation subsides. Based upon previously published mortality data, the use of systemic immunosuppressant therapy for scleritis in patients with rheumatoid arthritis may confer a better overall ocular and systemic prognosis. Without aggressive systemic treatment, patients with necrotizing scleritis and rheumatoid arthritis had a higher 5-year mortality rate from the onset of the systemic vasculitic manifestations [14, 45]. The physician administering immunosuppressive therapy should be trained to diagnose and manage the side effects and potential complications associated with these medications [33].

The alkylating agent cyclophosphamide is the most frequently used cytotoxic agent for severe scleritis, especially efficacious in scleritis associated with Wegener's granulomatosis. Patients are advised to drink 3 liters of fluid daily to decrease the risk of hemorrhagic cystitis. Other possible complications include bone marrow suppression, pancytopenia, and oncogenesis. The antimetabolite methotrexate can be used to treat collagen vascular disease-associated scleritis and may have complications of hepatotoxicity, skin and mucosal ulceration, bone marrow suppression, oncogenesis, and secondary infection. Azathioprine is another anti-metabolite that can be effective in relapsing polychrondritis-associated scleritis and has a similar side effect profile to methotrexate. For refractory severe scleritis, the antimicrobial cyclosporine may be effective and should be monitored for bone marrow suppression, hypertension, hirsutism, hyperglycemia, hyperlipidemia, tremor, nephrotoxicity, oncogenesis, and opportunistic infections. The immunosuppressant antibiotic tacrolimus has a similar mechanism of action to cyclosporine and may also be used in cases of refractory scleritis. Patients should be monitored for hyperglycemia, nephrotoxicity, neurotoxicity, and gastrointestinal dysfunction.

In general, topical non-steroidal anti-inflammatory agents and corticosteroids are insufficient to suppress scleral inflammation [43]. Lo-

cal use of corticosteroids as subconjunctival injections is controversial. Historically, subconjunctival injections have been contraindicated due to concerns of increased risk of scleral necrosis and perforation [43, 45]. Subconjunctival injections of triamcinolone acetonide may be a safe and effective treatment of anterior scleritis [47]. Additionally, use of orbital floor depots of corticosteroids have been reported. Local treatment may decrease the dosage requirement of systemic treatment for scleritis.

The previous recommendations do not apply to cases of infectious scleritis, where early diagnosis and treatment with the appropriate topical and intravenous anti-microbial medications are crucial [17, 24].

5.5 Surgical Management

Most cases of scleritis can be managed with medical therapy alone. In 5–10 % of cases of necrotizing scleritis, surgical intervention is to needed to cover scleral or corneal defects, to repair ruptured globes, or to support areas of uveal prolapse at risk for perforation [13]. Systemic immunosuppressive therapy needs to be implemented prior to or concomitant with such procedures to increase the likelihood of graft survival [3, 26, 31]. Homologous donor sclera tissue is the most commonly used graft material. Other materials that have been used with variable results include fascia lata, periosteum, aortic tissue, split-thickness dermal grafts, and auricular cartilage [26]. Similarly, lamellar corneal grafting may be needed for progressive corneal thinning or keratolysis. In conjunction with systemic immunosuppression, scleral patch grafting and lamellar corneal grafting can be useful in treating and preventing ocular perforation in progressive disease [31].

Surgical intervention for secondary complications, such as cataract and glaucoma, should be performed only when the inflammation is medically controlled. Additional immune suppression perioperatively may be beneficial in preventing a surgically induced relapse of scleritis.

Summary for the Clinician

- **Scleritis is generally medically managed in a stepwise manner with non-steroidal anti-inflammatory agents, corticosteroids, and steroid-sparing immunosuppressants, based upon therapeutic response and tolerance**
- **Due to the systemic nature of the medications, patients need to be followed closely for associated side effects**
- **Five to 10 % of scleritis cases require surgical intervention and success is highly dependent upon medical control of the inflammation**

References

1. Auer C, Herbort CP (1998) Indocyanine green angiographic features in posterior scleritis. Am J Ophthalmol 126:471–476
2. Bechrakis NE, Foerster MH, Bornfeld N (2002) Biopsy of indeterminate intraocular tumors. Ophthalmology 109:235–242
3. Benson WE (1988) Posterior scleritis. Surv Ophthalmol 32:297–316
4. Bernauer W, Buchi ER, Daicker B (1995) Immunopathological findings in posterior scleritis. Int Ophthalmol 18:229–231
5. Bloomfield SE, Becker CG, Christian CL, et al. (1980) Bilateral necrotising scleritis with marginal corneal ulceration after cataract surgery in a patient with vasculitis. Br J Ophthalmol 64:170–174
6. Boonman ZF, De Keizer RJ, Graniewski-Wijnands HS, et al. (2003) Orbital myositis in scleritis. Br J Ophthalmol 110:15–21
7. Bottazzo GF, Pujol-Borrell R, Hanafusa T, et al. (1983) Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. Lancet 2:1115–1119
8. Brawman-Mintzer O, Mondino BJ, Mayer FJ (1989) Distribution of complement in the sclera. Invest Ophthalmol Vis Sci 30:2240–2244
9. Calthorpe CM, Watson PG, McCartney ACE (1988) Posterior scleritis: a clinical and histological survey. Eye 2:267–277
10. Dougherty PJ, Binder PS, Mondino BJ, et al. (1994) Acanthamoeba sclerokeratitis. Am J Ophthalmol 117:475–479
11. Fauci AS, Haynes BF, Katz P (1978) The spectrum of vasculitis: clinical, pathologic, immunologic, and therapeutic considerations. Ann Intern Med 89:660–676

12. Fong LP, Sainz de la Maza M, Rice BA, et al. (1991) Immunopathology of scleritis. Ophthalmology 98:472–479
13. Foster CS, Sainz de la Maza M (1994) The sclera. Springer-Verlag, New York
14. Foster CS, Forstot SL, Wilson LA (1984) Mortality rate in rheumatoid arthritis patients developing necrotizing scleritis or peripheral ulcerative keratitis. Ophthalmology 91:1253–1263
15. Fraunfelder FT, Watson PG (1976) Evaluation of eyes enucleated for scleritis. Br J Ophthalmol 60:227–230
16. Harrison SA, Mondino BJ, Mayer FJ (1990) Scleral fibroblasts: HLA antigen expression and complement production. Invest Ophthalmol Vis Sci 31:2412–2419
17. Helm MD, Holland GN, Webster RG, Maloney RK, Mondino BJ (1997) Combination of intravenous ceftazidime and aminoglycosides in the treatment of pseudomonal scleritis. Ophthalmology 104:838–843
18. Hembry RM, Palyfair J, Watson PG, et al. (1979) Experimental model for scleritis. Arch Ophthalmol 97:1337
19. Karia N, Doran J, Watson SL, et al. (1999) Surgically induced necrotizing scleritis in a patient with ankylosing spondylitis. J Cataract Refract Surg 25:597–600
20. Legmann A, Foster CS (1996) Noninfectious necrotizing scleritis. Int Ophthalmol Clin 36:73–80
21. Livir-Rallatos C, El-Shabrawi Y, Zatirakis P, et al. (1998) Recurrent nodular scleritis associated with varicella zoster virus. Am J Ophthalmol 126: 594–595
22. McClusky PJ, Watson PG, Lightman S, et al. (1999) Posterior scleritis: clinical features, systemic associations, and outcome in a large series of patients. Ophthalmology 106:2380–2386
23. McGavin DDM, Williamson J, Forrester JV, et al. (1976) Episcleritis and scleritis: a study of their clinical manifestations and association with rheumatoid arthritis. Br J Ophthalmol 60:192–226
24. Mondino BJ, Phinney RB (1988) Treatment of scleritis with combined oral prednisone and indomethacin therapy. Am J Ophthalmol 106:472–479
25. Nieuwenhuizen J, Watson PG, Jager MJ, et al. (2003) The value of combining anterior segment fluorescein angiography with indocyanine green angiography in scleral inflammation. Ophthalmology 110:1653–1666
26. Nguyen QD, Foster CS (1999) Scleral patch graft in the management of necrotizing scleritis. Int Ophthalmol Clin 39:109–131
27. O'Donoghue E, Lightman S, Tuft S, et al. (1992) Surgically induced necrotizing sclerokeratitis (SINS) – precipitating factors and response to treatment. Br J Ophthalmol 76:17–21
28. Rao NA, Marak GE, Hidayat AA (1985) Necrotizing scleritis: a clinico-pathologic study of 41 cases. Ophthalmology 92:1542–1549
29. Rao NA, Phillips TM, Wong VG, et al. (1985) Etiology of scleritis. In: O'Connor GR, Chandler JW (eds) Advances in immunology and immunopathology of the eye. Masson, New York, pp 54–57
30. Riono WP, Hidayat AA, Rao NA (1999) Scleritis: a clinicopathologic study of 55 cases. Ophthalmology 106:1328–1333
31. Sainz de la Maza M, Tauber J, Foster CS (1989) Scleral grafting for necrotizing scleritis. Ophthalmology 96:306–310
32. Sainz de la Maza M, Foster CS (1991) Necrotizing scleritis after ocular surgery: a clinicopathologic study. Ophthalmology 98:1720–1726
33. Sainz del la Maza M. Jabbur NS, Foster CS (1993) An analysis of therapeutic decision for scleritis. Ophthalmology 100:1372–1376
34. Sainz del la Maza M, Jabbur NS, Foster CS (1994) Severity of scleritis and episcleritis. Ophthalmology 101:389–396
35. Sainz de la Maza M, Foster CS, Jabbur NS (1995) Scleritis associated with systemic vasculitis diseases. Ophthalmology 102:687–692
36. Sainz de la Maza M, Foster CS, Jabbur NS (1997) Scleritis-associated uveitis. Ophthalmology 104: 58–63
37. Sainz de la Maza M, Foster CS, Jabbur NS, Baltatzis M (2002) Ocular characteristics and disease associations in scleritis-associated peripheral keratopathy. Arch Ophthalmol 120:15–19
38. Salamon SM, Mondino BJ, Zaidman GW (1982) Peripheral corneal ulcers, conjunctival ulcers, and scleritis after cataract surgery. Am J Ophthalmol 93:334–33
39. Sevel D (1967) Necrogranulomatous scleritis: clinical and histologic features. Am J Ophthalmol 64:1125–1134
40. Singh G, Guthoff R, Foster CS (1986) Observations on long-term follow-up of posterior scleritis. Am J Ophthalmol 101:570–575
41. Sokoloff L, Bunim JJ (1957) Vascular lesions in rheumatoid arthritis. J Chronic Dis 5:668–687
42. Tuft SJ, Watson PG (1991) Progression of scleral disease. Ophthalmology 98:467–471
43. Watson PG (1980) The diagnosis and management of scleritis. Ophthalmology 87:716–720
44. Watson PG, Bovey E (1985) Anterior segment fluorescein angiography in the diagnosis of scleral inflammation. Ophthalmology 92:1–11

45. Watson PG, Hayreh SS (1976) Scleritis and episcleritis. Br J Ophthalmol 60:163–191
46. Young RD, Watson PG (1984) Microscopical studies of necrotising scleritis. Br J Ophthalmol 68: 770–780
47. Zamir E, Read RW, Smith RE, Wang RC, Rao NA (2002) A prospective evaluation of subconjunctival injection of triamcinolone acetonide for resistant anterior scleritis. Ophthalmology 109: 798–807

Clinical Aspects of MALT

6

Erich Knop, Nadja Knop, Uwe Pleyer

Core Messages

- Mucosa-associated lymphoid tissue (MALT) is an outpost of the immune system located at mucosal surfaces of the body. It can recognize antigens, generate specific effector cells and provide the mucosal organs with such cells
- MALT also occurs at the normal human ocular surface and appendage (lacrimal gland, conjunctiva and lacrimal drainage system), which together form an eye-associated lymphoid tissue (EALT)
- EALT makes an important contribution to the homeostasis of the ocular surface by maintaining the equilibrium between inflammatory immune reactions against pathogens and immune tolerance against non-pathogenic antigens
- Dendritic antigen presenting cells (DC) are a key regulator of the immune response by the promotion of different subpopulations of T-lymphocytes that act via specific sets of cytokines
- Various diseases of the ocular surface include an immune mediated inflammation with production of cytokines, chemokines, adhesion molecules and action of lymphocytes
- This is conceivably influenced by a dysregulation of EALT and can hence be therapeutically addressed by immune modulatory drugs
- Different types of dry eye disease contain an underlying immune modulated inflammation based on a deregulation of the physiological and normally protective mucosal immune system
- In chronic allergic and vernal keratoconjunctivitis (AKC and VKC) inflammatory cells and lymphocytes are activated by mast cell cytokines and result in an inflammatory infiltrate and corneal destruction
- Local as well as systemic T-cell mediated immune processes are involved in transplant rejection. CD4+ lymphocytes play an essential role in the immune response during corneal graft rejection and are the main target of immunomodulatory therapy

6.1 Introduction

The immune protection at the inner and outer mucosal surfaces of the body, including the ocular surface, is maintained by a part of the immune system termed the "mucosa-associated lymphoid tissue" (MALT). This is found in different mucosal organs where the lymphoid tissue for each is designated separately according to an international nomenclature composed of characteristic acronyms. MALT is most prominent in the gut (termed gut-associated lymphoid tissue or GALT) but is also found in the airways (termed bronchial-associated lymphoid tissue or BALT) or in the genitourinary system. Recently MALT was also described as a regular component of the normal human ocular surface and accordingly termed eye-associated lymphoid tissue (EALT).

One of the main functions of MALT is to establish a balance between immunity and tolerance in order to prevent destruction of the delicate mucosal tissues by constant inflammatory reactions, which applies in particular to the eye. This is maintained by an anti-inflammatory cytokine milieu in mucosal tissues and is most likely regulated by antigen presenting dendritic cells (DC) that act as key regulators of the immune system and normally favour anti-inflammatory T- or B-cell responses in mucosal locations. A major defence mechanism of MALT is the production of secretory immunoglobulins, mainly of the IgA and partly of the IgM isotype by differentiated B cells (plasma cells). In contrast to the IgG isotype that prevails in the blood, IgA has very little complement binding activity and therefore does not initiate inflammatory reactions during host defence.

The lymphoid cells of MALT migrate in a regulated fashion, guided by specialized vessels, adhesion molecules and soluble chemotactic factors. They migrate between the different mucosal organs, which are hence assumed to constitute a functionally interrelated mucosal immune system. By these migration pathways, MALT is also connected to the central immune system.

Since the mucosal immune system is a prominent source of professional immune regulating cells and soluble mediators that are constitutively present also at the normal human ocular surface, it is also involved in mucosal disease states as will be pointed out in the present paper. In fact research has indicated that deregulation of the physiological mucosal immune system may contribute as a primary or secondary pathogenetic factor to inflammatory diseases such as ocular allergy but also to conditions such as dry eye disease where an underlying inflammatory component is shown. The mucosal immune system is certainly also involved in the course of a corneal transplant and its potential rejection, which represents a T-cell-mediated process. This is regulated to a large extent through the mediation of conjunctival DC and the newly discovered central corneal DC.

Advances in the understanding of the different mechanisms of ocular mucosal immunity, concerning for example immune regulatory cells (e.g. T cells and DC), regulatory molecules or mechanisms of recirculation that guide the influx of cells into the respective tissues, may therefore be applied as future more effective strategies for some of today's not infrequently therapy resistant diseases. The successful application of immunosuppressive agents in certain cases of dry eye disease, for example, has indicated the rationality of this approach.

6.2 Structure and Function of MALT

6.2.1 Structure of MALT

6.2.1.1 Histology of the Mucosa

Mucosal tissues consist of two sheets (Fig. 6.1). The superficial sheet represents a unilayered or, at the ocular surface, a multilayered arrangement of epithelial cells. They usually have a strong mechanical connection by intercellular adherence junctions (e.g. desmosomes and zonulae adherentes) and are sealed by an apical tight junction complex that prevents entrance of foreign materials including potential antigens. Impairment of the epithelial integrity is a major reason for a deregulation of mucosal immunity and is observed in dry eye disease and in allergic eye disease but is also caused by the surgical trauma during corneal transplantation.

The epithelium is separated by a thin basement membrane from the underlying loose connective tissue of the lamina propria. The lamina propria not only has mechanical properties for anchorage of the epithelium but is highly vascularized to serve for metabolic purposes and to provide migratory pathways for lymphoid cells. Lymphoid cells can immigrate in a regulated fashion via specialized postcapillary high endothelial venules (HEV) or flat-lined vessels into the tissue and can leave from the tissue via afferent lymphatic vessels towards the regional lymph nodes and eventually into the blood circulation in order to recirculate (Fig. 6.1). The lamina propria is filled with a

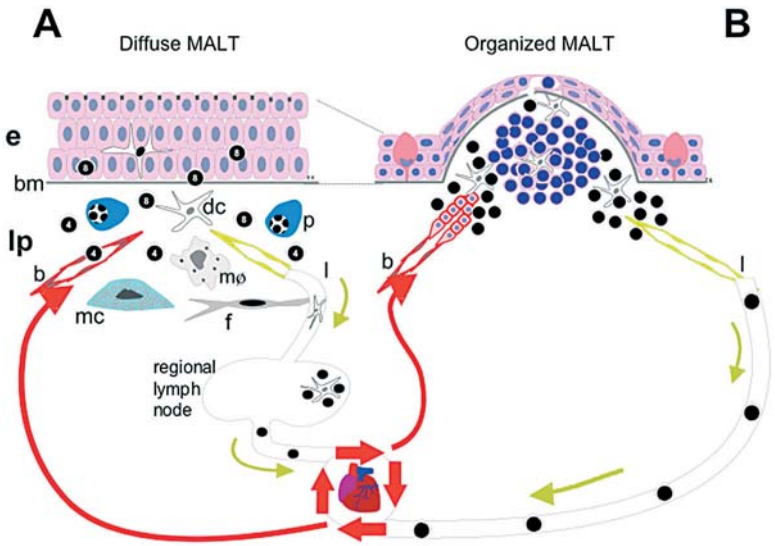

Fig. 6.1 A, B. Structure and function of the mucosal immune system. MALT consists of a diffuse lymphoid tissue (**A**) and of an organized follicular tissue (**B**), shown here at different enlargements. Mucosal tissues in general are composed of two sheets, the luminal epithelium (*e*) with its basement membrane (*bm*) and an underlying lamina propria (*lp*), which both contain lymphocytes. The lamina propria is composed of loose connective tissue with small blood vessels (*b*), afferent lymph vessels (*l*) and numerous cells including lymphoid cells [T-lymphocytes (*black*), B-lymphocytes (*blue*), plasma cells (*p*)]. Accessory cells occur like fibroblasts (*f*), macrophages (*mϕ*), mast cells (*mc*) or dendritic cells (*dc*). Intraepithelial lymphocytes are mainly CD8+ suppressor/cytotoxic T cells whereas in the lamina propria of the diffuse tissue (**A**) they occur in roughly equal numbers together with CD4+ T-helper cells. Follicular lymphoid tissue (**B**) is formed by accumulations of B-lymphocytes with parafollicular T-cell zones, vessels and an overlying specialized follicle-associated epithelium for antigen transport towards the follicle. Naïve lymphocytes enter follicular regions via blood vessels (*b*), come into contact with antigens, antigen-specific lymphocytes proliferate, differentiate and leave via lymphatics (*l*). They finally reach the blood circulation and may later emigrate to populate the same or other mucosal tissues as effector cells (T cells and plasma cells)

large number of different cell types and macromolecules that serve the purpose of nutrition and protection as maintained, e.g. by immunoglobulins and antibacterial peptides. The lamina propria also enables the communication of cells with each other and their extracellular matrix by different types of molecules. Cytokines, chemokines and adhesion molecules transfer (immunoregulatory) information, guide the migration by forming a gradient, act as traffic signals anchored to the extracellular matrix and cells or provide direct cell contacts.

Cells in the lamina propria consist of so-called fixed cells like fibroblasts that are responsible for the production and maintenance of the connective tissue itself and free cells (e.g. lymphocytes, plasma cells, macrophages, DC, eosinophils or mast cells) that can migrate in and partially out of the tissue and mainly have protective tasks. Lymphoid cells occur in both mucosal layers: in the connective tissue as lamina propria lymphocytes (LPL) and plasma cells and inside mainly the basal layers of the epithelium as intraepithelial lymphocytes (IEL). Antigen presenting DC also occur in the epithelium and lamina propria. The other free cell types normally only occur inside the lamina propria.

6.2.1.2 Conformations of MALT

MALT is divided into two forms [29] (Fig. 6.1). In the "organized" lymphoid tissue lymphocytes are organized into lymphoid follicles whereas the "diffuse" lymphoid tissue is composed of diffusely interspersed lymphoid cells along the mucous membranes and their associated glands.

Follicular MALT is regarded as the afferent arm of mucosal immunity where antigens are taken up from the environment by a specialized follicle-associated epithelium (FAE). Antigens can be presented to lymphocytes by antigen presenting cells in the parafollicular T-cell regions around follicles. This leads to lymphocyte activation, proliferation and eventual differentiation into effector cells of the T- or B-lineage. Contact with antigens and proliferation of B cells results in a transformation of the homogeneous primary follicle into a secondary follicle with a bright germinal centre of proliferating B cells. The mucosal antigen presentation to naïve T cells takes place in parafollicular regions in order to produce differentiated effector cells. This can happen in local follicles of MALT but antigens can also be transported by antigen presenting DC via the afferent lymphatic vessels into regional lymph nodes.

Diffuse lymphoid tissue is populated by the arising effector cells and represents the efferent arm of mucosal immunity. T-lymphocytes [61] that have differentiated into CD8-positive suppressor/cytotoxic cells either directly act against antigens and provide the cellular T-cell immune response or support immunosuppression. B cells in contrast that differentiate into immunoglobulin producing plasma cells, act indirectly by secreted immunoglobulins. In contrast to systemic immunity, plasma cells in mucosal tissues contribute to secretory immunity by the production of polymeric immunoglobulins that are transported through the overlying epithelium with the help of an epithelial transporter molecule (secretory component, SC) and build up a protective layer at the mucosal surface [4].

6.2.1.3 Lymphocytes and Accessory Cells

6.2.1.3.1 Lymphocytes

B-lymphocytes represent the majority of lymphocytes in the follicular zones whereas in the diffuse lymphoid tissue B cells are rare and T cells predominate together with various other cell types. CD8-positive suppressor/cytotoxic cells are usually more frequent in the mucosa than CD4-positive T-helper cells (CD8+) that regulate the differentiation of T- and B-lymphocytes. CD8+ cells strongly dominate in the epithelium and frequently bear the human mucosa lymphocyte antigen (HML-1), whereas in the lamina propria both populations occur in roughly equal amounts. Since there was indication that CD8+ suppressor/cytotoxic T cells may primarily be involved in immune suppression, it was supposed that their presence characterizes the ocular surface, similar to other parts of the mucosal immune system, as a highly immune regulated tissue that favours immune suppression rather than inflammation [6, 53]. However, T cells can also mediate inflammatory immune responses that represent basic pathological mechanisms in the types of ocular surface diseases considered in the present paper.

6.2.1.3.2 Antigen Presenting Cells

Antigen presentation to naïve T cells is performed by so-called professional antigen presenting cells (APC) that are composed of macrophages, B cells and dendritic cells, all of which occur in ocular MALT. Only the bone marrow derived dendritic cells (DC) can directly stimulate T-lymphocytes and are therefore the most important APC also at mucosal surfaces [2] for the initiation of a T-cell-mediated immune response. T-cell-mediated responses are important for most kinds of immunological reactions, including the humoral immunity by production of soluble antibodies which is influenced by T-helper cells, and they also determine the clinical conditions considered in this paper.

DC occur in the epithelium and also in the lamina propria of mucosal tissues. Immature DC prevail, which show an active uptake of antigens but a low surface expression of antigen presenting MHC-class-II molecules and co-stimulatory molecules, performing an ineffective presentation of antigens to T cells [2, 37]. They produce preferably anti-inflammatory cytokines such as interleukin 10 (IL-10) [19]. If these DC come into contact with T cells, they tend to inhibit inflammatory T-cell immune reactions and favour the humoral immune answer with the production of soluble immunoglobulins, as in fact mainly found at mucosal surfaces (IgA and IgM) [4], or they may initiate immune tolerance by induction of regulatory T cells [67].

6.2.1.4 Recirculation of Lymphoid Cells

Due to the enormous variety of potential antigens there is only a limited number of lymphocytes available with a given antigen specificity although the total number of lymphocytes is relatively high (for review see [26]). Therefore naïve lymphocytes continuously patrol through the body and stay in the blood only for a short time. They leave in large numbers from the blood into the tissues, and the term "recirculation" refers to the fact that they finally re-enter the blood circulation via lymphatic vessels, lymph nodes and the thoracic duct (Fig. 6.1). In the search for their specific antigen, naïve lymphocytes may follow this pathway several times and after contact with the cognate antigen they can undergo activation, clonal proliferation and differentiation into effector cells in order to mount a specific response. Antigen-specific memory cells await a second contact with the antigen to boost a rapid and forceful secondary immune response [5].

There is no a unified concept for the homing of lymphoid cells into tissues available so far. Several studies seem to show a cell type specific distribution of adhesion molecules (lymphocytes homing receptors) on lymphoid cells and a respective tissue selective distribution of binding molecules (vascular addressins) on the vascular endothelium of tissues in order to guide lymphocyte migration through the body [5, 61]. Together with previous results and other data on the isotype specific distribution of the secretory IgA response [4], this indicates a certain compartmentalization of the mucosal immune response and a certain tissue specificity of migration and homing in the mucosal immune system. Other authors have challenged this concept by lymphocyte migration studies using adoptive lymphocyte transfer in otherwise unmanipulated hosts and have found that events inside the tissue such as local lymphocyte retention, proliferation or apoptosis may contribute equally to the effective accumulation of lymphocytes inside certain tissues or their effective movement through these tissues (reviewed in [63]).

Summary for the Clinician

- **Mucosa-associated lymphoid tissue (MALT) is a part of the immune system that is located at mucosal surfaces of the body**
- **It consists of a diffuse lymphoid tissue populated by effector cells and accessory cells. T-lymphocytes provide cellular defence and differentiated B-lymphocytes (plasma cells) secrete soluble protective IgA immunoglobulins**
- **Interspersed organized lymphoid follicles allow antigen recognition, activation and differentiation of specific effector cells**

6.2.2 Eye-Associated Lymphoid Tissue (EALT)

There has been considerable controversy about the occurrence and normality of lymphoid cells at the ocular surface and appendage. Recent results in whole mounts of complete normal human ocular tissues have shown that lymphoid cells are a normal tissue constituent and in fact form a continuous mucosa-associated lymphoid tissue in the lacrimal gland, conjunctiva and lacrimal drainage system, termed eye-associated lymphoid tissue (EALT) [22, 23, 25, 28] (Fig. 6.2).

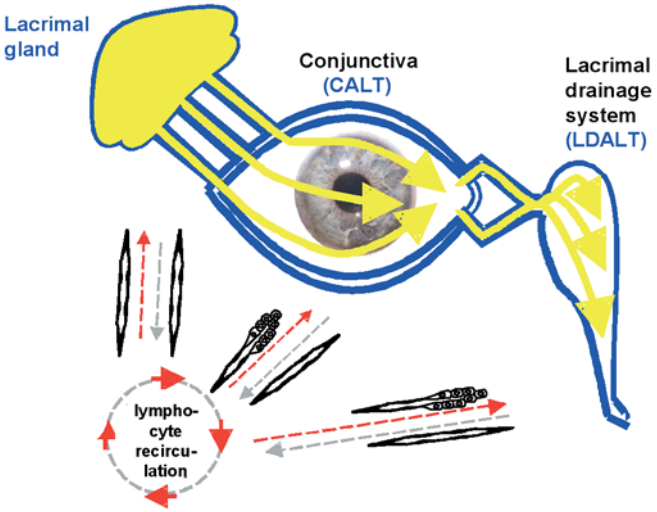

Fig. 6.2. Eye-associated lymphoid tissue (EALT). The ocular surface is an integral part of the mucosal immune system of the body. The diffuse lymphoid tissue with an effector function by lymphocytes and plasma cells is continuous as indicated by blue line from the lacrimal gland along the excretory ducts into the conjunctiva as conjunctiva-associated lymphoid tissue (CALT) and continues through the lacrimal canaliculi inside the lacrimal drainage system as lacrimal drainage-associated lymphoid tissue (LDALT). The lymphoid tissue of these three organs together constitutes EALT. Follicular tissue for the detection of ocular antigens occurs in CALT and LDALT. Effector cells that are primed in follicular tissue against ocular surface antigens can migrate in a regulated fashion via specialized vessels between the organs of EALT and the other parts of the mucosal immune system and can hence provide them with effector cells that are specifically directed against antigens that occur at the ocular surface

6.2.2.1 Organized Lymphoid Tissue in EALT

Organized lymphoid follicles in the human conjunctiva have been reported in different numbers in individuals with a macroscopically normal conjunctiva (as reviewed in [28]). Apart from the fact that most of these studies investigated only small tissue biopsies or selected conjunctival areas which do not represent the whole organ as found later, the amount of follicles also varies with age [44].

Recent results in normal whole-mount tissues of the human conjunctiva have shown that even in an old age population about 60 % of tissues contain organized lymphoid follicles with a distinct topographical distribution. They cumulate in the tarso-orbital zone and have a high (>80 %) bilateral symmetry [28]. In the lacrimal drainage system, a similar lymphoid tissue occurs and was termed, according to the international nomenclature, lacrimal drainage-associated lymphoid tissue (LDALT) [22] with lymphoid follicles in roughly half of the tissues (about 41 % [45], 44 % [22] or up to 56 % of old age body donors [24]).

6.2.2.2 Diffuse Lymphoid Tissue in EALT

Similar relations of lymphoid cell types as in other diffuse MALT were found in immunohistological studies on biopsies of the human conjunctiva [6, 18, 53] including the regular presence of mucosa-specific lymphocytes [6, 18]. However, there were different, partly conflicting, reports concerning the amount and location of lymphoid cells. This is probably due to the topographical distribution of these cells as found in studies on normal human conjunctival whole-mount tissues. Lymphoid cells in the subepithelial lamina propria form a lymphoid layer that can have local inhomogeneities but still shows an overall distinct topographical dis-

tribution with a preference for the tarso-orbital conjunctiva [28].

The components of the secretory immune system (lamina propria plasma cells positive for IgA and its transporter molecule SC in the epithelium) have not been found consistently at the normal human ocular surface except for the lacrimal gland, which therefore appeared as the sole source of specific immune protection, whereas the same plasma cells in the conjunctiva were addressed as inflammatory cells. The universal presence of a secretory immune system that reaches continuously from the lacrimal gland via the conjunctiva into the lacrimal drainage system was only recently verified at the normal human ocular surface by studies that combined the histological and immunohistological investigation of complete tissue whole mounts from normal human body donors [22, 23, 25, 28]. Together with ultrastructural results on the differentiation of the employed cell types and molecular-biological evidence for the presence of the mRNA of IgA and SC [21], this demonstrated the local production of secretory IgA in the conjunctiva and lacrimal drainage system.

6.2.2.3 Dendritic Cells in EALT

At the ocular surface the dendritic Langerhans cells occur as antigen presenting cells similar to the skin [42]. They have been described in a number of animal species and humans for several decades and have frequently been detected with antibodies against some of their antigen presenting surface molecules (e.g. MHC-class-I, MHC-class-II) in isolated epithelial sheets and in histology.

In contrast to the conjunctiva and the marginal cornea that both have relatively frequent MHC-class-II positive DC, it was found that the central cornea is almost free of these cells. Only in inflammatory conditions, for example after vascularization or after experimental wounding or irritation, were appreciable numbers of MHC-class-II positive DC observed in the central cornea. These observations seemed to fit relatively well with the known low percentage of rejection of corneal allografts compared to results with transplantation of other organs, e.g. skin, kidney or heart [50]. Only recently with new antibodies has it been possible to observe that even the central cornea in fact contains numerous DC that are immature and do not express antigen-presenting surface molecules [15] under normal conditions.

6.2.2.4 Recirculation to EALT

In EALT, there is only sparse information so far about homing mechanisms and regulating factors. The presence of high endothelial venules in the normal human conjunctiva has been shown (for review see [26]). The intestinal vascular addressin MAdACAM-1 is not observed on high endothelial venules but other adhesion molecules like VAP-1, ICAM-1, VCAM-1 and E-selectin have been found and showed a weak or sporadic staining. These addressins are thought to be possibly involved in extraintestinal homing but may also indicate an inflammatory response. The presence of ICAM-1 in the normal human conjunctiva was confirmed but ICAM-1, VCAM-1 and E-selectin were found to be inflammation dependent and strongly expressed only under inflammatory allergic conditions [1].

Summary for the Clinician

- **Mucosa-associated lymphoid tissue also occurs at the normal human ocular surface and appendage and is integrated into the mucosal immune system of the body as eye-associated lymphoid tissue (EALT)**
- **It is continuously expressed from the lacrimal gland throughout the conjunctiva (as conjunctiva associated lymphoid tissue, CALT) and inside the lacrimal drainage system (as lacrimal drainage-associated lymphoid tissue, LDALT)**
- **It has the machinery to recognize ocular surface antigens, generate effector cells (T-lymphocytes and plasma cells) that are specifically directed against them and can provide, via lymphocyte recirculation, the ocular effector tissues and other mucosal organs with such cells**

6.2.3 Basic Functions of MALT

MALT modulates between inflammatory immune protection and immune tolerance (Fig. 6.3). There was a historic misunderstanding of the function and significance of lymphoid cells at least at the ocular surface because they were usually considered an indication for an inflammatory infiltration of the mucosa. Consequently lymphocytes and plasma cells were frequently termed "inflammatory cells".

In contrast to this term, lymphoid cells have important functions for the preservation of the tissue integrity. Mucosal immunoglobulins (IgA) from local plasma cells are distinctly anti-inflammatory and perform "immune exclusion", i.e. the inhibition of antigen penetration into and the removal of penetrated antigens from the mucosal tissue [4]. Recent advances in immunology have furthermore shown that T-lymphocytes per se are not inflammatory cells but that there are different types of T-lymphocytes with differential functions (Fig. 6.4). Even those which support a cellular inflammatory immune answer require, in addition to the mere presence of antigen, distinct and highly regulated activation procedures in the context of accessory professional antigen presenting cells together with co-stimulatory signals (Fig. 6.4).

However, if deregulation of the physiological mucosal immune system occurs, lymphoid cells can also be involved as a primary or secondary pathogenetic factor in several forms of ocular surface disease as outlined below for some common ocular diseases.

In contrast to the systemic immunity in the blood and internal organs which favours the destruction of antigens, a main function of mucosal immunity appears to be the generation of immune tolerance (Fig. 6.3) against the multitude of non-pathological antigens at mucosal surfaces that are not supposed to cause constant immune activation [29]. This applies in particular to the ocular surface which is directly exposed to the environment.

6.2.3.1 Immune Regulation at Mucosal Surfaces and the Th1/Th2 Paradigm

To initiate immune responses, antigens must be recognized by naïve T cells in order to activate them to effector cells. According to the present concept, two signals are necessary for the activation of T cells. Besides the correct recognition of a presented antigen (signal 1) by the interaction of the specific T-cell receptor, the peptide antigen and the antigen presenting MHC-class-II molecule on the APC, the presence of co-stimulatory signals (signal 2) such as CD80/86, CD40 or ICAM-1 on the APC is required. This is necessary to initiate the production of a sufficient amount of the cytokine interleukin-2 (IL-2) by the T cell that is required for the autocrine stim-

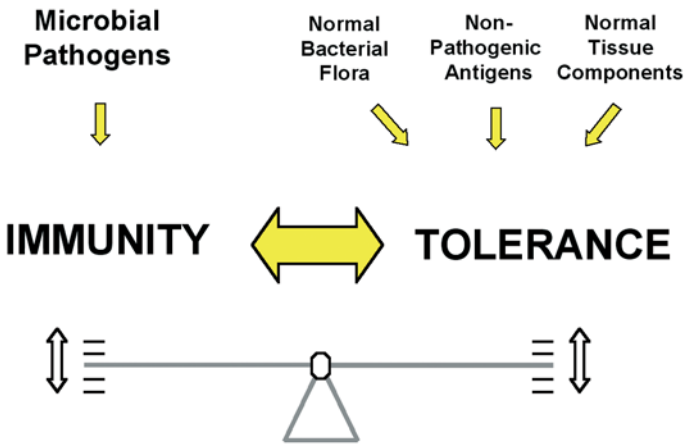

Fig. 6.3. Basic functions of the mucosal immune system. One of the main functions of the mucosal immune system is the maintenance of a fine equilibrium between inflammatory immune protection against microbial infection and the generation of tolerance to the majority of non-pathogenic antigens that occur at mucosal surfaces in order to prevent constant inflammatory reactions that are destructive not only for the antigen but also for the tissue itself

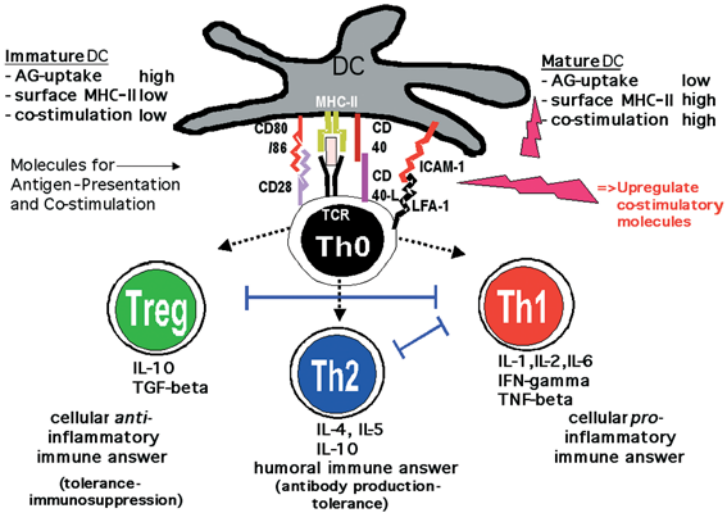

Fig. 6.4. Immune regulation by DC. Bone marrow derived antigen presenting dendritic cells (DC) are key regulators of mucosal immunity by initiating different types of effector T cells that act through different cytokine patterns. Normally DC are in an immature state that cannot efficiently present antigens to naïve T cells (*Th0*). Th0 cells hence develop into inactive anergic T cells or into immunosuppressive anti-inflammatory regulatory T cells (termed Th3 or Treg, according to different nomenclatures) that produce immunosuppressive cytokines (e.g. IL-10, TGF-β) or they support development of Th2 cells which act through promotion of B-cell maturation into immunoglobulin secreting plasma cells. When "danger signals" are introduced by microbial infection or tissue destruction the DC start their maturation, upregulate the antigen presentation molecule MHC-class-II at their surface together with co-stimulatory molecules (e.g. CD80/86, CD40, ICAM-1) and stimulate proinflammatory Th1 cells. Th1 cytokines inhibit Th2 and Treg cells and reverse in order to focus the immune response in a distinct direction

ulation of T-cell proliferation and differentiation. If co-stimulation is missing, non-reactivity (tolerance) is induced by an anergy or deletion of the respective antigen of the specific T-lymphocyte. The requirement of two signals is assumed to represent a control mechanism in peripheral tissues (peripheral tolerance) against accidental activation of autoreactive T cells that may have escaped the mechanisms of central tolerance in primary lymphatic organs (bone marrow and thymus) which produce the naïve lymphocytes.

DC have different functional states in order to modulate the antigen presentation [37]. The type and concentration of local cytokines (cytokine milieu) and other external factors influence the immune regulation by DC and the resulting differentiation of different types of T-helper (Th) cells, which in turn differ in the cytokine signals they produce themselves [40] and the immune reactions they initiate or favour.

In mucosal tissues immature DC prevail that show an active uptake of antigens but a low surface expression of MHC-class-II and co-stimulatory molecules [2] and also produce preferably interleukin 10 (IL-10) [19]. If such immature DC present antigens to T cells, they stimulate the differentiation of T-helper cell type 2 (Th2) that induce immunoglobulin production by plasma cells by their cytokines (IL-4, IL-5 and IL-10). Alternatively, newly (re-)discovered regulatory T cells (termed Treg or Th3) can arise that are more strongly immunosuppressive through the combined production of IL-10 and transforming growth factor β (TGF-β) and can therefore even inhibit the rejection of transplants [67].

Through the contact with maturation signals [37], immature DC differentiate into mature DC. Since the maturation signals are initiated via

unphysiologic events in the context of infection or tissue destruction (e.g. contact with microbial pathogens, inflammatory cytokines, transplant surgery), these signals are also termed "danger signals" [11]. Hereby DC are activated and mature by expression of high levels of surface MHC-class-II and co-stimulatory molecules (CD80, CD86, CD40). This allows an effective antigen presentation that leads to the initiation of a cellular proinflammatory immune answer by T-helper cells type 1 (Th1) and their cytokines (IL-2, IFN-γ, TNF-α).

Th1- and Th2-lymphocytes tend to inhibit each other by shifting the cytokine milieu in opposing directions and this Th1/Th2 paradigm is frequently used to explain the course of immune reactions. However, this paradigm may be oversimplified because other Th subtypes exist and because findings from the ocular surface, e.g. in allergy [38] and in dry eye disease (M.E. Stern, S.C. Pflugfelder, personal communication), indicate that both subtypes can be involved in inflammatory processes as shown below.

Summary for the Clinician

- **A basic function of MALT is the immune regulation at mucosal surfaces by balancing between an inflammatory immune defence of pathogens and a tolerance of the ubiquitous non-pathogenic antigens**
- **The preference is for a generation of tolerance mechanisms in order to avoid constant inflammatory destruction of the delicate mucosal surface**
- **Immune regulation is mainly performed by a special class of professional antigen presenting cells, dendritic cells (DC)**
- **Depending on external influences in the tissue (cytokine milieu, microbes, cell wounding, etc.), the function of DC is biased and leads to the stimulation of different types of T-helper (Th) cells that govern different directions of immune response by differential patterns of secreted immunomodulatory mediators (cytokines)**
- **Th1-lymphocytes maintain inflammatory defence, Th2 cells stimulate the mainly anti-inflammatory immunoglobulin production and Th3 (or regulatory T cells) act immunosuppressively**

6.3 Dry Eye Disease

6.3.1 Introduction

Recent findings have shown that dry eye disease (keratoconjunctivitis sicca, KCS), similar to other types of ocular surface disease, frequently contains an inflammatory component. This is regulated by immune modulators (e.g. cytokines and chemokines), can affect other parts of the integrating functional anatomy of the ocular surface [27] and eventually leads to a vicious circle of degenerative remodelling of the ocular surface. In other mucosal organs of the body it was shown that inflammatory mucosal disease is initiated by destruction of the surface epithelium which allows uncontrolled influx of antigens and leads to a deregulation of the cells of the mucosal immune system. This is characterized by a shift of the effector T cells in the direction of Th1. The main aspects of this pathogenesis have also been verified at the ocular surface in dry eye. Consequently, immunosuppressive therapy has proven to be effective in moderate to severe cases of dry eye disease.

6.3.2 Epidemiology, Definition and Characteristics of Dry Eye

Dry eye disease is a widespread disruption of the normal homeostasis of the ocular surface that affects, depending on the tests applied for diagnosis in various studies, up to 10–30% of the population [56]. It is not homogeneously distributed in the population but more likely affects elderly people and preferentially women, which may point to certain risk factors such as age or hormonal status. It is caused, according to a definition of the American National Eye Institute (NEI) [30], by an alteration of the tear film either due to aqueous deficiency or to increased evaporation. This condition leads to a disruption of the cellular and morphological integrity of the ocular surface and eventually

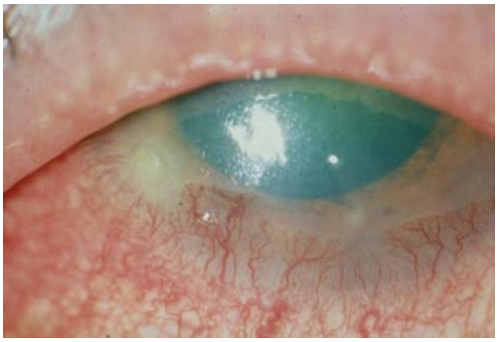

Fig. 6.5. Clinical photo of severe dry eye disease. A severe dry eye shows dryness of the ocular surface with epithelial staining (if different kinds of vital stains such as fluorescein or rose bengal are applied). The corneal reflex is disturbed and the transparency of the cornea is decreasing. The eye is severely inflamed and shows neovascularization with new vessels that grow from the limbus onto the cornea

causes the symptoms that are presented to the ophthalmologist [48]. Symptoms can range over a wide spectrum from mild discomfort and increased fatigue of the eye to redness, itching, burning and stinging sensations (Fig. 6.5). It can be associated with usually minor alterations of visual acuity but may in severe cases lead to severe inflammation, scarring and blinding.

The dry eye syndrome appears as a complex deregulation of the functional anatomy of the ocular surface [27] and deficiencies of the tear film can originate, for example, from alterations of the lid shape, blinking mechanism or innervation, from alterations of the endocrine network, from the presence of meibomian gland disease or from chronic mechanical irritation of any kind (e.g. contact lenses).

In recent years evidence has accumulated from intensive investigations that various forms of dry eye disease are associated with inflammatory alterations [41, 48, 58] of the ocular surface and appendage that are associated with inflammatory factors inside the tissue and tear film.

6.3.2.1 Lacrimal Gland Contribution to Dry Eye Disease – Sjögren's Syndrome

The lacrimal gland is an associated gland of the ocular surface that functionally and embryologically constitutes an integral part of the ocular surface. Similarly, from the viewpoint of mucosal immunology, it is an integral part of the ocular mucosal immune system (EALT) [23] together with the conjunctiva-associated lymphoid tissue (CALT) and the lacrimal drainage-associated lymphoid tissue (LDALT). It contains similar cell populations of T- and B-lymphocytes and DC [64].

T-cell-mediated inflammatory alterations of the lacrimal gland have been known for a long time; they appear to be associated with an impairment of the innervation that triggers the final release of aqueous secretion, and Sjögren's syndrome is a major cause for tear deficiency [7]. Alterations of B-lymphocytes are also described in Sjögren's syndrome. The aetiology of the disease is unknown, but it may originate from activation of the acinar epithelial cells due to viral infection by Epstein-Barr [52] or other viruses which stimulate a production of inflammatory cytokines and can lead to presentation of epithelial autoantigens by upregulated MHC-class-II and ICAM-1 production and expression at the epithelial surface. A number of respective autoantigens are characteristic for Sjögren's syndrome and can be used to support the diagnosis (e.g. SS-A, SS-B, α- and β-fodrin, M3 receptor). A distinct repertoire of antigen receptors was found on T and B cells in Sjögren's patients. This can lead to a breakdown of the physiological peripheral self-tolerance and results in an activation of lymphocytes that carry receptors for self antigens and happen to have escaped the central tolerance mechanisms in primary lymphoid tissues. An accumulation of mainly CD4+ T-helper cells, DC and smaller amounts of B cells in the salivary and lacrimal glands has been reported [46]. This results in the destruction of acinar epithelial tissue by binding of the self-intolerant cytotoxic T cells to acinar cells and lymphocyte induction of acinar apoptosis via release of cytotoxic molecules. The lymphocytes, and to a certain extent also

epithelial cells, produce a large amount of mainly inflammatory Th1-type cytokines (IL-2, IL-6, IFN-γ, TNF-α). Th2 cytokines (IL-4, IL-5, IL-10) are produced in smaller amounts and preferably in areas of occasional B-cell accumulations [43]. The presence of inflammatory cytokines induces a further influx of more lymphocytic cells by upregulation of adhesion molecules on glandular vessels and leads to an activation of stromal cells with release of matrix metalloproteinases that cause a degenerative remodelling of the extracellular matrix around the epithelial acini.

Since only about half of the secretory acinar cells are destroyed by this process, it appears likely that the remaining intact acinar cells are inhibited from secretion by negative interference with innervation [7, 8]. Suggested mechanisms include an observed reduction of density of innervating nerve fibres, the inhibition of release of neurotransmitters by inflammatory cytokines or the blockade of innervation effects in the epithelial cells by autoantibodies against their muscarinic M3 receptor [7].

Androgen deficiency is shown as an important predisposing factor for the initiation of inflammatory reactions as well as alterations of the secretion of the lacrimal and meibomian glands resulting in tear deficiency [60].

6.3.2.2 Conjunctival Contribution to Dry Eye Disease – Non-Sjögren's Dry Eye

Inflammatory affections in dry eye disease are not only found in the lacrimal gland. Even in the clinically inflammation free and primary tear deficient non-Sjögren's dry eye an elevation of inflammatory cytokines (IL1α, IL6, IL8, TNFα) is found in the tear film and inside the tissue of the conjunctiva [48]. The ability of conjunctival epithelial cells to release inflammatory cytokines has been reported [12]. This indicates a shift of the cellular immune response into the direction of an inflammatory Th1 response similar to the inflammatory affections in the lacrimal gland and may similarly lead to a destruction of the epithelium and the underlying extracellular matrix [48, 58]. The primary affection seems to lie in the epithelial cells, similarly to the lacrimal gland, but also in the conjunctiva an upregulation of inflammatory markers that indicate an activation of the mucosal lymphocytes was recently described [59].

Protective factors such as growth factors (EGF, HGF) that are responsible for the proliferation but even more for the mature differentiation of the tissue may at the same time be downregulated [48]. Consequently in dry eye syndromes a hyperproliferation of the conjunctival epithelium is observed combined with impaired differentiation. This is conceivably driven by the presence of inflammatory cytokines and the relative inhibition of cell differentiation is due to diminished growth factors. The conjunctival epithelium in dry eye shows an immature phenotype of the apical cells with a basal cell type cytokeratin pattern and an absence of integral epithelial surface mucins [48, 49], which in turn diminishes the adherence of the tear film to the ocular surface and hence reduces the tear film stability.

Elevated inflammatory cytokines further induce an upregulation of proteases (matrix metalloproteinases) in the tissue and tearfilm, which indicates additional degenerative remodelling of the connective tissue of the mucosal lamina propria at the ocular surface [13, 31, 36]. Inflammatory cytokines can also impair ocular surface innervation in the sense that they inhibit sensory information about ocular dryness to reach the central nervous system in order to elicit efferent secretomotor impulses in glandular tissue. The neural reflex arc is thereby interrupted, leading to a further decrease of secretion and potentially inducing neurogenic inflammation of the lacrimal gland also in primary non-Sjögren's dry eyes [58].

6.3.2.3 Common Mechanisms in Immune Mediated Dry Eye Disease

The starting point of immune mediated inflammation in the conjunctiva, similar to events in the lacrimal gland in Sjögren's syndrome, may lie in an alteration of the epithelial cells. In the case of the conjunctiva, this is caused by destructions that are observed in all kinds of dry eye due to mechanical abrasion via increased

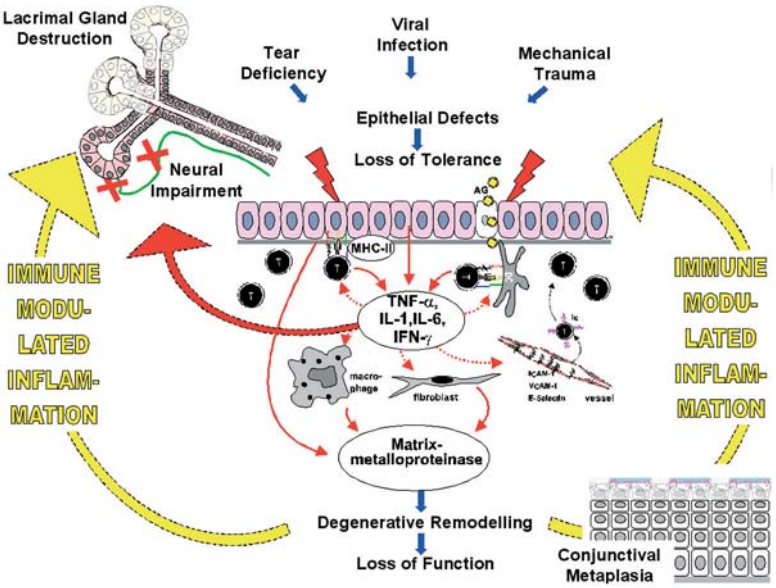

Fig. 6.6. Common mechanisms in immune mediated dry eye disease. Different types of dry eye disease share an immune modulated inflammatory process that can similarly occur in the lacrimal gland (e.g. in Sjögren's syndrome) and at the ocular surface. It appears to start from epithelial defects resulting in a loss of immunological tolerance. Epithelial cells produce inflammatory cytokines (e.g. TNF-α, IL-1, IL-6, IFN-γ), upregulate MHC class II and co-stimulatory molecules on their surface and allow uncontrolled antigen (*AG*) influx through defects. Together this leads to an uncontrolled activation of normal resident mucosal T cells into the inflammatory Th1 type that also produces inflammatory cytokines and hence amplifies the inflammatory cytokine milieu in the tissue. Further events include an impairment of innervation with a decrease of glandular secretion, and an activation of matrix metalloproteinases that results in a degenerative remodelling of the tissue with loss of function (e.g. destruction of secretory acini in the lacrimal gland or squamous metaplasia in the conjunctiva) and the risk of further epithelial defects. The events involved here can hence result in a vicious circle of tissue destruction (*solid arrows* indicate production of molecules; *interrupted arrows* indicate action of molecules or movement of cells)

friction of the eyelids on the ocular surface (Fig. 6.6). In addition, cell damage can also be caused by hyperosmolarity of the tear film [14]. In the case of the lacrimal gland, epithelial alterations may arise after viral infection [52].

If alteration of the epithelial barrier occurs due to such damage, antigens can achieve uncontrolled access to the tissue, which may be the dominating effect in the conjunctiva; or epithelial cells gain the ability to present autoantigens as observed in the lacrimal gland. In both affections the mucosal immune tolerance is likely to fail. Resident T-helper cells of the physiological mucosal immune system in the subepithelial connective tissue can then be activated and immunological reactions shifted towards inflammation [59], resulting in the further elevation of proinflammatory cytokines. Additional new T-cell can immigrated via an upregulation of adhesion molecule on the vascular endothelium. Similar events have been shown in inflammatory bowel disease (IBD) [34], which represents an inflammatory mucosal condition of the intestine where a large body of information is already acquired. At the ocular surface, the production of these cytokines is as yet mainly attributed to the epithelial cells. This may be due to the fact that the presence of a resident population of lymphocytes and plasma cells constituting a physiologic mucosal immune system at the normal ocular surface (EALT) was unknown until recently because lymphoid cells in general

were erroneously believed to be "inflammatory". However, in the intestine where the presence of a physiologic mucosal immune system has been accepted for a longer time, it has been verified that TNFα and IL-1β are also secreted by activated lamina propria lymphocytes promoting an inflammatory reaction and resulting in the production of matrix metalloproteinases by stromal cells [34]. A shift of the cytokine profile towards a TH-1 response has been reported in several inflammatory ocular surface diseases, as similarly found in IBD, and both disorders are reported to respond to immunosuppressive treatment.

6.3.3 Novel Therapeutic Approaches to Dry Eye Disease

Combining these results, it can be noted that the widespread dry eye syndrome is increasingly being recognized to include an inflammatory component [47, 59] and it thus resembles disorders in other mucosal organs which are governed by lymphocytes of the mucosal immune system [34]. Hence, the resident lymphatic population localized in the eye-associated lymphoid tissue of the ocular surface (EALT), which represents a potent source of professional cytokine producing cells, may also act as an important regulator of inflammatory ocular surface disease. Consequently the activation of T cells, which can be inhibited by different immunosuppressive strategies, is an interesting target for new therapeutic approaches [47].

Some compounds that interfere with the process of lymphocyte activation (Fig. 6.4), and hence act more specifically than for example glucocorticoids, are known from immunosuppression after transplantation of solid organs where they are administered systemically. An important step in lymphocyte activation is the production of IL-2 in T cells that is necessary for full activation. Cyclosporin A (CsA), like another agent (tacrolimus also known as FK506), prevents the transcription of the IL-2 gene by binding to the transcription factor calcineurin. Another compound, rapamycin, acts later in the activation cascade and blocks IL-2 peptide after production in the lymphocytes. Therefore CsA or FK506 can act synergistically in combination with rapamycin.

In inflammatory ocular surface disease, topical administration of immunosuppressive drugs has been attempted in order to achieve a high local concentration and to avoid systemic side effects. CsA has been the focus of interest in recent years. A CsA ophthalmic oil-in-water emulsion that was previously only available for veterinary use is now also approved for human therapy and has been tested in multicentre studies. It proved to be significantly better than placebo in reducing objective findings (corneal staining, Schirmer's test) and subjective symptoms when it was applied twice daily over a period of 3–6 months, with the best results at a concentration of 0.05 % CsA [54].

Other approaches successfully improved the underlying androgen deficiency, which increases susceptibility to ocular inflammation and negatively affects the secretion of ocular glands, with topical androgen therapy in animal [60] and human trials [65].

Summary for the Clinician

- **Different types of dry eye disease all contain an underlying immune modulated inflammatory component that is mediated by a deregulation of the physiological and normally protective mucosal immune system**
- **New causative topical treatment options are now available for a therapeutic approach to the immune mediated inflammation in moderate to severe dry eye disease**
- **Immunosuppression with CsA 0.05–0.1 % eyedrops, given twice daily for several months, is an effective treatment as tested in multicentre studies**
- **Topical androgen acts as a trophic and anti-inflammatory factor and normalizes glandular function. It was successfully used in animal models and tested in a case report on a human patient**

6.4 Ocular Allergy

6.4.1 Introduction

Allergy is characterized by an increased sensitivity against external factors that act as allergens and usually reach the ocular surface through the air. Characteristic is an IgE mediated degranulation of mast cells which leads to inflammatory events characterized by vasodilatation, edema and itching. Allergy is a hypersensitivity reaction and occurs in different forms. Seasonal and perennial allergic disease (SAC and PAC) are acute forms whereas vernal and atopic keratoconjunctivitis (VKC and AKC) are more severe and chronic. An iatrogenic form, giant papillary conjunctivitis (GPC), is caused by the introduction of artificial materials such as contact lenses or ocular prostheses onto the ocular surface.

Allergic eye disease shows signs of inflammation and appears to be modulated, apart from mast cells, by other inflammatory cells, such as eosinophils, and by T-lymphocytes. In contrast to inflammation in dry eye disease, effector T cells in allergy show a bias in the direction of either a Th2 or a Th1 immune answer, depending on the subtype of allergy. The mast cells and eosinophils can also produce both types of cytokines. Apart from substances that prevent the release or action of bioactive mediators from mast cells, a modulation of the mucosal immune system in the sense of application of antibodies or antimetabolites that interfere with cell migration, antigen presentation or T-cell activation has proven helpful in treating ocular allergy.

6.4.2 Epidemiology, Definition and Characteristics of Allergic Eye Disease

Ocular allergy is a widespread inflammatory process at the ocular surface that affects about 15–30 % of the population, with a higher incidence in industrialized countries. It is caused by an inappropriate reaction to external allergens that are able to crosslink IgE bound to the high affinity IgE receptor on mast cells. It leads to the release of bioactive substances from the mast cells and other cells and results in edema and inflammatory leukocyte infiltration [20, 57] (Fig. 6.7). Since the access of external antigens to intraconjunctival mast cells is normally prevented by an intact epithelial barrier at the healthy ocular surface, it has been assumed that patients suffering from allergic eye disease may have an underlying impairment of the epithelial integrity, and a respective increased uptake of fluorescein was found in patients [66].

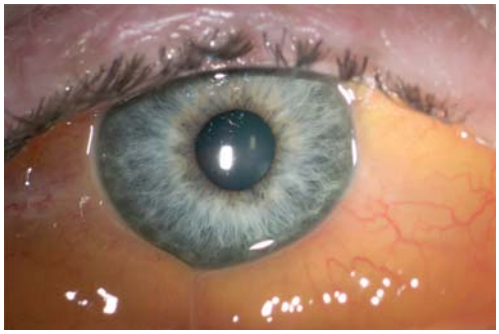

Fig. 6.7. Clinical photo of allergic eye disease. Acute allergic eye disease, as seen here, shows a distinct edema and hyperaemia of the conjunctiva due to degranulation of conjunctival mast cells with release of vasoactive substances (e.g. histamine). The cornea usually remains clear in acute allergic eye disease, and there is no ingrowth of vessels from the limbus. Chronic allergic eye disease, however, includes production of inflammatory cytokines and matrix metalloproteinases together with an inflammatory cell infiltrate. This causes a keratoconjunctivitis with corneal destruction and the beginning of impairment of vision

6.4.2.1 Mast Cells

Mast cells are mesenchymal cells that occur in the connective tissue of most organs and serve as host defence [62]. They have a varying shape that is influenced by the tissue microenvironment but generally shows prominent granules inside the cytoplasm on histological and electron microscopic examination. The granules

contain preformed bioactive agents, e.g. histamine and the enzymes tryptase and/or chymase. According to the content of tryptase and/or chymase, mast cells are divided into a connective tissue type containing tryptase and chymase (M_{TC}) and a mucosal type that contains only tryptase (M_T). In the normal human conjunctiva mast cells only occur in the lamina propria and their majority (95%) are positive for tryptase and chymase. In allergic eye disease the number of M_T mast cells increases and they can occur inside the epithelium and tear film [39]. They produce further signalling molecules such as cytokines [10] of the Th1 and Th2 type that act in an immune modulatory way on various cell types including leukocytes and epithelial cells and influence the course of ocular allergy.

6.4.2.2 Allergic Edema is Caused by Vasoactive Substances

The initial reaction of mast cells upon IgE mediated stimulation is the degranulation of the preformed substances like histamine, tryptase and chymase that leads to dilatation and increased permeability of vessels and results in connective tissue edema (Fig. 6.8). Cytokines of the Th2 type (IL-4, IL-5) and of the inflammatory Th1 type (IL-6, TNF-α) are also produced by mast cells and released as an answer to stimulation. Other secondary agents (e.g. leukotrienes, prostaglandins) are produced within several hours after stimulation [62]. Together these mediators initiate an inflammatory cascade the next step of which is the recruitment of inflammatory leukocytes (eosinophilic and basophilic granulocytes) and of T-lymphocytes into the edematous area.

6.4.2.3 Inflammatory Cytokines Induce a Leukocyte Infiltrate

The leukocyte immigration is mediated by chemoattractants such as the leukotrienes released from mast cells but also by chemotactic cytokines (chemokines) produced in epithelial cells and stromal fibroblasts after stimulation by mast cell cytokines, namely by the inflammatory cytokine TNF-α. TNF-α also initiates an upregulation of the cell adhesion molecule ICAM-1 on vascular endothelial cells and on epithelial cells. VCAM-1 and E-selectin were also found to be inflammation dependent and strongly expressed under allergic conditions [1].

Together this increases the local adhesion of intravascular leukocytes to endothelial cells and their immigration into the tissue as well as their chemotactic migration within the lamina propria and later adhesion inside the epithelium. Stromal fibroblasts activated by inflammatory cytokines appear to produce the chemokine eotaxin, which acts chemotactically on eosinophils and attracts them to the tissue.

After TNF-α stimulation epithelial cells produce a variety of chemokines (MCP, MIP-1, RANTES, IL-8) [9] that act as chemoattractants and conceivably regulate the further migration of immigrated leukocytes from the conjunctival lamina propria into the epithelium and eventually into the preocular tear film. Stimulated fibroblasts secrete Eotaxin. Mast cell derived TNF-α also leads to production of inflammatory cytokines and chemokines (IL-6, IL-8, TNF-α, GM-CSF), which are detected in the tissue and tear film, by epithelial cells and eosinophils [12]. Epithelial cells therefore reinforce the inflammatory pathomechanism and become an active player in allergic eye disease [17].

6.4.2.4 Activation of T-Lymphocytes by Cytokines

TNF-α upregulates ICAM-1, which is known to be an important factor in lymphocyte adhesion and can provide co-stimulation during their activation, not only on endothelial and epithelial cells but also on eosinophils. The activated lymphocytes and other cell types in turn produce further cytokines. Thereby mast cell derived cytokines interrelate the innate immune response with a specific T-cell-mediated immune answer. Since it is shown that the conjunctiva contains a population of resident lymphocytes [28] that belong to the physiological mucosal immune system, an activation of lymphocytes is possible without the previous necessity of lym-

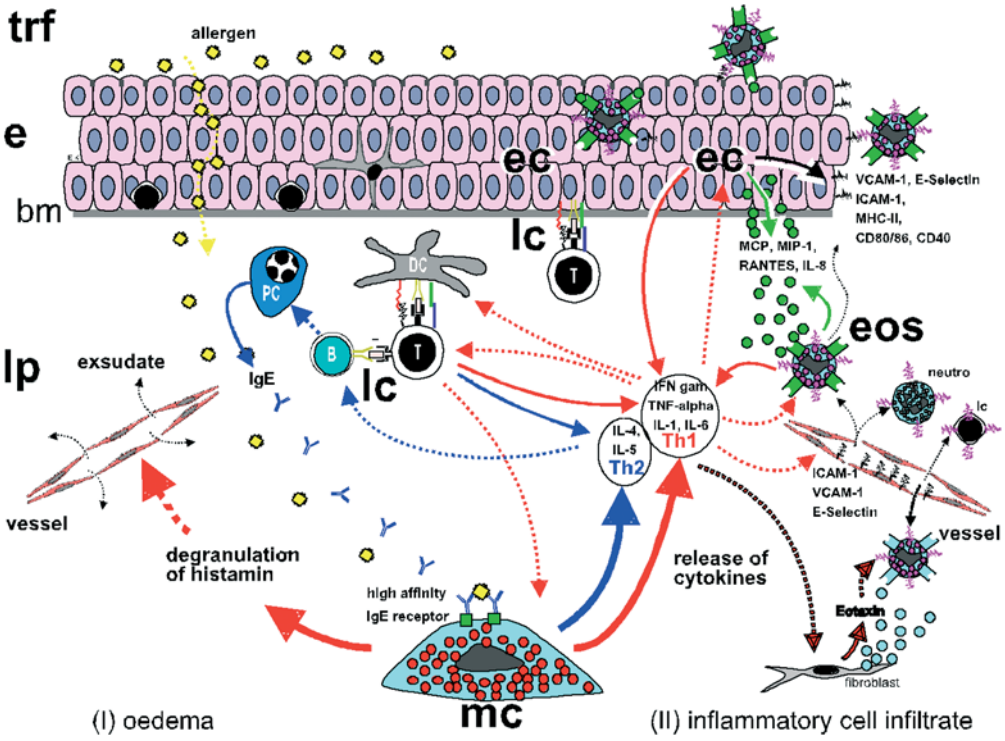

Fig. 6.8. Pathophysiological events in allergic eye disease. Allergic eye disease is an inflammatory process that starts with the activation of mast cells (*mc*) by allergens crosslinking IgE bound to the high affinity IgE receptor on mc. This initially leads to degranulation of mc with release of vasoactive substances resulting in vascular exudation and edema. In the chronic forms this is accompanied by a release of Th1 and Th2 cytokines by mc. These cytokines activate several other cell types such as (counterclockwise in the figure) stromal fibroblasts, vascular endothelial cells, eosinophils (*eos*), conjunctival epithelial cells (*ec*), dendritic cells (*dc*) and lymphocytes (*lc*). The activated cells in turn produce further mediators that reinforce the inflammatory process. Activated cells produce adhesion molecules (dendriform lines) like ICAM-1, VCAM-1, E-selectin and chemokines (small circles) and/or their receptors (dendriform lines and excavated squares) that allow binding and directed migration of cells. Endothelial adhesion molecules permit vascular arrest of leukocytes [eosinophils, neutrophils (*neutro*) and lymphocytes] and their immigration from the vessels into the tissue leading to the characteristic inflammatory cell infiltrate. Secreted chemokines produced by fibroblasts (e.g. eotaxin) and by the epithelium and eosinophils (e.g. MCP, MIP-1, RANTES, IL-8) guide the immigrated leukocytes with respective upregulated cell surface receptors into the lamina propria (*lp*) and from there into the epithelium (*e*) and tear film (*trf*). Upregulation of co-stimulatory molecules like ICAM-1, CD80/86, CD40 and the antigen presenting molecule MHC class II enables epithelial cells to potentially present antigens to T cells resulting in uncontrolled immune reactions (*solid arrows* indicate production of molecules, *interrupted arrows* indicate action of molecules or movement of cells)

phocyte immigration. If the inflammation process proceeds, an additional influx of lymphatic cells into the conjunctival tissue conceivably occurs. Besides the usual process of antigen presentation via DC, epithelial cells that have upregulated MHC-class-II and co-stimulatory ICAM-1 may be able to present antigens to T cells and reinforce the inflammatory process [17]. Investigation of T-lymphocyte cytokines in allergic ocular disease surprisingly showed that Th2-like cytokines prevail in some types such as VKC and partially GPC whereas AKC appears to have a predominant Th1 response [38]. Th2 cytokines act as stimulators of immunoglobulin

production by plasma cells and may therefore be involved in the upregulation of IgE that is observed in allergy. This indicates that inflammatory reactions are not solely mediated by Th1-type lymphocytes and questions the Th1/Th2 paradigm to some extent.

6.4.3 Course and Therapy Options in Allergic Ocular Disease

The inflammatory events that occur in allergic eye disease are moderate in the acute seasonal and perennial allergic disease, SAC and PAC, and mainly lead to edema, redness and itching, whereas the immigration of inflammatory cells is limited. However, in the more chronic allergic diseases such as vernal and atopic keratoconjunctivitis (VKC and AKC) there is a more pronounced immigration of inflammatory cells. In chronic allergic eye disease but not, or only weakly, in acute ocular allergy, activated matrix metalloproteinases occur in the tissue and tear film and may explain the occurrence of corneal destruction in the chronic forms. There the inflammatory process can lead to scarring and can have sight threatening complications, especially in AKC. The giant papillary type GPC causes tarsal conjunctival thickening and is of intermediate severity [20, 57].

Due to the multistep cascade with various involved factors from the bioactive content of mast cell granules to leukocyte immigration and T-cell-mediated immune processes there are a number of different therapeutic strategies. Apart from prevention of mast cell degranulation, the use of antihistamines and potential application of blocking antibodies to chemokines and cytokines is possible [3, 16]. Topical treatment with a 2% CsA solution over a period of 3 months has proven to have beneficial effects on the chronic AKC by reducing the number and activation of T cells and their production of the inflammatory Th1 cytokines IL-2 and IFN-γ. Although this treatment had no influence on the number of conjunctival mast cells and eosinophils, it is assumed that the immunosuppression may still modulate and normalize their function [16].

Summary for the Clinician

- Due to a pathological sensitivity, non-pathogenic environmental antigens act as allergens. They crosslink IgE that is bound to the mast cell through the high affinity IgE receptor and hence stimulate the cell
- This leads to a release of vasoactive (e.g. histamine) and immunomodulatory (e.g. cytokines and chemokines) substances from mast cells
- In acute (seasonal and perennial) ocular allergy the affection is mainly restricted to a conjunctival edema
- In chronic allergic and vernal keratoconjunctivitis (AKC and VKC) various inflammatory leukocytes (primarily eosinophils) but also lymphocytes of the mucosal immune system are activated by mast cell cytokines. A T-cell-mediated inflammatory processes arises that is associated with an inflammatory cell infiltrate and corneal destruction
- In addition to established antihistaminic therapy options, topical immunosuppression is effective in chronic allergic eye disease
- Two percent CsA eyedrops given over a period of 3 months improve objective findings (corneal destruction) and subjective symptoms in patients with severe AKC

6.5 Keratoplasty

6.5.1 Introduction

Keratoplasty, as an organ transplantation procedure, is enormously dependent on immunological mechanisms. The rejection rate in the otherwise normal and inflammation free ocular surface is relatively low because the normal cornea contains no blood vessels, no lymphocytes and only relatively few cells that express MHC class II. Furthermore, the anterior chamber appears to possess an immune privilege. This anterior chamber associated immune privilege (ACAID) induce stolerance against anti-

Fig. 6.9. Clinical photo of a penetrating keratoplasty with the beginning of rejection. The clinical picture of an eye where a penetrating keratoplasty was performed shows the graft tissue fixed by a continuous suture in the centre of the host cornea. The margin between donor and host tissue is demarcated by a *fine whitish line*. Beginnings of the process of immunological transplant rejection are indicated by a haze in the lower half of the graft tissue and result from swelling of the stroma due to destruction of the underlying corneal endothelium that is attacked by the host T cells

gens that are recognized in the anterior chamber of the eye.

In contrast to previous studies it has been shown in recent years that even the central cornea contains a large number of DC that are in a quiescent immature state but may upregulate MHC class II and respective antigen presentation capacity as soon as they are stimulated by the occurrence of antigen and other factors. DC represent components of the mucosal immune system and are key modulators of antigen presentation also at the ocular surface. They can, via the initiation of different types of T-helper cells, modulate between tolerance or rejection of a corneal transplant and hence determine the fate of a corneal graft (Fig. 6.9). Consequently they also represent an interesting target for future immunomodulatory therapeutic strategies.

6.5.2 Immunological Characteristics of Keratoplasty

In order to undergo corneal graft rejection, three processes have been implicated [50]. The donor antigen has to be released, recognized and transported to lymphoid tissue that is present in the form of the organized follicular conjunctival CALT, on the ocular surface itself and in the draining lymph nodes (afferent arm). Alloantigens have to be processed so that a specific cellular immune response might be generated (central stage). Finally in the efferent arm cellular and humoral effector mechanisms are delivered to the graft and cause its destruction.

6.5.2.1 Antigen Presenting Cells

6.5.2.1.1 Langerhans Cells

With regard to the role of antigen presentation ocular DC, Langerhans cells (LHC), are considered as a "key" element of the "afferent" immune process. These dendritic cells play a dominant role in processing and presentation of antigens and carry MHC-class-II antigens that are important stimulators of T and B cells. The distribution of LHC is compartmentally localized within specific regions of the ocular surface. The central cornea is normally devoid of LHC that are positive of MHC class II, but a number of stimuli may induce their immigration. However, recently it has been shown in the mouse that even the central cornea contains DC in an immature state and their potential precursors [15] that can be activated by the presence of antigens as in corneal transplantation.

6.5.2.1.2 Macrophages

In contrast to LHC that have been extensively studied, the role of macrophages (Mø) is less clear. Mø are a heterogeneous cell population that may exert multiple functions. They regularly occur in the conjunctiva and macrophage-like cells are also described in the cornea [15]. Beside their phagocytic activity they produce a number of highly active mediators such as tumour necrosis factor alpha (TNF-α) and nitric oxide (NO) displaying immunomodulatory functions. It is of interest that Mø are also present intraocularly in high density and provide a close network together with DC in the anterior

chamber, in particular the iris [35]. The potential role of Mø in the process of corneal graft rejection has been demonstrated by depletion studies. Following topical application of clodronate liposomes that selectively eliminate Mø, a highly significant prolonged survival of experimental keratoplasty could be observed. It is very likely that Mø play an as yet underestimated role in the afferent arm of the immune response following corneal transplantation.

6.5.2.2 Antigen Presentation

Two pathways of antigen presentation, either "direct" or "indirect", have been proposed in alloantigen recognition [55]. Donor APC can present antigen to host T cells via the direct pathway, thereby inducing a strong immune response. The emigration of antigen presenting dendritic cells with upregulated MHC-class-II molecules from the donor cornea into the host tissue has recently been shown in the mouse model of corneal transplantation [15, 32]. On the other hand, host APC are able to present corneal alloantigens via the indirect pathway of antigen presentation. The direct pathway is a specific feature of the response towards alloantigens, whereas the indirect pathway represents the "normal" mechanism for the generation of an immune response. Even when the indirect pathway is considered less effective, experimental studies have shown that it may result in prompt corneal graft rejection [33].

6.5.2.3 Allograft Rejection and Immune Modulatory Therapy

The critical role of T cells in allograft rejection is well established. The prevailing view is that a specific T-cell response against HLA antigens is initiated through CD4+ cells. Further potentiation of the reaction then takes place via cytokine-dependent co-stimulation (e.g. by CD80/86) is necessary for full activation of the lymphocyte as indicated by high production of the cytokine IL-2 and its receptor. The respective involved molecules are important targets for immunomodulation and immunosuppression especially after corneal transplantation (indicated by *blue text* and *arrows* for inhibition approaches)

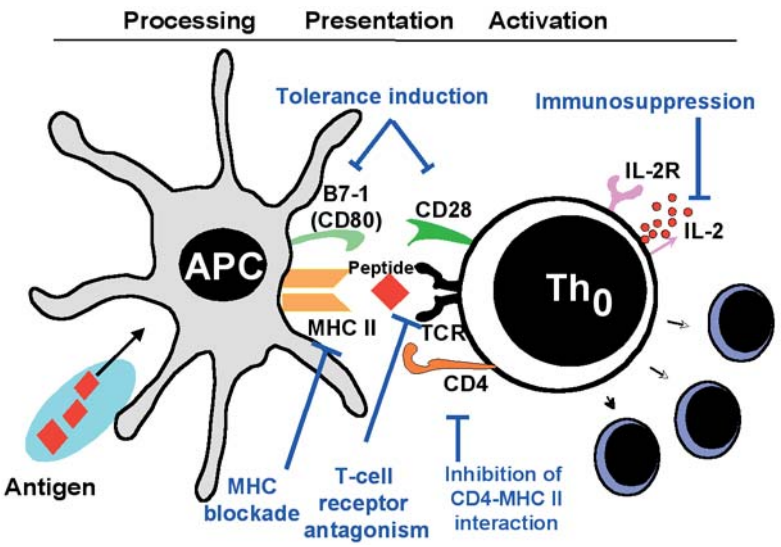

Fig. 6.10. Antigen presentation and potential targets for immune modulation. Antigen presentation is performed by antigen presenting cells (*APC*) after intracellular antigen processing. Processed peptides are loaded on MHC-class-II molecules and presented to the T-cell receptor (*TCR*) on naive T-helper cells. CD4 on the T cell acts as an accessory molecule and addi-

kine release with activation, proliferation and differentiation of other lymphocytes. It is obvious that new methods to prevent allograft rejection must take place in this early phase of graft reaction before activation of the T cell. The respective molecules involved in the processing and presentation of antigens as well as in the activation of lymphocytes represent promising targets for immune modulation and immunosuppression (Fig. 6.10). Much attention has therefore been paid in particular to the recognition behaviour of CD4+ cells (T-cell receptor) and the interaction with the target antigen and cytokines that are responsible for expansion of the immune response.

Cells of the physiological mucosal immune system are involved in the afferent antigen recognition phase (mainly conjunctival and corneal dendritic cells) and also in the efferent effector phase (performed by T cells). Besides systemic immunosuppression as similarly performed in solid organ transplantation, topical immunosuppression is possible after keratoplasty. In addition to immunosuppression with CsA or similar agents that suppress T-cell activation, it is also possible to inhibit mechanisms of antigen presentation (as indicated in Fig. 6.10) before rejection is initiated, for example by immunosuppressive cytokines, antibodies against co-stimulatory molecules (e.g. CD80/86, CD40) or against accessory molecules such as CD4. In addition to potential topical therapy which is possible in keratoplasty, in contrast to the transplantation of solid organs, the protective factors can also be applied by a gene therapeutic approach. In this case the DNA for the respective product is transfected into host cells with the help of different kinds of vectors (e.g. liposomes, viruses) and is then produced directly by the cells of the transplant or by surrounding tissues [51].

Summary for the Clinician

- **Transplant rejection remains the single most important cause of graft failure following penetrating keratoplasty**
- **Local as well as systemic immune processes are involved in transplant rejection and may allow development of more specific preventive and therapeutic options**
- **CD4+ lymphocytes play an essential role in the immune response in corneal graft rejection and are the main target of immunomodulatory therapy**
- **The relative importance of CD4+ subtypes classified as "Th1/Th2" is still a matter of debate but may allow new immunomodulatory strategies, such as gene therapy**

References

1. Abu El-Asrar AM, Geboes K, al-Kharashi S, Tabbara KF, Missotten L, Desmet V (1997) Adhesion molecules in vernal keratoconjunctivitis. Br J Ophthalmol 81:1099–1106
2. Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245–252
3. Bielory L, Mongia A (2002) Current opinion of immunotherapy for ocular allergy. Curr Opin Allergy Clin Immunol 2:447–452
4. Brandtzaeg P, Baekkevold ES, Morton HC (2001) From B to A the mucosal way. Nat Immunol 2: 1093–1094
5. Butcher EC, Picker LJ (1996) Lymphocyte homing and homeostasis. Science 272:60–66
6. Dua HS, Gomes JA, Jindal VK, Appa SN, Schwarting R, Eagle RC Jr, Donoso LA, Laibson PR (1994) Mucosa specific lymphocytes in the human conjunctiva, corneoscleral limbus and lacrimal gland. Curr Eye Res 13:87–93
7. Fox RI (1998) Sjogren's syndrome. Pathogenesis and new approaches to therapy. Adv Exp Med Biol 438:891–902
8. Fox RI, Stern M (2002) Sjogren's syndrome: mechanisms of pathogenesis involve interaction of immune and neurosecretory systems. Scand J Rheumatol Suppl 2002:3–13
9. Fukagawa K, Tsubota K, Simmura S, Saito H, Tachimoto H, Akasawa A, Oguchi Y (1998) Chemokine production in conjunctival epithelial cells. Adv Exp Med Biol 438:471–478
10. Galli SJ, Gordon JR, Wershil BK (1993) Mast cell cytokines in allergy and inflammation. Agents Actions Suppl 43:209–220
11. Gallucci S, Matzinger P (2001) Danger signals: SOS to the immune system. Curr Opin Immunol 13:114–119
12. Gamache DA, Dimitrijevich SD, Weimer LK, Lang LS, Spellman JM, Graff G, Yanni JM (1997) Secretion of proinflammatory cytokines by human conjunctival epithelial cells. Ocul Immunol Inflamm 5:117–128
13. Garrana RM, Zieske JD, Assouline M, Gipson IK (1999) Matrix metalloproteinases in epithelia

from human recurrent corneal erosion. Invest Ophthalmol Vis Sci 40:1266–1270
14. Gilbard J (1985) Tear film osmolarity and keratoconjunctivitis sicca. In: Holly FJ (ed) Proc 1 Int Tear Film Symposium, Lubbock, Texas, pp 127–139
15. Hamrah P, Zhang Q, Liu Y, Dana MR (2002) Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells. Invest Ophthalmol Vis Sci 43:639–646
16. Hingorani M, Calder VL, Buckley RJ, Lightman S (1999) The immunomodulatory effect of topical cyclosporin A in atopic keratoconjunctivitis. Invest Ophthalmol Vis Sci 40:392–399
17. Hingorani M, Calder VL, Buckley RJ, Lightman SL (1998) The role of conjunctival epithelial cells in chronic ocular allergic disease. Exp Eye Res 67: 491–500
18. Hingorani M, Metz D, Lightman SL (1997) Characterisation of the normal conjunctival leukocyte population. Exp Eye Res 64:905–912
19. Iwasaki A, Kelsall BL (1999) Mucosal immunity and inflammation. I. Mucosal dendritic cells: their specialized role in initiating T cell responses. Am J Physiol 276:G1074–G1078
20. Katelaris CH (2003) Ocular allergy: implications for the clinical immunologist. Ann Allergy Asthma Immunol 90:23–27
21. Knop E, Claus P, Knop N (2003) Eye-associated lymphoid tissue (EALT): RT-PCR verifies the presence of mRNA for IgA and its transporter (secretory component) in the normal human conjunctiva. Invest Ophthalmol Vis Sci 44:S3801
22. Knop E, Knop N (2001) Lacrimal drainage associated lymphoid tissue (LDALT): a part of the human mucosal immune system. Invest Ophthalmol Vis Sci 2001:566–574
23. Knop E, Knop N (2002) A functional unit for ocular surface immune defense formed by the lacrimal gland, conjunctiva and lacrimal drainage system. Adv Exp Med Biol 506:835–844
24. Knop E, Knop N (2002) Human lacrimal drainage-associated lymphoid tissue (LDALT) belongs to the common mucosal immune system. Adv Exp Med Biol 506:861–866
25. Knop E, Knop N (2003) [Eye-associated lymphoid tissue (EALT) is continuously spread throughout the ocular surface from the lacrimal gland to the lacrimal drainage system]. Ophthalmologe 100: 929–942
26. Knop E, Knop N (2004) Lymphocyte homing in the mucosal immune system to the eye-associated lymphoid tissue (EALT). In: Zierhut M, Sullivan DA, Stern ME (eds) Immunology of the ocular surface and tearfilm. Swets & Zeitlinger, Amsterdam
27. Knop E, Knop N, Brewitt H (2003) [Dry eye disease as a complex dysregulation of the functional anatomy of the ocular surface. New impulses to understanding dry eye disease]. Ophthalmologe 100:917–928
28. Knop N, Knop E (2000) Conjunctiva-associated lymphoid tissue in the human eye. Invest Ophthalmol Vis Sci 41:1270–1279
29. Kraehenbuhl JP, Neutra MR (1992) Molecular and cellular basis of immune protection of mucosal surfaces. Physiol Rev 72:853–879
30. Lemp MA (1995) Report of the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes. CLAO J 21:221–232
31. Li DQ, Lokeshwar BL, Solomon A, Monroy D, Ji Z, Pflugfelder SC (2001) Regulation of MMP-9 production by human corneal epithelial cells. Exp Eye Res 73:449–459
32. Liu Y, Hamrah P, Zhang Q, Taylor AW, Dana MR (2002) Draining lymph nodes of corneal transplant hosts exhibit evidence for donor major histocompatibility complex (MHC) class II-positive dendritic cells derived from MHC class II-negative grafts. J Exp Med 195:259–268
33. Liu Z, Sun YK, Xi YP, Maffei A, Reed E, Harris P, Suciu-Foca N (1993) Contribution of direct and indirect recognition pathways to T cell alloreactivity. J Exp Med 177:1643–1650
34. MacDonald TT, Bajaj-Elliott M, Pender SL (1999) T cells orchestrate intestinal mucosal shape and integrity. Immunol Today 20:505–510
35. McMenamin PG, Crewe J, Morrison S, Holt PG (1994) Immunomorphologic studies of macrophages and MHC class II-positive dendritic cells in the iris and ciliary body of the rat, mouse, and human eye. Invest Ophthalmol Vis Sci 35:3234–3250
36. Meller D, Li DQ, Tseng SC (2000) Regulation of collagenase, stromelysin, and gelatinase B in human conjunctival and conjunctivochalasis fibroblasts by interleukin-1 beta and tumor necrosis factor-alpha. Invest Ophthalmol Vis Sci 41: 2922–2929
37. Mellman I, Steinman RM (2001) Dendritic cells: specialized and regulated antigen processing machines. Cell 106:255–258
38. Metz DP, Hingorani M, Calder VL, Buckley RJ, Lightman SL (1997) T-cell cytokines in chronic allergic eye disease. J Allergy Clin Immunol 100: 817–824
39. Morgan SJ, Williams JH, Walls AF, Church MK, Holgate ST, McGill JI (1991) Mast cell numbers and staining characteristics in the normal and allergic human conjunctiva. J Allergy Clin Immunol 87:111–116

40. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348–2357
41. Nelson JD, Helms H, Fiscella R, Southwell Y, Hirsch JD (2000) A new look at dry eye disease and its treatment. Adv Ther 17:84–93
42. Novak N, Siepmann K, Zierhut M, Bieber T (2003) The good, the bad and the ugly – APCs of the eye. Trends Immunol 24:570–574
43. Ohyama Y, Nakamura S, Matsuzaki G, Shinohara M, Hiroki A, Fujimura T, Yamada A, Itoh K, Nomoto K (1996) Cytokine messenger RNA expression in the labial salivary glands of patients with Sjogren's syndrome. Arthritis Rheum 39:1376–1384
44. Osterlind G (1944) An investigation into the presence of lymphatic tissue in the human conjunctiva, and its biological and clinical importance. Acta Ophthalmol Copenh Suppl 23:1–79
45. Paulsen FP, Paulsen JI, Thale AB, Schaudig U, Tillmann BN (2002) Organized mucosa-associated lymphoid tissue in human naso-lacrimal ducts. Adv Exp Med Biol 506:873–876
46. Pepose JS, Akata RF, Pflugfelder SC, Voigt W (1990) Mononuclear cell phenotypes and immunoglobulin gene rearrangements in lacrimal gland biopsies from patients with Sjogren's syndrome. Ophthalmology 97:1599–1605
47. Pflugfelder SC (2004) Antiinflammatory therapy for dry eye. Am J Ophthalmol 137:337–342
48. Pflugfelder SC, Solomon A, Stern ME (2000) The diagnosis and management of dry eye: a twenty-five-year review. Cornea 19:644–649
49. Pflugfelder SC, Tseng SC, Yoshino K, Monroy D, Felix C, Reis BL (1997) Correlation of goblet cell density and mucosal epithelial membrane mucin expression with rose bengal staining in patients with ocular irritation. Ophthalmology 104:223–235
50. Pleyer U, Dannowski H, Volk HD, Ritter T (2001) Corneal allograft rejection: current understanding. 2. Immunobiology and basic mechanisms. Ophthalmologica 215:254–262
51. Pleyer U, Ritter T (2003) Gene therapy in immune-mediated diseases of the eye. Prog Retin Eye Res 22:277–293
52. Rhodus NL (1999) Sjogren's syndrome. Quintessence Int 30:689–699
53. Sacks EH, Wieczorek R, Jakobiec FA, Knowles DM (1986) Lymphocytic subpopulations in the normal human conjunctiva. A monoclonal antibody study. Ophthalmology 93:1276–1283
54. Sall K, Stevenson OD, Mundorf TK, Reis BL (2000) Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. Ophthalmology 107:631–639
55. Sayegh MH, Turka LA (1998) The role of T-cell costimulatory activation pathways in transplant rejection. N Engl J Med 338:1813–1821
56. Schaumberg DA, Sullivan DA, Dana MR (2002) Epidemiology of dry eye syndrome. Adv Exp Med Biol 506:989–998
57. Stahl JL, Cook EB, Barney NP, Graziano FM (2002) Pathophysiology of ocular allergy: the roles of conjunctival mast cells and epithelial cells. Curr Allergy Asthma Rep 2:332–339
58. Stern ME, Beuerman RW, Fox RI, Gao J, Mircheff AK, Pflugfelder SC (1998) The pathology of dry eye: the interaction between the ocular surface and lacrimal glands. Cornea 17:584–589
59. Stern ME, Gao J, Schwalb TA, et al. (2002) Conjunctival T-cell subpopulations in Sjogren's and non-Sjogren's patients with dry eye. Invest Ophthalmol Vis Sci 43:2609–2614
60. Sullivan DA, Edwards JA (1997) Androgen stimulation of lacrimal gland function in mouse models of Sjogren's syndrome. J Steroid Biochem Mol Biol 60:237–245
61. von Andrian UH, Mackay CR (2000) T-cell function and migration. Two sides of the same coin. N Engl J Med 343:1020–1034
62. Wedemeyer J, Tsai M, Galli SJ (2000) Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol 12:624–631
63. Westermann J, Engelhardt B, Hoffmann JC (2001) Migration of T cells in vivo: molecular mechanisms and clinical implications. Ann Intern Med 135:279–295
64. Wieczorek R, Jakobiec FA, Sacks EH, Knowles DM (1988) The immunoarchitecture of the normal human lacrimal gland. Relevancy for understanding pathologic conditions. Ophthalmology 95:100–109
65. Worda C, Nepp J, Huber JC, Sator MO (2001) Treatment of keratoconjunctivitis sicca with topical androgen. Maturitas 37:209–212
66. Yokoi K, Yokoi N, Kinoshita S (1998) Impairment of ocular surface epithelium barrier function in patients with atopic dermatitis. Br J Ophthalmol 82:797–800
67. Zelenika D, Adams E, Humm S, Lin CY, Waldmann H, Cobbold SP (2001) The role of CD4+ T-cell subsets in determining transplantation, rejection or tolerance. Immunol Rev 182:164–179

Immunogenetics of Ocular Inflammatory Disease

7

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Core Messages

- Immunogenetics is helpful to the clinician in evaluating patients with uveitis. The immunogenetics of disease also has implications for disease pathogenesis and nosology, which are important for designing both basic science and clinical research
- Human leukocyte antigen (HLA) associations are particularly strong in birdshot retinochoroidopathy, tubulointerstitial nephritis and uveitis syndrome, Vogt-Koyanagi-Harada disease in Asian populations, and acute anterior uveitis
- Additional, non-HLA genetic associations have been described and hold promise for providing insights into disease mechanisms
- Difficulties in studying immunogenetics in uveitis include the small numbers of patients and the lack of families with disease, although some progress is being made in HLA-B*27 associated uveitis
- Inflammation plays a role in diseases other than uveitis, including macular degeneration, glaucoma and diabetic retinopathy. Few immunogenetic studies have been performed for these diseases

7.1 Introduction

Insights gained from the study of immunogenetics play a role in clinical medicine and promise to provide additional tools for the clinician in the future. In this chapter the immunogenetics of ocular inflammatory disease will be reviewed, and pertinent terminology and concepts that are helpful in reading the literature will be discussed.

Most studies of the immunogenetics of uveitis have described human leukocyte antigen (HLA) associations with disease. Some of the HLA associations with uveitis are spectacularly robust. For example, the association of HLA-A29 with birdshot retinochoroidopathy is so compelling that anyone suspected of having birdshot retinochoroidopathy without that allele should be carefully scrutinized for another cause of multifocal choroiditis. Immunogenetics will be useful to the ophthalmologist in additional ways as research progresses. The presence of a gene may be used to predict an individual's risk for development of disease. The predictive positivity of an allele known to confer risk for disease in a relative of a patient with uveitis has not been established, but ongoing research into families with HLA-B*27 associated uveitis and birdshot retinochoroidopathy may provide useful guidelines physicians can use to advise patients and their families. Research to evaluate whether specific alleles or genomic markers are useful in predicting response to therapy will be particularly important as we use more biological disease modifying agents, such as anticytokine antibodies or vaccines; predicting the response to these agents based on the patient's genetic profile would be of great clinical utility. Genetic markers also provide evidence for the appropriate nosology of diseases or syndromes, which is important for designing clinical studies.

Understanding the genetics of ocular inflammatory disease is useful for gaining insight into disease processes. It is difficult to explore the pathogenesis of uveitis in part because tissue is

Table 7.1. HLA and other genetic associations with ocular inflammatory disease

Disease	HLA association	Other genetic associations	Comments
Birdshot retino-choroidopathy	A29 B44(12) by linkage disequilibrium		Strongest HLA association with human disease (>96% of patients, RR as high as 224)
Idiopathic retinal vasculitis	A29		
AAU	B27 B8 in blacks	MICA may be by linkage disequilibrium	1/2 to 3/4 of AAU; ongoing family studies for other genes
Behçet's disease	B51	MIC, LMP-2, factor V Leiden, TNF	B51 subtypes may vary in different populations
MEWDS	B51		
Serpiginous, APMPPE	B7		Weak association, but implies may be similar processes
Pars planitis	DR2/DR15 B51, B8		May vary in different populations; similar DR associations with multiple sclerosis, optic neuritis
VKH disease	DR1, DR4 in Mestizos, DR4 (DRB1*0405) in Asians. DQ associations also reported		DR associations much stronger for specific alleles in Asians than Mestizos
Sympathetic ophthalmia	DRB1*0405 in Asians, DRB1*0404 in Caucasians		May be similar to VKH disease
TINU syndrome	DRB1*01 (DRB1*0102) DQA*01,DQB*05		DRB1*0102 high RR but DR associations may be by linkage disequilibrium with DQ
JRA uveitis	DP2.1 (DPB1*0201) DR1 protective	IL1	DR11(5) in one study, not in a second
ARN	DQw7, Bw62, DR4		DR9 correlated with severity
Toxoplasmosis	Bw62		DQ3 with CNS involvement, DQ1 may protect
POHS	DRw2		Associated with subretinal neovascular membranes
Leprosy (uveitis)	DR2		DR4 may be decreased
Sjögren's syndrome	Shared DQB1 reactivity		
Stevens-Johnson	Bw44		
Mooren's ulcer	DR17(3), DQ2		DR1 in rheumatoid ulcers
Ocular cicatricial pemphigoid	B12		
GCA	DR1, DR4, DQB1*03	IL1, IL6, mannose-binding lectin, TNF	

Table 7.1. (Continued)

Disease	HLA association	Other genetic associations	Comments
Graves' disease	DRB1*0301, DRB1*08	CTLA-4	
Blau syndrome		NOD2	NOD2 polymorphisms not found in sarcoid uveitis
Diabetic retinopathy	DQB1*0201, 0302		Varies in different study populations and disease subtypes
	B62 Cw4/DR4/DQ4		
POAG	DRB1*0407, DQB1*0302 in Mestizos	TNF in Chinese	

not often available. Indeed, the only eye from a patient with clearly documented birdshot retinochoroidopathy to be examined histologically was published over 20 years after the description of the disease [25]. Animal models are helpful, but may not reflect important aspects of human disease. The strong HLA associations with uveitis presented in this chapter are a challenge; until research into the basic mechanisms of uveitis explains these observations we are missing what may be critical pieces of the puzzle (Table 7.1).

Researchers have begun to evaluate nonclassical HLA genes, genes involved with antigen processing for HLA molecules, cytokine and chemokine genes, and genes involved in processes and pathways important in the cell biology of ocular inflammation. There is an ongoing study based at the University of Oregon examining genetic markers in families in which multiple individuals have HLA-B*27 associated uveitis. It has been difficult to perform genome based linkage studies because of the relatively small numbers of patients available for such studies for many uncommon forms of uveitis, and more importantly because there are very few families with multiple members who have uveitis. As the human genome is better understood and more powerful techniques for doing such research are developed many of these limitations will be overcome.

7.2 Human Leukocyte Antigens

The study of the genomic contribution to the immune system was initiated by the description of immune response genes. The genes involved in determining whether an animal was a high or low responder to specific antigens were mapped to a region that became known as the major histocompatibility complex (MHC) on chromosome 6 of the human. Although the MHC contains many genes involved in the immune system, the gene products primarily responsible for the immune response became known in the human as human leukocyte antigens as they were characterized by evaluating an individual's serologic reactivity to allogenic leukocytes. There are hundreds of allelic variations of HLA molecules and each individual generally has 12 alleles of the classical HLA genes (three Class I and three Class II alleles from each parent). Such heterogeneity allows an individual to present a wide range of pathogen-derived peptides to T cells in order to initiate the adaptive immune response, as well as peptides derived from self proteins to maintain tolerance and immune responsiveness. The allelic variation between individuals protects the population from being destroyed by a novel pathogen.

7.2.1 HLA Nomenclature

Historically, an individual's HLA type was determined serologically, and each new type described was given a number, for example HLA-B*27. A workshop number indicated by a "w" would be given when the HLA type was in the process of being established, for example HLA-DRw4. A subtype would be indicated by a decimal point, for example HLA-A29.1 and HLA-A29.2. Since the advent of polymerase chain reaction technology, DNA sequencing and other techniques to determine HLA genotypes, a different but related nomenclature has been used. The HLA type, such as HLA-B, is followed by an asterisk (*), then four to six digits. The first two digits are often the same as the serologic designation, and the next two digits represent the subtype. The remaining two digits are used for pseudogenes or single base differences when present. Several groups of serologically defined HLA types have been reclassified using newer techniques as well. For example, HLA-B*44 consists of subtypes HLA*B4401 though HLA-B*4411; HLA*B44 is the genomic equivalent of HLA-B*44(12). The designation HLA-B*44(12) indicates that HLA-B*12 and HLA-B*44 in older reports were different serologic designations of the products of the same HLA gene (HLA split products).

7.2.2 Class I HLA

Class I HLA (or Class I MHC; either term can be used for humans) glycoproteins, and the genes that code for them, are divided into HLA-A, HLA-B and HLA-C. Class I molecules are composed of a Class I alpha chain (which determines whether the molecule is A, B or C) and a non-covalently bound invariant beta-2 (B2) microglobulin, coded for on chromosome 15 in humans. Class I HLA proteins are expressed on the surface of almost every nucleated cell. Class I HLA molecules are stable on the cell surface only as a heterotrimer consisting of a class I alpha chain, a B2 microglobulin and a peptide in the binding cleft. Antigenic peptides bind non-covalently to a cleft formed by the alpha chain in Class I HLA molecules. The bound peptide antigens are usually eight to nine amino acids long and are usually derived from intracellular proteins, although extracellular peptides may also be presented by Class I molecules. The peptides that will bind to a given HLA subtype are determined by the charges, size and orientation of the amino acids of the peptide and the corresponding binding pocket of the HLA molecule (this also applies to peptide binding to Class II HLA molecules). For class I HLA molecules the amino acid in the second position of the peptide, the "anchor", is particularly important. For example, HLA-B*27 is remarkable for preferring a positively charged arginine in the anchor position. A major pathway of generating peptides for binding to class I HLA molecules is through enzymatic cleavage of proteins in the proteosome and subsequent transport into the endoplasmic reticulum. Several of the genes for this pathway are also in the MHC. Class I HLA molecules and peptide interact with T cell receptors on CD8+ lymphocytes. Some class I molecules also interact with NK receptors, and in this interaction may be altered by the peptide in the binding cleft.

Class IB molecules, including MHC class I chain-related (MIC) A and B genes and HLA-E, also combine with B2 microglobulin but are considered "non-classical" HLA molecules. MICA and MICB are less heterogenic than the classical HLA molecules. The MIC proteins have variable expression on the cell surface, but may be upregulated when the cell is under stress during infection or inflammation. MIC interact with NK receptors and gamma delta T cells, but the role of all of these components in ocular inflammation is poorly understood. HLA-E molecules present leader sequences from other HLA molecules, and may be one means by which NK cells and other components of the immune response are instructed as to the level of HLA turnover in a cell.

7.2.3 Class II HLA

Class II HLA molecules were serologically defined as HLA-DP, HLA-DQ and HLA-DR. They are composed of alpha and beta chains, both coded for by genes in the MHC. The beta chains are generally more heterogeneic; in fact, in some cases there is only one HLA alpha chain for a given Class II HLA type, in which case variations in the beta chain alone confer differences in subtypes and peptide binding repertoires. The class II HLA molecules are normally found primarily on antigen presenting cells such as dendritic cells, B cells and macrophages. During an inflammatory response other cell types may be induced to express Class II HLA antigens, including retinal pigment epithelial cells and vascular endothelial cells. Unlike class I MHC molecules, the binding cleft formed by the alpha and beta chains of Class II MHC molecules is open at both ends, allowing presentation of peptides containing 12–24 amino acids. The peptides bound to class II molecules are usually derived from proteins found in the extracellular fluid. The Class II molecules and peptide interact primarily with T-cell receptors on CD4+ lymphocytes.

7.2.4 HLA Haplotypes and Linkage Disequilibrium

Because the Class I and Class II HLA genes are so close on chromosome 6 in the MHC, there is little likelihood of recombination between parental chromosomes during meiosis. This results in linkage disequilibrium, with groups of HLA genes being consistently inherited together. Such a cluster of HLA genes is a "haplotype". As a group of genes found on either the maternal or paternal chromosome, a haplotype can only be determined with certainty by family studies. Many haplotypes are well described in a given population, however, and in some cases a reasonable assumption of the haplotype can be made from the HLA types found without knowing the HLA types of family members. For the same reason, it is often difficult to determine whether the HLA type that appears to confer risk for disease may be no more than a genetic marker, linked to a more critical gene, either another HLA gene or a non-HLA gene in the MHC. In addition, more than one HLA type may contribute to disease pathogenesis, further complicating the assessment of the genetic risk conferred by a specific HLA allele. For example, there is evidence for presentation of HLA-DQ derived peptide by HLA-DR molecules that confer risk for rheumatoid arthritis [101]. Interestingly, these are the same HLA types associated with Vogt-Koyanagi-Harada (VKH) disease.

7.2.5 HLA and Disease Pathogenesis

The HLA system has been associated with disease for over 30 years, but the precise role of HLA molecules in disease pathogenesis is not well established [16]. While microbial pathogens may play a more direct role than currently appreciated even in diseases we now consider due to autoimmunity, it is likely that in many cases HLA associated uveitis is at least in part an autoimmune process resulting from loss of tolerance to self antigens. While a discussion of the proposed mechanisms of loss of tolerance is beyond the scope of this chapter, several observations are pertinent. Despite the known role of HLA molecules in antigen presentation and the establishment and maintenance of tolerance there is no direct evidence that antigen presentation by the HLA molecule is critical to disease pathogenesis. It may be that presentation of ocular peptides in the thymus plays a role in the susceptibility to uveitis. In one study that did suggest that the HLA molecule itself was critical, a mouse transgenic for HLA-A*2902 developed posterior uveitis [105]. Recent work has also highlighted the importance of the kinetics of HLA glycoprotein [91] as well as the HLA molecule and peptide in the immune response [36]. HLA molecules may directly influence disease pathogenesis by mechanisms other than antigen presentation. HLA molecules interact with NK receptors, have been found in soluble form in the serum, may be taken up with cell

membranes by cells of the immune system, may themselves be presented as antigens, and may function as growth factors.

It is important to keep in mind that no HLA type is sufficient or necessary for disease. For example, HLA-A29 is very strongly associated with birdshot retinochoroidopathy but is also found in 7% of the Caucasian population; indeed patients with lymphomas or sarcoidosis can be HLA-A29 positive.

Summary for the Clinician

- **The role of HLA molecules in presenting antigens for the adaptive immune response is well established**
- **Class I and II HLA molecules have different roles**
- **The role of HLA in putative autoimmune diseases such as uveitis remains unclear**
- **Some HLA associations may be due to linkage disequilibrium, and the HLA allele may act as a genetic marker**
- **HLA types associated with disease will often contribute to making the diagnosis in a patient with uveitis, but do not make the diagnosis**

7.3 HLA Associations with Ocular Inflammatory Disease

7.3.1 Class I HLA Associations with Ocular Inflammatory Disease

The association of the HLA-B*27 serotype with acute anterior uveitis (AAU) was first described 30 years ago. HLA-B*27 is also associated with ankylosing spondylitis, Reiter's syndrome, psoriatic arthritis, and inflammatory bowel disease. Multiple studies have confirmed that between half and two-thirds of individuals with acute anterior uveitis are HLA-B*27 positive; and a significant number of patients with AAU will have a spondyloarthropathy [100]. The relative risk for developing AAU for an HLA-B*27 positive individual compared to an HLA-B*27 negative individual is about 10. Even though AAU is the most common form of uveitis in the community [66], as about 7% of Caucasians are HLA-B*27 positive, it has been estimated that only about 1% of HLA-B*27 positive individuals will develop uveitis. HLA-B*8 was associated with AAU in African Americans [81].

In other diseases associated with HLA-B*27, such as ankylosing spondylitis, the presence of disease in a first-degree family member increases the risk of an individual developing disease, but this has not been examined for AAU. HLA-B*27 associated uveitis is not limited to AAU, and the uveitis may also have different characteristics in patients with different systemic diseases [100], implying a role for other genetic or environmental risk factors in determining disease phenotype.

The Class II genes HLA-DRB1*0101 and DQB1*0501 alleles were increased in patients with ankylosing spondylitis and anterior uveitis compared to HLA-B*27 positive controls [50]. This is interesting as these are the same Class II associations with Tubulointerstitial nephritis and uveitis (TINU) syndrome, which is also characterized primarily by anterior uveitis. It may be, however, that the Class II HLA associations with HLA-B*27 associated uveitis were due to linkage disequilibrium with the HLA-B*27 subtypes HLA-B**2704 and HLA-B*2705, which were most strongly associated with uveitis [50].

The proteosome is particularly adept at generating peptides that will bind to the HLA-B*27 molecule. Genes coding for proteins that assist in processing peptides for presentation by class I HLA molecules have been examined. Low molecular weight polypeptide-2 gene polymorphisms have been associated with extraspinal disease, including uveitis [60, 61], although this has not been found in all studies [37]. Transporter associated with antigen processing gene polymorphisms in patients with ankylosing spondylitis were not associated with AAU [49].

The MICA and MICB genes have little polymorphism but may play a role in presenting antigens to gamma delta T cells. This is intriguing because these T cells are found in the gastrointestinal lymphoid tissues and HLA-B*27 disease may be precipitated by mucosal infections. A strong environmental component does appear to play a role [55]. The theory of "molec-

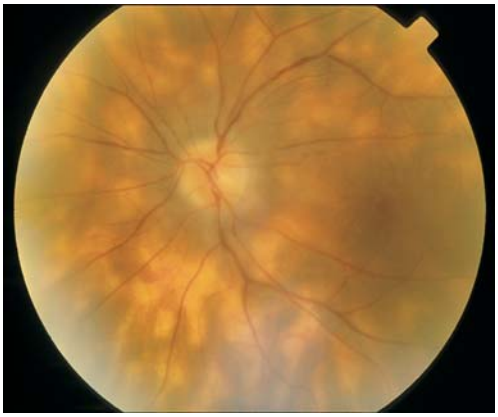

Fig. 7.1. Birdshot retinochoroidopathy is strongly associated with HLA-A29

ular mimicry" is that bacterial antigens at mucosal surfaces may in the context of clinical or subclinical inflammation result in an immune response to similar ocular antigens [100]. MICA alleles were associated with AAU in Japanese [31] and Caucasian patients [32], but the association with MICA genes may be due to linkage disequilibrium with HLA-B*27.

The association of HLA-A29 with birdshot retinochoroidopathy is one of the strongest of any disease with an HLA type (Fig. 7.1). At least 95% of patients with BSR are HLA-A29 positive with a relative risk estimated to be as high as 224; the sensitivity and specificity of HLA-A29 as a diagnostic test for BSR are over 90% [54]. Interestingly, patients with idiopathic retinal vasculitis also appear to have an increased prevalence of HLA-A29, implying that there may be a spectrum of HLA-A29 associated posterior uveitis [10].

There has been some controversy as to whether specific HLA subtypes are particularly associated with disease. Although HLA-29.1 is less common than HLA-A29.2 in patients with birdshot retinochoroidopathy, it was also less common in controls [54]. Although the disease is rare in Asians and the HLA-A29.1 subtype is more common in Asians, all subtypes of HLA-A29 are rare in some Asian populations. Further, birdshot retinochoroidopathy is rare in blacks, but the frequency of HLA-A*2902 subtype is similar to Caucasians. It is likely that other genes are important in the different incidence of disease in different populations. The amino acids in these subtypes do not differ in the area of peptide binding or where the HLA molecule binds to the T-cell receptor; additional evidence from the subtype may not be important. At this point this issue remains unresolved.

The HLA-B*44(12) split product has been associated with birdshot retinochoroidopathy, but that is most likely due to linkage disequilibrium with HLA-A29. No HLA-DR serotype was found to be associated in the only study to look at this [83]. Other Class II antigens were not explored and no one has repeated the study using genetic techniques.

There is evidence provided by a transgenic animal model that the HLA-A29 molecule itself is involved in the pathogenesis of birdshot retinochoroidopathy, rather than genes in linkage disequilibrium with the HLA-29 allele [105]. Investigators used constructs derived from complementary HLA-A*2902 DNA to create transgenic HLA-A29 mice. No regulatory or other human genomic material was thought to have been part of the transgene. The transgenic animals did develop a spontaneous bilateral posterior uveitis after 6 months of age. The expression of the HLA-A29 protein in murine cells was low, and no further studies have been published exploring whether the HLA-A29 molecule was acting to present antigenic peptides or may have played a different role in disease pathogenesis. Other evidence for a direct role of the HLA-A29 molecule in birdshot retinochoroidopathy includes the presence of CD8+ T cells in a postmortem specimen from a patient with birdshot retinochoroidopathy [25], consistent with the involvement of a class I HLA molecule in disease pathogenesis.

Behçet's disease is associated with HLA-B*51, but with a relatively low relative risk of about five. Researchers have examined the role of HLA-B*51 subtypes. Behçet's disease is associated with HLA-B*51 and HLA-B*52 alleles [88]. HLA-B*51 has been divided into 21 subtypes. The HLA-B*5101 subtype was found in 56 of 57 Japanese patients [72] and 33 of 36 Iranian patients [74]; however, all 18 Japanese HLA-B*51 positive controls had the HLA-B*5101 allele and no subtype predominated in the Iranian pa-

tients compared to controls. HLA-B*5108 as well as HLA-B*5101 was also found in Greek [73] and Israeli patients [88]. In Israeli patients HLA-B*52 was found well as HLA-B*51, and no subtype reached statistical significance compared to controls [73, 88]. These patients comprised individuals of Jewish and Arab origin; HLA-B*5101 was more common in patients of Jewish origin.

Other genes have been associated with Behçet's disease. The MIC genes may be associated with disease by linkage disequilibrium; studies have found that using microsatellite markers the HLA-B region was the strongest candidate for disease association in Iranian [76], Japanese, Greek or Italian patients [75]. T-cell proliferation in response to retinal S antigen and to an HLA-B derived peptide that is found in HLA-B*51 as well as HLA-B*27 (B27PD) was increased in Behçet's disease patients with posterior uveitis compared to patients with Behçet's disease but no uveitis, patients with anterior uveitis not associated with Behçet's disease, and healthy controls [51]. This may have implications for inducing tolerance as the HLA-B*27PD has been suggested as a therapeutic agent [109]. Such studies must be interpreted with caution as reactivity to S antigen is not infrequent in patients with posterior uveitis. Even if such reactivity is not primary to the pathogenesis of disease, however, it may be exploited therapeutically (a "bystander effect").

White dot syndromes are a heterogeneous group of posterior uveitides, although some overlap between certain entities is apparent. No HLA association was found with multifocal choroiditis and uveitis in two small studies [80, 102]. There was evidence for a weak association for HLA-B*7 with acute posterior multifocal placoid pigment epitheliopathy and serpiginous choroiditis, suggesting these white dot syndromes may be related, as has been suggested on clinical grounds, although again these studies had small numbers of patients [52, 116]. HLA-B*51 was found to have a relative risk of about six for MEWDS in another small study [11], similar to the relative risk for Behçet's disease.

Summary for the Clinician

- **HLA-B*27 is associated with AAU and a high frequency of spondyloarthropathies**
- **HLA-A29 is so strongly associated with birdshot retinochoroidopathy that the diagnosis should be questioned if the patient is HLA-A29 negative on repeat testing; patients with other forms of ocular inflammation may be HLA-A29 positive, however, and there may occasionally be patients with birdshot retinochoroidopathy who are HLA-A29 negative**
- **Behçet's disease is associated with HLA-B*51; the presence of this HLA type provides additional evidence for the diagnosis but does not make the diagnosis**
- **HLA types in some white dot syndromes provide evidence for shared pathogenesis**

7.3.2 Class II HLA Associations with Ocular Inflammatory Disease

Pars planitis has been associated with HLA-DR2 split products, in particular HLA-DR15 [86, 92, 107], as well as HLA-B*51 and HLA-B*8 [62], although not in all studies [33]. Similarly HLA-DR15 and HLA-DR2 have been associated with multiple sclerosis and optic neuritis, but also with differences in different studies and populations [62, 114].

Vogt-Koyanagi-Harada disease is strongly associated with HLA-DRB1*0405 in Asians [41, 47, 97, 99]. T cells from Asian indi-viduals with VKH disease have been found to be stimulated by putative auto-antigens presented on these molecules [118], implying a direct role for the HLA-DRB1*0405 molecule. In light of the similarities between sympathetic ophthalmia (SO) and VKH disease, it is interesting that SO is also associated with HLA-DR4, and in particular with HLA-DRB1*0405 in Asians [98] and HLA-DRB1*0404 in Caucasians [46].

In Mexican Mestizo patients both HLA-DR1 and HLA-DR4 confer risk for disease, however, and both with a low relative risk (Fig. 7.2) [4, 8, 115]. Further, the HLA-DRB1*0405 molecule was not found more often in patients than controls

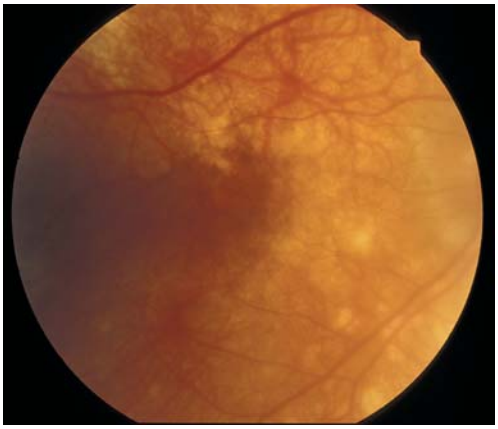

Fig. 7.2. Peripheral retina of a Mestizo individual with Vogt-Koyanagi disease in the chronic phase showing retinal pigment epithelial changes. This patient had the HLA-DRB1*0102 allele

[4]. It may be that multiple HLA-DR1 and HLA-DR4 subtypes can confer risk for VKH disease, perhaps by binding similar antigenic peptides, and the specific alleles present in the population at risk will determine the relative risk of a given allele. HLA-DQ associations with VKH disease have also been described [41, 47, 97, 99], and it has been difficult to establish whether these are due to linkage disequilibrium with either HLA-DR associated alleles or other, unknown, genes in the MHC, or if they may play an additional role in disease pathogenesis as has been suggested for rheumatoid arthritis [12].

Tubulointerstitial nephritis and uveitis syndrome is strongly associated with HLA-DRB1*01, in particular HLA-DRB1*0102. Both HLA-DQA*01 and DQB*05 were also associated with TINU syndrome [56]. The relative risk of HLA-DRB1*0102 was 167.1; however, this was based on published controls. The relative risk was around 20 for the other HLA-DRB and HLA-DRQ associations found. Seventeen of 18 patients were HLA-DQA*01 positive, with 14 having the HLA-DQA1*0101 allele. Previous reports of HLA types in patients with TINU syndrome consisted of smaller numbers of patients, but the various different HLA-DR types found were in linkage disequilibrium with the critical HLA-DQA and HL-DQB alleles found in this larger study. These findings imply that the HLA-DQ molecules may be more important than HLA-DR in conferring risk for TINU syndrome. Interestingly, the patients were older in this larger study than in previous reports [56], but the similarities in HLA associations imply that this is a single nosologic entity.

HLA-DR5 was associated with chronic systemic sarcoidosis, and HLA-DR1 and HLA-DR4 were thought to be protective, but uveitis was not specifically examined [18, 23]. A recent report implies HLA-B*7 may play a role in chronic sarcoidosis, although again uveitis was not specifically examined [34].

Juvenile rheumatoid arthritis (JRA) is the term traditionally used in the United States for what may be a heterogeneous group of arthritides in children. Arthritis and uveitis associated with HLA-B*27 can rarely start in childhood, but the chronic arthritis of children referred to as JRA is found in HLA-B*27 negative individuals. The presence of antinuclear antibodies, lack of positive serology for rheumatoid factor, female sex, and oligoarticular onset are associated with increased risk for the development of uveitis. The highest risk for developing uveitis was associated with the presence of HLA-DR5 and HLA-DP2.1 and HLA-DPw8 and the lack of HLA-DR1; individuals without HLA-DR5 and HLA-DP2.1 but who were positive for HLA-DR1 had the lowest risk for developing uveitis [27]. It is interesting that these HLA associations are similar to those reported in systemic sarcoidosis. HLA-DR5 has subsequently been redefined and now is classified as HLA-DR11. HLA-DR11 did not correlate with prognosis in more recent studies [35, 87], but HLA-DRB1*01 was again thought to be protective [35]. The HLA-DP associations remain intriguing as HLA-DPB1*0201 was associated with chronic anterior uveitis in Greek children with JRA [90].

Summary for the Clinician

- **HLA associations with pars planitis vary in different populations but appear similar to those for optic neuritis and multiple sclerosis, as expected clinically**
- **HLA-DRB1*0405 is strongly associated with VKH disease in Asian patients, but not Mexican Mestizo patients, for whom both HLA-DR1 and HLA-DR4 confer a low relative risk**

- **HLA-DRB1*01, HLA-DQA*01 and DQB*05 are associated strongly with TINU syndrome. Diverse populations with TINU syndrome have a common genetic risk**
- **JRA associated uveitis has HLA-DP associations that have been replicated and HLA-DR1 is protective**

7.3.3 HLA Associations with Ocular Infections

While infections are clearly due to exogenous agents and therefore an environmental factor par excellence for ocular inflammatory disease, it is similarly clear that the host response is critical in determining disease outcome. An elegant animal model of herpes simplex virus (HSV) stromal keratitis that illustrates this, as well as the complexities of HLA in disease, has been reviewed elsewhere [16]. An HSV coat protein, UL-6, shares a seven amino acid sequence with IgG2a. Mice were protected from stromal keratitis if they had a certain IgG2a allele, IgG2a b. When mice were infected with HSV, it was thought that a corneal peptide that cross reacted with the specific IgG2a b allele and viral UL-6 was "unmasked"; if mice had the IgG2a b allele they would have developed central tolerance and an immune response was not elicited.

Acute retinal necrosis (ARN) is due to infection with either herpes zoster or simplex viruses. As for other manifestations of disease secondary to herpes viruses, it is not clear why some otherwise healthy individuals develop this devastating infection. In one study in HLA-DQw7, Bw62 and DR4 was associated with ARN [38] and in a second study HLA-DR9 was correlated with severity of ARN [64].

Two studies found no HLA association with toxoplasmosis [85, 82]. There are other studies that indicate that HLA may be important. Severe ocular involvement was associated with HLA-Bw62 [68], and HLA-DQ3 may be associated with hydrocephalus secondary to congenital toxoplasmosis [59] as well as with toxoplasmic encephalitis in individuals with AIDS [104], whereas HLA-DQ1 may be protective [103, 104]. In addition, T-cell clones that were HLA-DR restricted were derived from the vitreous of patients with ocular toxoplasmosis [21].

Presumed ocular histoplasmosis is not associated with overt active inflammation clinically, and loss of vision is most often a result of macular choroidal neovascular membranes. One study found that macular damage from macular choroidal neovascular membranes was associated with HLA-DRw2 [69].

Uveitis in patients with leprosy was associated with HLA-DR2, but HLA-DR4 was decreased in patients with uveitis [43]. In the same study HLA-BRB1*1501 was also found in increased frequency in patients with uveitis and HLA-DRB1*0405 was decreased.

7.3.4 HLA Associations with External Ocular Inflammation

Class II HLA genes sharing a DQB1 reactivity were associated with primary Sjögren's syndrome [44]. Ocular involvement was more common in patients with Stevens-Johnson syndrome if they were HLA-Bw44 positive [77]. Mooren's ulcer was associated with HLA-DR17(3) and/or HLA-DQ2 in patients of Asian or African descent, but the number of patients was small and comparing to appropriate controls is difficult with a group of individuals with differing descent [108]. As expected, among a group of patients with sterile corneal ulcers, individuals with rheumatoid arthritis were more likely to be HLA-DR1 positive [26]. This does imply that corneal ulcers from rheumatoid arthritis are a separate nosologic entity from Mooren's ulcer. HLA-B*12 was more common in controls than in patients with ocular cicatricial pemphigoid [78].

7.3.5 HLA Associations with Extraocular Disease

Giant cell arteritis (GCA) has been associated with HLA-DRB1*04 in most studies, and in some patient populations with HLA-DR1 genotypes [29]. In Italian patients, HLA-DQB1*0302 was weakly associated with GCA [94]. While

these HLA associations are similar to what has been found in individuals with VKH disease, it is interesting that, unlike VKH disease, biopsy proven GCA appears to be uncommon in Hispanics in Southern California [58], implying a large role for other genetic or environmental factors. Additional genetic associations have been found including an IL-6 promoter polymorphism [30], mannose-binding lectin alleles [42] and tumour necrosis factor and IL-1 polymorphisms [65], but not intercellular adhesion molecule-1 [6].

Graves' disease has been associated with HLA-DRB1*0301 as well as HLA-DRB1*08 in early onset disease in Caucasians [14, 121], but not in Taiwanese patients, in whom HLA-A*0207 was weakly associated [39]. HLA-DQ associations were thought to be by linkage disequilibrium [121]. A mouse transgenic for HLA-DRB1*0301 developed inflammatory thyroid disease when immunized with a plasmid encoding human thyrotropin receptor [22]. In a review of studies involving genome wide scans in autoimmune thyroid disease, the author felt the HLA associations with Graves' disease most likely were modulating genes, increasing risk but not primary to disease pathogenesis [110]. Another gene important in the immune response that was implicated was the gene for CTLA-4 and several other candidate loci were described [110].

7.4 Non-HLA Genes Associated with Ocular Inflammatory Disease

The most exciting non-MHC association with uveitis has been the gene for the nucleotide oligomerization domain (NOD) 2 protein [93]. Mutations of this protein are found in systemic diseases associated with uveitis, Crohn's disease [40, 84], and Blau syndrome (familial juvenile systemic granulomatosis, or Jabs disease) [70, 112]. A mutation in another domain, the capsase recruitment domain (CARD) of the same protein, has been associated with another rare disease that includes uveitis, NOMID/CINCA (neonatal-onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, articular syndrome) [3]. This fascinating protein is involved in the NF-kB and apoptosis pathways, and so efforts have been made to find mutations in other inflammatory disease, but no association was found with either uveitis in patients with sarcoidosis [63] or Wegener's granulomatosis [79].

Another strategy is to look for genes that may predispose to complications of disease. Recently Behçet's patients with retinal vascular occlusive disease were found to have a higher frequency of factor V Leiden, but not prothrombin gene G20210A mutations [9].

Tumour necrosis factor (TNF) plays a role in Behçet's disease and other forms of uveitis. The TNF inhibitor, infliximab, has been reported to be successful in treating uveitis in small case series. The TNF allele TNFB*2 may be associated with a poor prognosis in ocular Behçet's disease [111], but a role for TNF polymorphisms in Behçet's disease has not been clearly established. While one study found TNF-1031C allele to be independently associated in Caucasians [2], a second study found no TNF polymorphism to be associated independently with disease in Korean patients [53]. Research into whether TNF polymorphisms or other genomic markers may predict response to TNF inhibition in systemic inflammatory disease is being actively pursued. Polymorphisms of the promoter/enhancer region of the TNF gene were associated with uveitis from the human T-cell lymphotrophic virus (HTLV) type I [96].

Interleukin (IL)-1 polymorphisms have also been thought to be associated with juvenile rheumatoid arthritis [67], but not in all studies [17]. Homozygosity for specific IL-1 polymorphisms did appear to increase the risk of Behçet's disease [45]. These studies did not look at uveitis specifically. Using single nucleotide polymorphisms a recent study found polymorphisms in chemokine genes differed between men and women with Behçet's disease [15].

Summary for the Clinician

- **The recent finding of NOD2 associated with a rare familial granulomatous disease has been promising, although so far has not been found to be associated with other granulomatous uveitides**

- **Finding TNF associated polymorphisms provides hope that further research will provide a means to predict who will respond best to specific therapeutic agents**

7.5 Other Ocular Diseases

Inflammation is involved in the pathogenesis of many ocular diseases, not only those thought to be due to primarily to autoimmunity or infection. Such diverse entities as dry eye, macular degeneration [5, 7, 89, 95], diabetic retinopathy [24] and glaucoma [113, 119] have been shown to have an inflammatory component. The prognosis of patients with choroidal melanoma may also be in part determined by HLA and other genetic risk factors, as may be expected by the role of the immune system in controlling malignancies.

No immunogenic studies of macular degeneration have been published, although manipulation of genes involved in the immune response has resulted in the development of a murine model of disease [5].

Despite the putative involvement of T cells [106], cytokines [19, 120], IL-6 [48], and chemokines [13, 20] in diabetic eye disease, a consistent HLA association has not been found. HLA-DQB1*0201 and HLA-DQB1*0302 were thought to be associated with severe retinopathy, with HLA-DR3 and HLA-DR4 being involved by linkage disequilibrium in one study [1]. HLA-DR3 and HLA-DR4 were not associated with the diabetic retinopathy in a more recent study [117], while an association with HLA-B*62, HLA-Cw4 and HLA-DQ4, and in particular with the HLA-Cw4/DR4/DQ4 haplotype in patients with type 2 diabetes, was described [71]. These discrepancies are not surprising; multiple environmental and genetic factors contribute to the development of diabetic retinopathy, and different patient populations have been studied. It may be that genes related to these other mediators of inflammation will be found that are more critical in the inflammatory response in diabetic retinopathy.

Primary open angle glaucoma was associated with the HLA-DRB1*0407-DQB1*0302 in Mexican Mestizos [28]. A TNF gene polymorphism was found in Chinese patients with primary open angle glaucoma [57].

Summary for the Clinician

- **Macular degeneration, diabetic retinopathy, glaucoma and dry eye have an inflammatory component; future research in the immunogenetics of these diseases may provide insights into pathogenesis**

7.6 Conclusion

Genetic associations are useful for providing supportive evidence for the diagnosis of specific entities in the work-up of patients with uveitis. No specific HLA type makes the diagnosis, however. Immunogenetic research has presented us with the profound challenge of trying to understand the reasons for the impressive HLA associations with ocular inflammatory disease. This would have implications for non-ocular diseases as well. As our understanding of the human genome becomes more sophisticated and our research techniques more robust, we will be able to evaluate additional genetic contributions to the pathogenesis not only of uveitis, but other ocular diseases including macular degeneration, diabetic retinopathy, glaucoma and intraocular tumours. This will allow a better understanding of disease mechanisms and the design of novel therapies.

References

1. Agardh D, Gaur LK, Agardh E, et al. (1996) HLA-DQB1*0201/0302 is associated with severe retinopathy in patients with IDDM. Diabetologia 39:1313–1317
2. Ahmad T, Wallace GR, James T, et al. (2003) Mapping the HLA association in Behçet's disease: a role for tumor necrosis factor polymorphisms? Arthritis Rheum 48:807–813
3. Aksentijevich I, Nowak M, Mallah M, et al. (2002) De novo CIAS1 mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new member of the expanding family of pyrin-associated autoinflammatory diseases. Arthritis Rheum 46:3340–3348

4. Alaez C, del Pilar MM, Arellanes L, et al. (1999) Strong association of HLA class II sequences in Mexicans with Vogt-Koyanagi-Harada's disease. Hum Immunol 60:875–882
5. Ambati J, Anand A, Fernandez S, et al. (2003) An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. Nat Med 9:1390–1397
6. Amoli MM, Shelley E, Mattey DL, et al. (2001) Lack of association between intercellular adhesion molecule-1 gene polymorphisms and giant cell arteritis. J Rheumatol 28:1600–1604
7. Anderson DH, Mullins RF, Hageman GS, Johnson LV (2002) A role for local inflammation in the formation of drusen in the aging eye. Am J Ophthalmol 134:411–431
8. Arellanes-Garcia L, Bautista N, Mora P, et al. (1998) HLA-DR is strongly associated with Vogt-Koyanagi-Harada disease in Mexican Mestizo patients. Ocul Immunol Inflamm 6:93–100
9. Batioglu F, Atmaca LS, Karabulut HG, Beyza SD (2003) Factor V Leiden and prothrombin gene G20210A mutations in ocular Behçet disease. Acta Ophthalmol Scand 81:283–285
10. Bloch-Michel E, Frau E (1991) Birdshot retinochoroidopathy and HLA-A29+ and HLA-A29– idiopathic retinal vasculitis: comparative study of 56 cases. Can J Ophthalmol 26:361–366
11. Borruat FX, Herbort CP, Spertini F, Desarnaulds AB (1998) HLA typing in patients with multiple evanescent white dot syndrome (MEWDS). Ocul Immunol Inflamm 6:39–41
12. Buckner JH, Nepom GT (2002) Genetics of rheumatoid arthritis: is there a scientific explanation for the human leukocyte antigen association? Curr Opin Rheumatol 14:254–259
13. Capeans C, De Rojas MV, Lojo S, Salorio MS (1998) C-C chemokines in the vitreous of patients with proliferative vitreoretinopathy and proliferative diabetic retinopathy. Retina 18:546–550
14. Chen QY, Huang W, She JX, et al. (1999) HLA-DRB1*08, DRB1*03/DRB3*0101, and DRB3*0202 are susceptibility genes for Graves' disease in North American Caucasians, whereas DRB1*07 is protective. J Clin Endocrinol Metab 84:3182–3186
15. Chen Y, Vaughan RW, Kondeatis E, et al. (2004) Chemokine gene polymorphisms associate with gender in patients with uveitis. Tissue Antigens 63:41–45
16. Davey MP, Rosenbaum JT (2000) The human leukocyte antigen complex and chronic ocular inflammatory disorders. Am J Ophthalmol 129: 235–243
17. Donn RP, Farhan AJ, Barrett JH, et al. (1999) Absence of association between interleukin 1 alpha and oligoarticular juvenile chronic arthritis in UK patients. Rheumatology (Oxford) 38:171–175
18. du Bois RM (2002) The genetic predisposition to interstitial lung disease: functional relevance. Chest 121:14S–20S
19. Elner SG, Elner VM, Jaffe GJ, et al. (1995) Cytokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. Curr Eye Res 14: 1045–1053
20. Elner SG, Strieter R, Bian ZM, et al. (1998) Interferon-induced protein 10 and interleukin 8. C-X-C chemokines present in proliferative diabetic retinopathy. Arch Ophthalmol 116:1597–1601
21. Feron EJ, Klaren VN, Wierenga EA, et al. (2001) Characterization of *Toxoplasma gondii*-specific T cells recovered from vitreous fluid of patients with ocular toxoplasmosis. Invest Ophthalmol Vis Sci 42:3228–3232
22. Flynn JC, Rao PV, Gora M, et al. (2004) Graves' hyperthyroidism and thyroiditis in HLA-DRB1*0301 (DR3) transgenic mice after immunization with thyrotropin receptor DNA. Clin Exp Immunol 135:35–40
23. Foley PJ, McGrath DS, Puscinska E, et al. (2001) Human leukocyte antigen-DRB1 position 11 residues are a common protective marker for sarcoidosis. Am J Respir Cell Mol Biol 25:272–277
24. Gardner TW, Antonetti DA, Barber AJ, et al. (2002) Diabetic retinopathy: more than meets the eye. Surv Ophthalmol 47 Suppl 2:S253–S262
25. Gaudio PA, Kaye DB, Crawford JB (2002) Histopathology of birdshot retinochoroidopathy. Br J Ophthalmol 86:1439–1441
26. Geggel HS, Nettles JW, Nepom BS (1992) HLA-DR alleles and sterile ulcerative keratitis. Cornea 11:505–509
27. Giannini EH, Malagon CN, Van Kerckhove C, et al. (1991) Longitudinal analysis of HLA associated risks for iridocyclitis in juvenile rheumatoid arthritis. J Rheumatol 18:1394–1397
28. Gil-Carrasco F, Vargas-Alarcon G, Zuniga J, et al. (1999) HLA-DRB and HLA-DQB loci in the genetic susceptibility to develop glaucoma in Mexicans. Am J Ophthalmol 128:297–300
29. Gonzalez-Gay MA, Amoli MM, Garcia-Porrua C, Ollier WE (2003) Genetic markers of disease susceptibility and severity in giant cell arteritis and polymyalgia rheumatica. Semin Arthritis Rheum 33:38–48
30. Gonzalez-Gay MA, Hajeer AH, Dababneh A, et al. (2002) IL-6 promoter polymorphism at position –174 modulates the phenotypic expression of polymyalgia rheumatica in biopsy-proven giant cell arteritis. Clin Exp Rheumatol 20:179–184
31. Goto K, Ota M, Ando H, et al. (1998) MICA gene polymorphisms and HLA-B*27 subtypes in Japanese patients with HLA-B*27-associated acute anterior uveitis. Invest Ophthalmol Vis Sci 39:634–637

32. Goto K, Ota M, Maksymowych WP, et al. (1998) Association between MICA gene A4 allele and acute anterior uveitis in white patients with and without HLA-B*27. Am J Ophthalmol 126:436–441
33. Greiner KH, Wilson DW, Thomson J, et al. (2003) Genetic polymorphism of HLA DR in a Scottish population of patients with pars planitis. Eur J Ophthalmol 13:433–438
34. Grunewald J, Eklund A, Olerup O (2004) Human leukocyte antigen class I alleles and the disease course in sarcoidosis patients. Am J Respir Crit Care Med 169:696–702
35. Haas JP, Truckenbrodt H, Paul C, et al. (1994) Subtypes of HLA-DRB1*03, *08, *11, *12, *13 and *14 in early onset pauciarticular juvenile chronic arthritis (EOPA) with and without iridocyclitis. Clin Exp Rheumatol 12 Suppl 10:S7–14
36. Hall FC, Rabinowitz JD, Busch R, et al. (2002) Relationship between kinetic stability and immunogenicity of HLA-DR4/peptide complexes. Eur J Immunol 32:662–670
37. Hohler T, Schaper T, Schneider PM, et al. (1997) No primary association between LMP2 polymorphisms and extraspinal manifestations in spondyloarthropathies. Ann Rheum Dis 56:741–743
38. Holland GN, Cornell PJ, Park MS, et al. (1989) An association between acute retinal necrosis syndrome and HLA-DQw7 and phenotype Bw62, DR4. Am J Ophthalmol 108:370–374
39. Huang SM, Wu TJ, Lee TD, et al. (2003) The association of HLA -A, -B, and -DRB1 genotypes with Graves' disease in Taiwanese people. Tissue Antigens 61:154–158
40. Hugot JP, Chamaillard M, Zouali H, et al. (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411:599–603
41. Islam SM, Numaga J, Matsuki K, et al. (1994) Influence of HLA-DRB1 gene variation on the clinical course of Vogt-Koyanagi-Harada disease. Invest Ophthalmol Vis Sci 35:752–756
42. Jacobsen S, Baslund B, Madsen HO, et al. (2002) Mannose-binding lectin variant alleles and HLA-DR4 alleles are associated with giant cell arteritis. J Rheumatol 29:2148–2153
43. Joko S, Numaga J, Maeda H (1999) Immunogenetics of uveitis in leprosy. Jpn J Ophthalmol 43:97–102
44. Kang HI, Fei HM, Saito I, et al. (1993) Comparison of HLA class II genes in Caucasoid, Chinese, and Japanese patients with primary Sjogren's syndrome. J Immunol 150:3615–3623
45. Karasneh J, Hajeer AH, Barrett J, et al. (2003) Association of specific interleukin 1 gene cluster polymorphisms with increased susceptibility for Behçet's disease. Rheumatology (Oxford) 42:860–864
46. Kilmartin DJ, Wilson D, Liversidge J, et al. (2001) Immunogenetics and clinical phenotype of sympathetic ophthalmia in British and Irish patients. Br J Ophthalmol 85:281–286
47. Kim MH, Seong MC, Kwak NH, et al. (2000) Association of HLA with Vogt-Koyanagi-Harada syndrome in Koreans. Am J Ophthalmol 129:173–177
48. Kojima S, Yamada T, Tamai M (2001) Quantitative analysis of interleukin-6 in vitreous from patients with proliferative vitreoretinal diseases. Jpn J Ophthalmol 45:40–45
49. Konno Y, Numaga J, Mochizuki M, et al. (1998) TAP polymorphism is not associated with ankylosing spondylitis and complications with acute anterior uveitis in HLA-B*27-positive Japanese. Tissue Antigens 52:478–483
50. Konno Y, Numaga J, Tsuchiya N, et al. (1999) HLA-B*27 subtypes and HLA class II alleles in Japanese patients with anterior uveitis. Invest Ophthalmol Vis Sci 40:1838–1844
51. Kurhan-Yavuz S, Direskeneli H, Bozkurt N, et al. (2000) Anti-MHC autoimmunity in Behçet's disease: T cell responses to an HLA-B-derived peptide cross-reactive with retinal-S antigen in patients with uveitis. Clin Exp Immunol 120:162–166
52. Laatikainen L, Erkkila H (1981) A follow-up study on serpiginous choroiditis. Acta Ophthalmol (Copenh) 59:707–718
53. Lee EB, Kim JY, Lee YJ, et al. (2003) TNF and TNF receptor polymorphisms in Korean Behçet's disease patients. Hum Immunol 64:614–620
54. Levinson RD, Gonzales CR (2002) Birdshot retinochoroidopathy: immunopathogenesis, evaluation, and treatment. Ophthalmol Clin North Am 15: 343–350
55. Levinson RD, Greenhill LH (2002) The monthly variation in acute anterior uveitis in a community-based ophthalmology practice. Ocul Immunol Inflamm 10:133–139
56. Levinson RD, Park MS, Rikkers SM, et al. (2003) Strong associations between specific HLA-DQ and HLA-DR alleles and the tubulointerstitial nephritis and uveitis syndrome. Invest Ophthalmol Vis Sci 44:653–657
57. Lin HJ, Tsai FJ, Chen WC, et al. (2003) Association of tumour necrosis factor alpha-308 gene polymorphism with primary open-angle glaucoma in Chinese. Eye 17:31–34
58. Liu NH, LaBree LD, Feldon SE, Rao NA (2001) The epidemiology of giant cell arteritis: a 12-year retrospective study. Ophthalmology 108:1145–1149
59. Mack DG, Johnson JJ, Roberts F, et al. (1999) HLA-class II genes modify outcome of *Toxoplasma gondii* infection. Int J Parasitol 29:1351–1358
60. Maksymowych WP, Jhangri GS, Gorodezky C, et al. (1997) The LMP2 polymorphism is associated with susceptibility to acute anterior uveitis in

HLA-B*27 positive juvenile and adult Mexican subjects with ankylosing spondylitis. Ann Rheum Dis 56:488–492
61. Maksymowych WP, Tao S, Vaile J, et al. (2000) LMP2 polymorphism is associated with extraspinal disease in HLA-B*27 negative Caucasian and Mexican Mestizo patients with ankylosing spondylitis. J Rheumatol 27:183–189
62. Malinowski SM, Pulido JS, Goeken NE, et al. (1993) The association of HLA-B*8, B51, DR2, and multiple sclerosis in pars planitis. Ophthalmology 100:1199–1205
63. Martin TM, Doyle TM, Smith JR, et al. (2003) Uveitis in patients with sarcoidosis is not associated with mutations in NOD2 (CARD15). Am J Ophthalmol 136:933–935
64. Matsuo T, Matsuo N (1991) HLA-DR9 associated with the severity of acute retinal necrosis syndrome. Ophthalmologica 203:133–137
65. Mattey DL, Hajeer AH, Dababneh A, et al. (2000) Association of giant cell arteritis and polymyalgia rheumatica with different tumor necrosis factor microsatellite polymorphisms. Arthritis Rheum 43:1749–1755
66. McCannel CA, Holland GN, Helm CJ, et al. (1996) Causes of uveitis in the general practice of ophthalmology. UCLA Community-Based Uveitis Study Group. Am J Ophthalmol 121:35–46
67. McDowell TL, Symons JA, Ploski R, et al. (1995) A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. Arthritis Rheum 38:221–228
68. Meenken C, Rothova A, de Waal LP, et al. (1995) HLA typing in congenital toxoplasmosis. Br J Ophthalmol 79:494–497
69. Meredith TA, Smith RE, Duquesnoy RJ (1980) Association of HLA-DRw2 antigen with presumed ocular histoplasmosis. Am J Ophthalmol 89: 70–76
70. Miceli-Richard C, Lesage S, Rybojad M, et al. (2001) CARD15 mutations in Blau syndrome. Nat Genet 29:19–20
71. Mimura T, Funatsu H, Uchigata Y, et al. (2003) Relationship between human leukocyte antigen status and proliferative diabetic retinopathy in patients with younger-onset type 1 diabetes mellitus. Am J Ophthalmol 135:844–848
72. Mizuki N, Ota M, Katsuyama Y, et al. (2001) HLA-B*51 allele analysis by the PCR-SBT method and a strong association of HLA-B*5101 with Japanese patients with Behçet's disease. Tissue Antigens 58:181–184
73. Mizuki N, Ota M, Katsuyama Y, et al. (2002) Sequencing-based typing of HLA-B*51 alleles and the significant association of HLA-B*5101 and -B*5108 with Behçet's disease in Greek patients. Tissue Antigens 59:118–121
74. Mizuki N, Ota M, Katsuyama Y, et al. (2001) HLA class I genotyping including HLA-B*51 allele typing in the Iranian patients with Behçet's disease. Tissue Antigens 57:457–462
75. Mizuki N, Ota M, Yabuki K, et al. (2000) Localization of the pathogenic gene of Behçet's disease by microsatellite analysis of three different populations. Invest Ophthalmol Vis Sci 41:3702–3708
76. Mizuki N, Yabuki K, Ota M, et al. (2002) Analysis of microsatellite polymorphism around the HLA-B locus in Iranian patients with Behçet's disease. Tissue Antigens 60:396–399
77. Mondino BJ, Brown SI, Biglan AW (1982) HLA antigens in Stevens-Johnson syndrome with ocular involvement. Arch Ophthalmol 100:1453–1454
78. Mondino BJ, Brown SI, Rabin BS (1979) HLA antigens in ocular cicatricial pemphigoid. Arch Ophthalmol 97:479
79. Newman B, Rubin LA, Siminovitch KA (2003) NOD2/CARD15 gene mutation is not associated with susceptibility to Wegener's granulomatosis. J Rheumatol 30:305–307
80. Nolle B, Faul S, Jenisch S, Westphal E (1998) Peripheral multifocal chorioretinitis with panuveitis: clinical and immunogenetic characterization in older patients. Graefes Arch Clin Exp Ophthalmol 236:451–460
81. Nussenblatt RB, Mittal KK (1981) Iridocyclitis in black Americans: association with HLA B8 suggests an autoimmune aetiology. Br J Ophthalmol 65:329–332
82. Nussenblatt RB, Mittal KK, Fuhrman S, et al. (1989) Lymphocyte proliferative responses of patients with ocular toxoplasmosis to parasite and retinal antigens. Am J Ophthalmol 107:632–641
83. Nussenblatt RB, Mittal KK, Ryan S, et al. (1982) Birdshot retinochoroidopathy associated with HLA-A29 antigen and immune responsiveness to retinal S-antigen. Am J Ophthalmol 94:147–158
84. Ogura Y, Bonen DK, Inohara N, et al. (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411:603–606
85. Ohno S, O'Connor GR, Kimura SJ (1977) HLA antigens and toxoplasmic retinochoroiditis. Tohoku J Exp Med 123:91–94
86. Oruc S, Duffy BF, Mohanakumar T, Kaplan HJ (2001) The association of HLA class II with pars planitis. Am J Ophthalmol 131:657–659
87. Paroli MP, Speranza S, Marino M, et al. (2003) Prognosis of juvenile rheumatoid arthritis-associated uveitis. Eur J Ophthalmol 13:616–621
88. Paul M, Klein T, Krause I, et al. (2001) Allelic distribution of HLA-B*5 in HLA-B*5-positive Israeli patients with Behçet's disease. Tissue Antigens 58:185–186

89. Penfold PL, Madigan MC, Gillies MC, Provis JM (2001) Immunological and aetiological aspects of macular degeneration. Prog Retin Eye Res 20:385–414
90. Pratsidou-Gertsi P, Kanakoudi-Tsakalidou F, Spyropoulou M, et al. (1999) Nationwide collaborative study of HLA class II associations with distinct types of juvenile chronic arthritis (JCA) in Greece. Eur J Immunogenet 26:299–310
91. Radosevich M, Ono SJ (2003) Novel mechanisms of class II major histocompatibility complex gene regulation. Immunol Res 27:85–106
92. Raja SC, Jabs DA, Dunn JP, et al. (1999) Pars planitis: clinical features and class II HLA associations. Ophthalmology 106:594–599
93. Rosenbaum JT, Planck SR, Davey MP, et al. (2003) With a mere nod, uveitis enters a new era. Am J Ophthalmol 136:729–732
94. Salvarani C, Boiardi L, Mantovani V, et al. (1999) HLA-DRB1, DQA1, and DQB1 alleles associated with giant cell arteritis in northern Italy. J Rheumatol 26:2395–2399
95. Seddon JM, Gensler G, Milton RC, et al. (2004) Association between C-reactive protein and age-related macular degeneration. JAMA 291:704–710
96. Seki N, Yamaguchi K, Yamada A, et al. (1999) Polymorphism of the 5'-flanking region of the tumor necrosis factor (TNF)-alpha gene and susceptibility to human T-cell lymphotropic virus type I (HTLV-I) uveitis. J Infect Dis 180:880–883
97. Shindo Y, Inoko H, Yamamoto T, Ohno S (1994) HLA-DRB1 typing of Vogt-Koyanagi-Harada's disease by PCR-RFLP and the strong association with DRB1*0405 and DRB1*0410. Br J Ophthalmol 78:223–226
98. Shindo Y, Ohno S, Usui M, et al. (1997) Immunogenetic study of sympathetic ophthalmia. Tissue Antigens 49:111–115
99. Shindo Y, Ohno S, Yamamoto T, et al. (1994) Complete association of the HLA-DRB1*04 and -DQB1*04 alleles with Vogt-Koyanagi-Harada's disease. Hum Immunol 39:169–176
100. Smith JR (2002) HLA-B*27-associated uveitis. Ophthalmol Clin North Am 15:297–307
101. Snijders A, Elferink DG, Geluk A, et al. (2001) An HLA-DRB1-derived peptide associated with protection against rheumatoid arthritis is naturally processed by human APCs. J Immunol 166:4987–4993
102. Spaide RF, Skerry JE, Yannuzzi LA, DeRosa JT (1990) Lack of the HLA-DR2 specificity in multifocal choroiditis and panuveitis. Br J Ophthalmol 74:536–537
103. Suzuki Y (2002) Host resistance in the brain against *Toxoplasma gondii*. J Infect Dis 185 Suppl 1:S58–S65
104. Suzuki Y, Wong SY, Grumet FC, et al. (1996) Evidence for genetic regulation of susceptibility to toxoplasmic encephalitis in AIDS patients. J Infect Dis 173:265–268
105. Szpak Y, Vieville JC, Tabary T, et al. (2001) Spontaneous retinopathy in HLA-A29 transgenic mice. Proc Natl Acad Sci U S A 98:2572–2576
106. Tang S, Le Ruppert KC (1995) Activated T lymphocytes in epiretinal membranes from eyes of patients with proliferative diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol 233:21–25
107. Tang WM, Pulido JS, Eckels DD, et al. (1997) The association of HLA-DR15 and intermediate uveitis. Am J Ophthalmol 123:70–75
108. Taylor CJ, Smith SI, Morgan CH, et al. (2000) HLA and Mooren's ulceration. Br J Ophthalmol 84:72–75
109. Thurau SR, Diedrichs-Mohring M, Fricke H, et al. (1999) Oral tolerance with an HLA-peptide mimicking retinal autoantigen as a treatment of autoimmune uveitis. Immunol Lett 68:205–212
110. Tomer Y (2002) Genetic dissection of familial autoimmune thyroid diseases using whole genome screening. Autoimmun Rev 1:198–204
111. Verity DH, Wallace GR, Vaughan RW, et al. (1999) HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behçet's disease. Tissue Antigens 54:264–272
112. Wang X, Kuivaniemi H, Bonavita G, et al. (2002) CARD15 mutations in familial granulomatosis syndromes: a study of the original Blau syndrome kindred and other families with large-vessel arteritis and cranial neuropathy. Arthritis Rheum 46:3041–3045
113. Wax MB, Tezel G (2002) Neurobiology of glaucomatous optic neuropathy: diverse cellular events in neurodegeneration and neuroprotection. Mol Neurobiol 26:45–55
114. Weinshenker BG, Santrach P, Bissonet AS, et al. (1998) Major histocompatibility complex class II alleles and the course and outcome of MS: a population-based study. Neurology 51:742–747
115. Weisz JM, Holland GN, Roer LN, et al. (1995) Association between Vogt-Koyanagi-Harada syndrome and HLA-DR1 and -DR4 in Hispanic patients living in southern California. Ophthalmology 102:1012–1015
116. Wolf MD, Folk JC, Panknen CA, Goeken NE (1990) HLA-B*7 and HLA-DR2 antigens and acute posterior multifocal placoid pigment epitheliopathy. Arch Ophthalmol 108:698–700
117. Wong TY, Cruickshank KJ, Klein R, et al. (2002) HLA-DR3 and DR4 and their relation to the incidence and progression of diabetic retinopathy. Ophthalmology 109:275–281

118. Yamaki K, Gocho K, Hayakawa K, et al. (2000) Tyrosinase family proteins are antigens specific to Vogt-Koyanagi-Harada disease. J Immunol 165:7323–7329
119. Yang J, Patil RV, Yu H, et al. (2001) T cell subsets and sIL-2R/IL-2 levels in patients with glaucoma. Am J Ophthalmol 131:421–426
120. Yuuki T, Kanda T, Kimura Y, et al. (2001) Inflammatory cytokines in vitreous fluid and serum of patients with diabetic vitreoretinopathy. J Diabetes Complications 15:257–259
121. Zamani M, Spaepen M, Bex M, et al. (2000) Primary role of the HLA class II DRB1*0301 allele in Graves disease. Am J Med Genet 95:432–437

Immune Mechanisms in Uveitis

Ling Chen, Lynn K. Gordon

Core Messages

- The eye is a site of immune privilege that results from local structural features, local expression of soluble immunosuppressive agents, and cell surface expression of negative immunoregulators
- Despite the bias against active inflammation, immune mediated disease of the eye is responsible for significant morbidity and permanent visual loss in affected patients
- Animal uveitis models help identify important immune mechanisms that may regulate disease in humans
- Experimental autoimmune uveitis (EAU) is induced in genetically susceptible animal species and is produced by CD4+ T lymphocytes bearing a Th1 phenotype
- Cytokines such as interleukin 2 (IL-2), interleukin 12 (IL-12), and interferon gamma (IFNγ) play central roles in EAU pathogenesis
- Adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and P-selectin, are important to leukocyte migration into the eye in cases of experimental uveitis
- Immune pathogenesis in human uveitis likely results from genetic susceptibility factors, for example HLA-B*27 and HLA-A29, in the setting of appropriate proinflammatory stimuli
- Behçet's disease is associated with neutrophil activation, vasculitis, and T-lymphocyte-mediated immunity
- Evidence supports a role for antigen-specific T lymphocytes directed against tyrosinase family members in the pathogenesis of Vogt-Koyanagi-Harada (VKH) syndrome

8.1 Introduction

Immune privilege refers to an anatomical site in an immunocompetent host in which there is dampening of the immune response. In comparison to conventional sites, immune privilege confers preferential maintenance of allogeneic material without eliciting significant rejection [1]. The eye is a classic example of an immune-privileged site in which multiple factors, including local structural anatomy and tissue specific gene expression of secreted and cell surface proteins, are believed to play important roles.

Tissue features that bias against immunologic reactivity in the eye include (1) blood tissue barriers formed by tight endothelial cell junctions of the retinal and uveal vasculature, (2) tight junctions between epithelial cells of retina, iris and ciliary body, (3) limited afferent delivery of local antigen to regional lymph nodes, and (4) an immunosuppressive ocular microenvironment [1–3]. The immunosuppressive ocular microenvironment is also maintained through multiple soluble and cell surface mechanisms. Soluble negative immunoregulators in the aqueous humor include transforming growth factor-beta (TGF-β), vasoactive intestinal polypeptide (VIP), α-melanocyte stimulating hormone (α-MSH), macrophage migration inhibitory factor (MIF), and IL-1 receptor antagonist (IL-1ra). Constitutive expression of Fas ligand on intraocular cells also contributes to ocular immune privilege through the promotion of site-specific apoptosis [4]. Anterior chamber associated immune deviation (ACAID), characterized by a selective antigen-specific downregulation of delayed hypersensitivity and a simultaneous induction of the serum antibod-

ies, is a well-defined example of ocular immune privilege that is believed to protect the eye from deleterious immune responses.

Despite these multiple mechanisms for immunologic downregulation, clinically relevant immune-mediated ocular disease produces significant patient morbidity as well of loss of visual function [5]. This chapter reviews both the current state of animal models used in the study of intraocular inflammation and selected immunologic mechanisms of specific forms of human uveitis.

8.2 Animal Uveitis Models

Development of animal models of uveitis permits definition of immunopathogenetic mechanisms that are likely relevant to human disease. These models provide useful tools to help define basic studies of immune mechanisms in ocular inflammation and genetic factors predisposing to uveitis, and serve as templates for development of new therapeutic strategies.

8.2.1 Experimental Autoimmune Uveitis (EAU)

EAU is an animal model of posterior uveitis, which can be induced in genetically susceptible animal species by immunization with uveitogenic retinal antigens or by the adoptive transfer of antigen-specific T cells [6]. The EAU course is characterized by photoreceptor damage, vasculitis, choroiditis and vitritis, serous retinal detachment and retinal folding. EAU histopathology strikingly resembles lesions of ocular sarcoidosis, Vogt-Koyanagi-Harada (VKH) syndrome, sympathetic ophthalmia, Behçet's disease, and birdshot retinochoroidopathy, a group of non-infectious uveitis syndromes in humans that have a suspected autoimmune aetiology. EAU also serves as an animal model for organ-specific, T-lymphocyte-mediated autoimmunity.

8.2.1.1 Cellular Responses in EAU

Various lines of experimental evidence point to a central role for T lymphocytes in EAU. Uveitogenic antigens are classically presented by antigen presenting cells (APCs) to T lymphocytes. The presentation, in conjunction with MHC and costimulatory molecules, results in T-cell activation and, in susceptible animal strains, the T cells produce tissue-specific disease. Once stimulated, the activated T lymphocytes traffic to the target tissue, produce proinflammatory cytokines, and result in local damage(Fig. 8.1) [6]. Effector T cells are often characterized as either T-helper cell type 1 or type 2 (Th1 or Th2) by their selective patterns of cytokine secretion. Classically, the Th2 phenotype is observed in allergy or in conferring protection from autoimmune diseases whereas the Th1 phenotype has been linked to numerous autoimmune diseases [7]. The central role of CD4+ T cell polarized towards Th1 in EAU pathogenesis is confirmed by adoptive transfer experiments. In these studies, the tissue specific EAU is transferred to susceptible naive syngeneic recipients by uveitogenic, antigen-specific CD4+ T lymphocytes. Furthermore, EAU susceptibility was associated with a high Th1 effector response, whereas resistance was associated with a Th1-low response [8]. The critical role for T cells is also provided by experiments with athymic rats which are resistant to EAU.

Dynamic changes in lymphocyte subsets are observed through immunohistopathological studies of EAU involved eyes [9]. Early stages of S-Ag induced EAU is associated with an early T-helper phenotype. In contrast, T-suppressor cell abundance is very low during the initial inflammatory phases (less than 20 % of the total T lymphocytes), but continually increases in the recovery phase (50–67 % of the total T lymphocytes). These changes in the ratios between T-helper and T-suppressor cells during the different stages of EAU likely reflect the kinetics and regulation of the inflammatory response in autoimmune diseases.

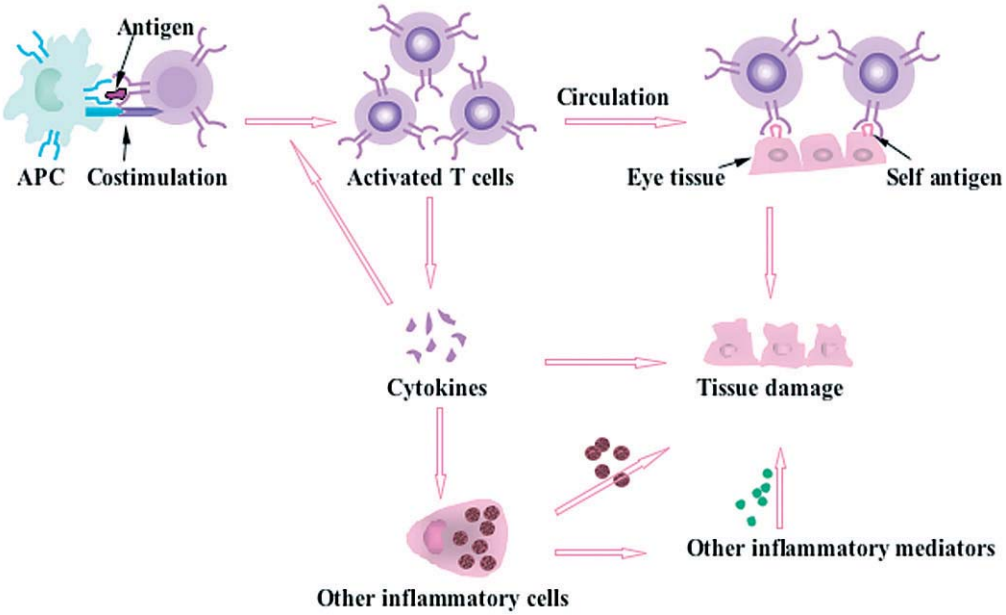

Fig. 8.1. Schematic of ocular tissue damage in uveitis

8.2.1.2 Uveitogenic Antigens in EAU

Multiple uveitogenic antigens are identified which induce EAU in susceptible animal species. Although the uveitogenicity of these proteins varies in different species, the ocular inflammatory disease, as observed both clinically and by histopathology, shares the essential features.

Retinal Soluble Antigen (S-Ag). S-Ag, the first purified uveitogenic antigen, is a 48-kDa intracellular protein localized to photoreceptor cells, and involved in the visual process [10]. Its function is believed to mediate rhodopsin-catalysed adenosine triphosphate binding and quench cyclic guanosine monophosphate phosphodiesterase (PDE) activation. It binds to photoactivated phosphorylated rhodopsin, preventing the transducin-mediated activation of PDE. Immunization with native S-antigen produces a severe inflammatory response which results in the complete destruction of the photoreceptor cell layer of the retina.

Interphotoreceptor Retinoid-Binding Protein (IRBP). IRBP is a large glycolipoprotein found in the interphotoreceptor matrix between the neural retina and retinal pigment epithelium. IRBP, which binds multiple retinoid and fatty acid ligands, is believed to play a role in retinoid transport between retinal photoreceptors and pigment epithelial cells [11]. This protein is highly uveitogenic in rats, mice and monkeys and multiple specific peptide fragments are also capable of inducing disease. In contrast to S-Ag induced EAU, IRBP immunization is associated with a more chronic disease with less vitreous inflammation.

Rhodopsin. Rhodopsin, a membrane protein of rod photoreceptor cells, is central to the visual pathway. This 40-kDa rod visual pigment and its less uveitogenic form, opsin, induces EAU in rats [12]. In high doses, rhodopsin produces a severe bilateral uveoretinitis which results in complete elimination of the photoreceptor cells.

Recoverin. Recoverin, a 23-kDa calcium-binding protein present in vertebrate photoreceptor cells, is believed to be involved in the termination

of the phototransduction cascade. It is the target of antibodies in the cancer-associated retinopathy syndrome [12, 13]. Immunization with recoverin can induce a severe panuveitis which closely resembles those induced by S-Ag [14]. EAU can be transferred to naive animals by lymphocytes from recoverin-immunized animals.

Phosducin. Phosducin is a 33-kDa phosphoprotein present in retinal photoreceptor cells which plays an important role in visual phototransduction. In high quantities, it is able to induce EAU, resembling the inflammation induced by S-Ag and recoverin [12].

RPE 65. RPE 65 is a 61-kDa protein, specifically and abundantly expressed in the retinal pigment epithelium (RPE) and associated with the microsomal fraction. RPE 65 appears to play an essential role in vitamin A metabolism. Mutations of RPE 65 were identified to be associated with Leber congenital amaurosis and retinitis pigmentosa. Immunization with this antigen induces an acute and severe ocular inflammation in rat, similar to that induced by the S-Ag [15].

8.2.1.3 Susceptibility to EAU

Genetic association of human uveitis with histocompatibility antigens, ethnicity, familial background, or gender suggests a genetic susceptibility factor. In animal uveitis models, susceptibility to EAU is strain associated, indicating an important genetic influence on disease development. EAU expression in mice or rats requires the presence of both a susceptible MHC haplotype and a "permissive" genetic background. Non-MHC genetic influences including T-cell repertoire, hormonal regulation, cytokine regulation and mast cell mediators are thought to play an important role in development of EAU and determine the immunoresponsive "permissiveness". Caspi and coworkers found that MHC control of susceptibility in H-2k mice is dependent on the I-A subregion of class II genes [8]. Their results showed that the presence of a susceptible I-A subregion (functional equivalent of the human HLA-DR) was both necessary and sufficient to permit EAU expression. These findings might help explain the complexity of uveitis susceptibility in association with specific HLA haplotypes.

8.2.1.4 Cytokines in EAU

Cells polarized towards a Th1 phenotype secrete interleukin-2 (IL-2) and interferon-γ (IFNγ), which are thought to be primarily responsible for cell-mediated inflammatory reactions, delayed-type hypersensitivity (DTH), and tissue injury in autoimmune diseases (Fig. 8.2). In contrast, Th2 cells produce IL-4, IL-5, IL-6, IL-9 and IL-10 and are efficient promoters of antibody responses. The imbalance between these two types of response is considered to be involved in the pathogenesis of autoimmune disease. Both susceptible as well as resistant animal strains initially mount a balanced Tho type response to the uveitopathogenic antigen [16]. However, EAU susceptibility is characterized by polarization of the early Tho response toward Th1 in susceptible strains and Th2 in resistant strains. Since cytokine production may play a critical role in the mechanism of human uveitis and biologic agents that interfere with cytokine action are becoming available as potential therapeutic agents, it is important to understand the role of various Th1 and Th2 cytokines in pathogenesis of EAU.

IFNγ. IFNγ is a homodimeric protein produced by NK cells, CD4+Th1 cells, and CD8+ T cells. It is the signature cytokine of the Th1 subset. IFNγ promotes the differentiation of naive CD4+ T cells to the Th1 subset and inhibits the proliferation of Th2 cells [17]. IFNγ can also stimulate expression of MHC class I, MHC class II, and costimulatory molecules on antigen presenting cells. Elevation of IFNγ was observed in eyes affected by EAU which may be directly responsible for the observed ocular overexpression of MHC class II molecules during experimental uveitis. Additionally, levels of IFNγ expression following antigenic challenge are higher in EAU-susceptible as compared to EAU-resistant species.

Further evidence for the importance of this molecule in uveitis is provided by studies using transgenic rats with constitutive overexpression

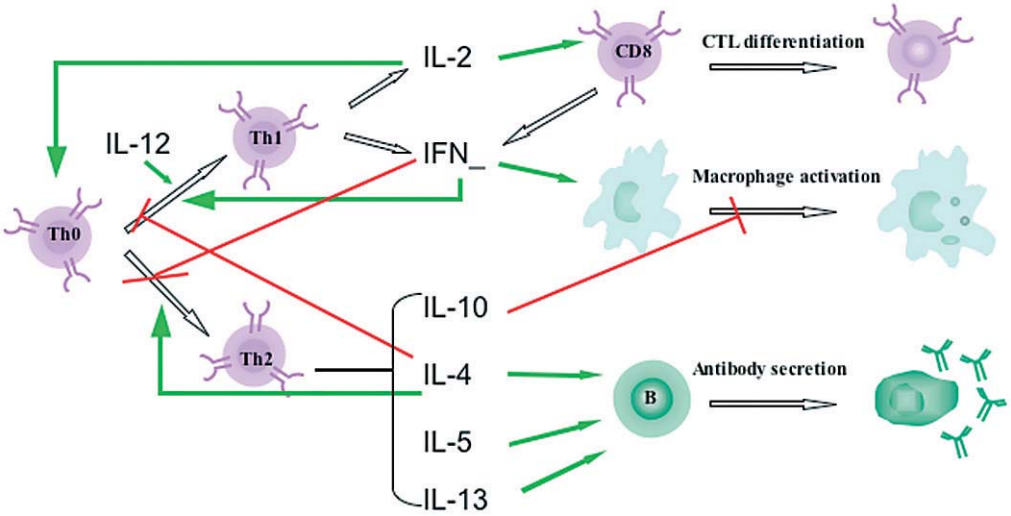

Fig. 8.2. Simplified diagram of major immunopathogenic mechanisms and cytokines in immune-mediated disease

of IFNγ in the eye [18]. In these transgenic rats IFNγ increases both the severity and acceleration of the onset of experimental autoimmune uveitis. However, studies using cytokine deficient mouse strains provide conflicting evidence for the role of IFNγ in EAU pathogenesis. However, studies using IFNγ knockout mice(IFNγ−/−) provide evidence for additional pathogenic pathways in uveitis development. This mouse strain does not promote the development of a Th1 response, yet it may develop EAU following an appropriate antigenic stimulus. Strong MHC class II expression is apparent in the eyes of IFNγ−/− mice with EAU, indicating alternative pathways for MHC upregulation. IFNγ−/− mice exhibited an antigen-specific effector response with upregulation of IL-5, IL-6 and TNF-α in the uveitic eyes. There was no significant increase in IL-4 and no upregulation of inducible nitric oxide synthase (iNOS) in these studies. Therefore, tissue damage in the IFNγ−/− mice appears to be mediated by a different mechanism than in the wild-type [19]. These seemingly conflicting results in the overexpressing rat strain and the knockout mouse strain may indicate distinct roles for IFNγ in immunomodulatory pathways in mice and rats during uveitis or may simply reflect redundancy of the proinflammatory pathway.

IL-2. IL-2 is a growth factor for antigen-stimulated T lymphocytes and is responsible for T-cell clonal expansion after antigen recognition. IL-2 is produced by activated CD4+ T lymphocytes and, in lesser amounts, by CD8+ T cells. IL-2 production is transient, with peak secretion occurring about 8–12 h after activation. The IL-2 receptors (IL-2R) consist of three non-covalently associated proteins: α, β and γ. The α and β chains are involved in cytokine binding, and β and γ chains are involved in signal transduction. Expression of functional IL-2R is enhanced by antigen stimulation. In EAU, upregulation of the IL-2R is observed and believed to be an important step in the pathogenesis of disease. Therapeutic use of antibodies against the IL-2R in EAU results in the decrease of acute ongoing inflammation in the eye. In addition, use of low dose IL-2 with small quantities of antigen may enhance the induction of oral tolerance in the EAU model [20].

IL-4. IL-4 is the major stimulus for the production of Ig E antibodies, development of Th2 cells from naive CD4+ T cells, and functions as an autocrine growth factor for differentiated Th2 cells. In EAU, an inverse relationship between IL-4 and IFNγ is observed and it is hypothesized

that IL-4 may exert a dose-dependent differential effect on the induction of immune responses and on autoimmunity. Experimentally, administration of IL-4 shifts an autoimmune response towards a non-pathogenic Th2 pathway [21]. In addition, evidence for a role of IL-4 in suppressing autoimmunity is supported by increased IL-4 mRNA in orally tolerized mice.

IL-6. In animal models of EIU, conflicting evidence supports the role of IL-6 as a proinflammatory mediator in ocular inflammatory disease [22, 23]. Upregulation of IL-6 in the anterior chamber is reported during EIU and levels of IL-6 positively correspond to cellular infiltration; however, IL-6 was not sufficient to induce uveitis and neither did the absence of IL-6 prevent uveitis induction. Studies of anterior chamber-associated immune deviation (ACAID) demonstrate reduced ACAID after exposure to IL-6 and increased aqueous humor immunosuppression following IL-6 depletion [24].

IL-10. IL-10 inhibits activated macrophages and dendritic cells and is involved in the control of cell-mediated immunity. IL-10 can inhibit the production of IL-12, and expression of both costimulatory and MHC class II molecules on macrophages and dendritic cells. Studies indicate that endogenous expression IL-10 limits development of EAU and, reciprocally, IL-10-deficient mice are susceptible to EAU [21, 25, 26]. Both IL-4 and IL-10 are required for induction of protective oral tolerance, providing additional evidence for the protective role of IL-10 in EAU.

IL-12. IL-12 is a key inducer of cell-mediated immunity. The principal sources of IL-12 are activated mononuclear phagocytes and dendritic cells. Although IL-12 was originally identified as an activator of NK cell cytolytic function, one of its critical actions is to stimulate IFNγ production by T cells and NK cells and to polarize differentiation of CD4+ helper T lymphocytes into IFNγ-producing Th1 cells. An elevated level of IL-12 is observed in the ocular tissues of EAU animal species, supporting a role for IL-12 in EAU pathogenesis. Studies to evaluate the role for endogenous IL-12 were performed using IL-12 p40-deficient mice [27]. These mice were resistant to EAU; however, they were able to develop a Th2 polarized antigen-specific response to the inciting uveitogenic antigen. In contrast, EAU developed in these animals after receipt of syngeneic, antigen-specific cells that had been incubated with antigen in the presence of IL-12. These findings support the critical role for IL-12 in the induction phase of EAU and that resistance to EAU may involve the inability to develop a pathogenic Th1 response in response to antigenic stimuli.

IL-13. IL-13 is a pleiotropic cytokine produced by Th2 CD4+ T cells. IL-13 inhibits the synthesis of proinflammatory cytokines IL-1, IL-6, IL-8 and TNF-α, and induces B-cell proliferation, IgE production, and expression of certain adhesion molecules on endothelial cells. IL-13 does not exhibit an autocrine affect and therefore has no direct action on the T cells. However, by inhibiting the production of IL-12 and IFNγ, IL-13 may indirectly prevent the active development of the Th1 lymphocyte pathway. Use of IL-13 in uveitis therapy was studied in a monkey model of EAU. At the onset of EAU in monkey, IL-13 was injected subcutaneously once a day for 28 days [28]. Dramatic improvement of the inflammatory signs of uveitis was observed in the IL-13 treated group as compared to the controls. This study suggests a potential therapeutic role for IL-13.

Th1 and Th2 cytokines have been implicated in pathogenesis, recovery, and resistance of EAU. Many experimental evidences point to an important role of a Th1 type response in the pathogenesis of autoimmune uveitis. However, the Th2 type response may contribute to the spontaneous termination of EAU and resistance to autoimmune response. New approaches for the immunotherapy of inflammatory autoimmune disease are based on the observations in these experimental systems in which polarization of the pathogenic Th1 response towards an immunosuppressive, Th2 response is beneficial in disease prevention or reduction.

8.2.1.5 Adhesion Molecules in EAU

Cell adhesion molecules are surface proteins that mediate cell binding, and expression of these molecules can promote leukocyte migration into areas of ocular inflammation [29, 30]. Cell adhesion molecules are divided into three structural groups; selectins, integrins, and immunoglobulin gene superfamily. Selectins appear to mediate the initial adhesion of inflammatory cells to the vascular endothelium, leading to a rolling of the cells along the vascular wall. Integrins and members of the immunoglobulin gene superfamily interact to form a more firm adherence between the leukocytes and vascular endothelium, leading to transendothelial immigration of the cells into the inflamed tissue. Cell adhesion molecules have been shown to play a critical role in the pathogenesis of EAU.

Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family and is expressed on the cornea, retinal pigment epithelium, capillary endothelium of the iris, ciliary body, choroid, and retinal glial cells. Lymphocyte function-associated antigen-1 (LFA-1), the counterreceptor for ICAM-1, is expressed on leukocytes and is involved in lymphocyte trafficking. An important role of ICAM-1 and LFA-1 in EAU is suggested by experimental studies [31, 32]. ICAM-1 is strongly expressed on the vascular endothelium of ciliary body and retina within 7 days following immunization, thus preceding definitive histopathologic evidence of inflammatory cells in the eye by 4 days. Levels of adhesion molecules both increase progressively during EAU development and correlate with the histologic grade of ocular inflammation. Localization and upregulation of the adhesion molecules ICAM-1 and P-selectin in retinal vein and venules in EAU may contribute to the specific adhesion of activated leukocytes in these vessels and subsequently the breakdown of the blood-retinal barrier (BRB), promoting intraocular migration of inflammatory cells. Experimental evidence for a fundamental role for adhesion molecules in EAU is suggested by studies using monoclonal antibodies against cell adhesion molecules. In vivo treatment with antibodies against ICAM-1 and LFA-1 reduced or prevented disease development in both IRBP and S-antigen-induced EAU models. Mechanistically there is evidence for EAU inhibition through interference with immunization and antigen sensitization as well as inhibition of leukocyte migration into the eye [31].

8.2.1.6 Mechanisms of Tissue Damage

Histopathologic and immunopathologic studies indicate that both antigen-specific and nonspecific inflammatory cells penetrate ocular tissue in EAU. T-cell infiltration is the dominant feature in the posterior segment tissue lesions; however, the total cellular infiltrate in EAU varies according to both the species and antigens used. In rats, an acute inflammatory infiltrate characterized by a prominent early neutrophil influx is often observed, whereas a more chronic, granulomatous inflammation is typically observed in monkeys and mice. Cell recruitment depends on local production of T-cell production of lymphokines, which induce adhesion molecules on retinal vascular endothelium and chemoattractants, thus establishing a chemotactic gradient. Recruited cells then amplify this process by contributing their own products, thus fueling an escalating inflammatory cascade [33]. Central to recruitment of circulating inflammatory cells in EAU is the BRB [29]. Early in EAU, upregulation of adhesion molecule expression facilitates the BRB breakdown with resultant leukocyte migration into the eye. An alternative source of BRB breakdown are mast cell derived mediators, which are thought to contribute to the pathogenesis of EAU.

Active oxygen products are believed to play an important role in the tissue damage observed in EAU. Retinal lipid peroxidation products, measured at various stages of EAU, are closely related to both the cellular infiltrate and to retinal tissue damage. Peroxynitrite is observed to concentrate in the photoreceptors but is also present in some inner retinal areas, including ganglion cell layers, nerve fibre layers, and retinal blood vessels, correlating with the

local pathologic oxidation [34]. Nitric oxide is an important mediator of inflammation and iNOS is found to be strongly expressed by infiltrating macrophages during the acute inflammatory stages of EAU. Experimentally, the critical role for iNOS in EAU pathogenesis is suggested by decreased EAU susceptibility after inhibition of iNOS in rats. However, the iNOS knockout mice are fully susceptible to EAU, suggesting alternative pathways in disease pathogenesis [35]. These findings underscore the concept of redundant pathways in mechanisms of oxidative tissue damage.

Summary for the Clinician

- **EAU is an animal model of posterior uveitis**
- **Retinal S-Ag and IRBP are the main uveitogenic antigens in EAU**
- **EAU expression requires the presence of both a susceptible MHC haplotype and a "permissive" genetic background**
- **CD4+ T-lymphocyte-mediated immune response play an important role in the pathogenesis of EAU**
- **Th1 pathway cytokines such as IL-2, IL-12, and IFNγ play central roles in EAU pathogenesis**
- **Intraocular infiltration of inflammatory cells, BRB breakdown, and active oxygen products are involved in the tissue damage in EAU**
- **Determination of relevant immunopathogenic mechanisms of EAU may help identify potential therapeutic targets for treatment of human uveitis syndromes**

8.2.2 Endotoxin-Induced Uveitis (EIU)

EIU, an acute monophasic inflammatory response, is induced by the systemic injection of endotoxin in the rat and mouse, and has been used as an animal model for the human acute anterior uveitis. Within 6 h following endotoxin exposure, an influx of cells and protein is seen within the aqueous humor. This reaction increases, achieving a peak at 24 h, with gradual resolution. Histopathologic analysis reveals a prominent iridocyclitis, characterized by macrophages, polymorphonuclear cells, and lymphocytes near the iris-ciliary body and the vitreous body [36]. Recently, a biphasic ocular inflammatory response to endotoxin was observed in a C3H/HeN mouse model [37]. In this model, the first inflammatory wave appears at day 1, subsides by day 3, and a second inflammatory wave is observed beginning on day 5. The initial influx is characterized by neutrophils and sparse macrophages in the posterior vitreous and iris. This is accompanied by a sharp peak of ocular IL-6 mRNA, serum IL-6 levels, and elevation of the aqueous protein level. This inflammation resembles an acute form of human anterior uveitis. The second wave is characterized by significant macrophage infiltration present in the anterior segment, accompanied by neutrophils and a few cytotoxic CD8+ T cells. This is similar to the cellular profile of human subacute anterior uveitis. High levels of ocular TNF-α, IL-1α, and GM-CSF message are found in this second wave. The level of aqueous protein is lower and IL-6 is absent, indicating that two inflammatory phases of EIU are mediated by different cytokines and inflammatory cells.

8.2.3 Experimental Melanin-Protein-Induced Uveitis (EMIU)

EMIU (previously termed experimental autoimmune anterior uveitis) can be effectively induced by immunization with bovine ocular melanin protein in Lewis rats. EMIU is a CD4+ T-cell-mediated autoimmune uveitis, resembling non-infectious recurrent iridocyclitis and choroiditis in humans [38]. The most remarkable feature of EMIU in contrast to other animal uveitis models is that it involves the choroid and iris, but usually spares the retina, thus mimicking relevant forms of human anterior uveitis. At the onset of EMIU, lymphocytes virtually constitute the predominant infiltrating cells in the uvea, the anatomic site of melanocytic antigen expression. Choroiditis is variable and ranges from mild to moderate; however, the iritis is typically severe. The typical course of EMIU has an acute-phase anterior uveitis and choroiditis

2–3 weeks after immunization, and subsides within 1 month. Spontaneous recurrence may occur in some animals.

8.2.4 Experimental Autoimmune Pigment Epithelial Membrane Protein-Induced Uveitis (EAPU)

EAPU occurs following immunization with uveitogenic pigment epithelial polypeptide (PEP)-65, PEP-43, or PEP28/30 isolated from the microsomal fraction of the RPE cells. The histopathological pattern of onset and progression of EAPU differ markedly from EAU and EMIU. EAPU is mainly characterized by a pigment epithelitis. Accumulation of a massive number of macrophages is observed along both sides of the RPE-Bruch's membrane layer, which is destroyed by the inflammatory response. However, inflammatory foci within the neuroretina are virtually absent. The immunopathogenesis of EAPU appears to be macrophage dependent whereas those in EMIU and EAU are not. The macrophages can secrete many products that may play multiple roles in non-specific inflammatory reactions and may lead to damage of the normal cellular structures. Effector molecules produced by activated macrophages include eicosanoids, reactive oxygen species, proteinases, IL-1, IL-6, and TNF-α. These agents are capable of damage to the blood-ocular barrier and attract and activate immunocompetent cells [39].

8.3 Immune Mechanisms in Human Uveitis

Studies of specific human uveitis syndromes provide additional support for the complex immunopathogenic mechanisms in uveitis. Although multiple significant studies identify candidate uveitogenic antigens as well as the cellular and cytokine components of active inflammation, this chapter focuses on mechanisms in HLA-associated uveitis and the specific uveitis syndromes of Behçet's disease and VKH syndrome.

8.3.1 HLA-Associated Human Uveitis

The major histocompatibility complex, MHC, produces two major categories of proteins, called class I and class II and represented in the human by the HLA antigens. MHC class I molecules, HLA-A, HLA-B, and HLA-C, are ubiquitously expressed in all cells and are responsible for presenting antigens to CD8+ lymphocytes. MHC class II molecules, HLA-DR, HLA-DQ, and HLA-DP, have a limited expression profile and present antigen to CD4+ T cells. Genetic susceptibility to a variety of immune-mediated diseases is linked to specific HLA alleles.

8.3.1.1 HLA-B*27-Associated Uveitis

HLA-B*27-associated uveitis, typically an acute anterior uveitis (AAU), is frequently associated with systemic diseases including ankylosing spondylitis, reactive arthritis, psoriatic arthritis, and inflammatory bowel disease. Approximately 50 % of patients with AAU will be HLA-B*27 positive, which is a predictor of disease severity. HLA-B*27 consists of 24 subtypes, which are encoded by 26 different alleles. The HLA-B*27 subtypes vary in prevalence among different ethnic populations, and some subtypes are more highly associated with risk of eye disease. HLA-B*2705 is definitely associated with uveitis, and is the most common associated type in North America. HLA-B*2704 is the more common subtype in Chinese and Japanese populations, and is also associated with uveitis. However, some subtypes, for example HLA-B*2706 (predominant in Asians) and HLA-B*2709 (more common in Sardinians), may not be associated with uveitis. Studies in HLA-B*27 transgenic animals have not supported a role for HLA-B*27 in susceptibility to infectious uveitis in a rat model and decreased the incidence of but increased the severity of IRBP-induced uveitis in a mouse strain [40, 41]. Susceptibility to uveitis is complex and likely reflects the presence of the specific HLA-B*27 subtypes, in combination with other genetic and environmental factors.

8.3.1.2 HLA-A29 and Birdshot Retinochoroidopathy

The strongest association between HLA and human disease is that of HLA-A29 and birdshot retinochoriodopath [42]. The HLA-A29 phenotype is found in more than 90 % of individuals with birdshot retinochoroidopathy and suggests a primary and direct role for the HLA-A29 molecule in disease pathogenesis. The sensitivity and specificity of HLA-A29 phenotype in this uveitis syndrome have been estimated as high as 96 % and 93 %, respectively. In support for a direct role of HLA-A29 and uveitis pathogenesis, HLA-A29 transgenic mice developed a spontaneous retinopathy, showing a striking resemblance to birdshot retinochoriodopathy [43].

8.3.1.3 HLA-B*51

Although the aetiology and pathogenesis of Behçet's disease remains unknown, HLA typing of affected individuals revealed a strong association with HLA-B*51 [44, 45]. HLA-B*51 was detected in 50–80 % of patients with Behçet's disease in many ethnic populations from the Middle East to Japan, including Turkish, Greek, Italian, French, Tunisian, Saudi Arabian, Israeli, Chinese, Korean, and Japanese, as compared to its presence in only 10–20 % of unaffected controls. Studies using HLA-B*51 transgenic mice demonstrate immune dysregulation characterized by a heightened neutrophil response with increased production of superoxide, therefore supporting a possible role in immune pathogenesis [46]. However, uveitis and more specifically the typical lesions observed in Behçet's disease are not observed in these transgenic animals. It is likely that HLA-51 in combination with other genetic and environmental factors may predispose to ocular inflammatory and systemic autoimmune diseases.

8.3.1.4 HLA-DQ and HLA-DR Alleles and Tubulointerstitial Nephritis and Uveitis Syndrome (TINU)

Although TINU is uncommon, several HLA associations have been reported [47]. HLA-A24 is found in 67 % of Spanish patients and 75 % of Japanese patients. TINU has also been associated with specific HLA class II HLA-DR and -DQ alleles. In a study of 18 affected patients, the HLA-DQA1*01/DQB1*05/DRB1*01 haplotype was found in 13 TINU patients, suggesting the high association of the HLA-DQA1*01, -DQB1*05, and -DRB1*01 alleles with TINU. This observation suggests an importance for HLA class II antigens, expressed in both renal epithelial cells and inflamed uvea, in autoimmune pathogenesis.

8.3.1.5 Immunogenetics in Vogt-Koyanagi-Harada Syndrome

Strong HLA associations are observed in the VKH syndrome. In affected Japanese patients, 88 % of patients demonstrated the HLA-DR4 antigen, and HLA-B*53 was present in all the patients [48]. HLA-DRB1*0405 and HLA-DRw53 are also associated with multiple ethnic groups.

Summary for the Clinician

- **HLA-B*27-associated uveitis has frequent associations with systemic disease**
- **HLA-A29 is the strongest association of a specific HLA with a human disease syndrome, birdshot retinochoroidopathy**
- **HLA-B*51 is strongly linked with Behçet's disease**
- **Both HLA class I and HLA class II antigens may be susceptibility markers for human inflammatory diseases**

8.3.2 Behçet's Disease

Behçet's disease is a refractory multisystemic autoimmune inflammatory disorder characterized by recurrent and episodic uveitis, oral ulcers, genital ulcerations and skin lesions [45]. The prevalence of ocular involvement in Behçet's disease is high, occurring in 50–70 % of affected men and 20–30 % of affected women, and it is responsible for significant morbidity. Behçet's disease may produce permanent loss of vision in up to 20 % of affected individuals [49]. Although the pathogenesis is uncertain, recent studies suggest a role for neutrophil hyperfunction, vasculitis with endothelial injuries, and aberrant T-lymphocyte responses in its pathophysiology.

The histopathologic appearance in Behçet's disease is characterized by a non-granulomatous inflammation with perivascular T lymphocytes and neutrophil infiltration and upregulation of expression of multiple adhesion molecules. The neutrophil infiltrate is hypersensitive and believed to be "primed" for inflammation. There is rapid activation observed to proinflammatory signals and increased expression of proinflammatory cytokines, including TNF-α, IL-1β and IL-8 [50]. The neutrophil hypersensitivity is believed to result from genetic factors such as HLA-B*51, which as noted appears to be associated with intrinsic abnormalities of neutrophils [46]. Vascular involvement with accompanying thrombosis is a Behçet's disease characteristic that is not typically seen in other uveitis syndromes [51]. Local expression of proinflammatory cytokines, increased circulating immune complexes (CICs), endothelial dysfunction, and an abnormal coagulation system are important to pathogenesis in the Behçet's disease-associated vasculitis.

8.3.2.1 Cellular Immunological Abnormalities

T-cell-mediated immune responses play a central role in the pathogenesis of Behçet's disease. Evidence suggests a decrease in the CD4+ T cells and an increase in CD8+ T cells, therefore decreasing the CD4+/CD8+ T cell ratio and the presence of a suppressor regulatory defect. Studies of circulating antibodies in patients with Behçet's disease demonstrate increased levels of HSP-specific antibodies. T-cell stimulation studies, using multiple different antigens including heat shock protein (HSP)-derived peptides, S-antigen, and IRBP, used peripheral blood lymphocytes from affected patients and controls [52]. These studies reveal increased T-cell reactivity against the 60-kDa HSP, which has significant homology with the mycobacterial 65-kDa HSP, in a disease subset of patients with Behçet's disease. This result is intriguing as it raises the possibility for molecular mimicry, or an immunologic cross-reaction between human and microbial HSP, in disease pathogenesis.

The autoimmune lymphocytes in patients with Behçet's disease are reported to be resistant to Fas-mediated apoptosis [53]. Apoptosis of these inflammatory cells, mediated by the expression of Fas and FasL, is considered as an important mechanism leading to prompt rapid resolution of inflammation in the ocular microenvironment. Disturbed expression of Fas and FasL may contribute to T-cell longevity in Behçet's disease and may play an important role in the chronic and recurrent intraocular inflammation.

8.3.2.2 Cytokines

It has been hypothesized that polarization towards a Th1-type T-lymphocyte response is involved in the pathogenesis of Behçet's disease based on observations of upregulated expression of proinflammatory, Th1-type cytokines. However, other studies support a role for a Th2 response in Behçet's disease pathogenesis. Increased levels of IL-4, IL-10 and IL-13 are observed in the sera of patients with active Behçet's disease [54, 55]. IFNγ, TNF-α, TNF receptor (TNFR-75), IL-1, IL-2, soluble IL-2 receptor (sIL-2R), IL-8, and IL-12 are all reported to be significantly higher in the sera of affected patients [50]. In addition, a subset of cytokines, including sIL-2R, IL-8, IL-12 and TNFR-75, may serve as markers of disease activity [56]. In

studies of lymphocyte apoptosis, recombinant IL-12 decreased Fas-induced lymphocyte apoptosis and enhanced the proliferation of Th1 T lymphocytes [57]. These findings, both from in vivo and in vitro studies, suggest that high IL-12 levels increase both the survival and proliferation of autoreactive Th1 lymphocytes leading to disease progression. Exposure of peripheral blood lymphocytes to IFNγ produced decreases in IL-4 and IL-10 and an increase in IL-12, evidence for the Th1 polarization of the immune response.

8.3.2.3 Clinical Therapeutic Trials

Clinical trials can provide in vivo evidence for the importance of specific immunologic mechanisms in ocular inflammation diseases. Type I interferons (IFNα/β), which possess immunomodulatory, anti-proliferative, and immunosuppressive properties, have been studied in Behçet's disease. Recombinant human interferon alpha-2a, administered for severe ocular involvement, achieved a rapid clinical response within 2–4 weeks [58]. Although a successful response to therapy was observed in 92 % of treated patients, morbidity of treatment included depression, flu-like symptoms, alopecia, and leucopenia. These adverse effects were both dose dependent and reversible. Other studies confirmed significant efficacy of interferon alpha-2a in severe and refractory Behçet's disease-associated uveitis [59]. Following intramuscular IFNα/β administration, decreased levels of both intraocular IFNγ and IL-10 are observed, suggesting suppression of both Th1 and Th2 phenotypes. TNF-α is believed to play a pivotal role in Th1 T-cell-mediated disease. Direct use of TNF inhibitors also demonstrates clinical success in chronic, resistant Behçet's disease [60]. These clinical studies help support the role for Th1 T lymphocytes in disease pathogenesis of Behçet's disease.

Summary for the Clinician

- **Behçet's disease is characterized by a non-granulomatous inflammation with perivascular T lymphocytes and neutrophil activation**
- **T-cell-mediated immune responses play a central role in the pathogenesis of Behçet's disease**
- **Interferon alpha-2a and TNF inhibitors are two biologic agents that have been used in clinical therapy for refractory and severe Behçet's disease**

8.3.3 Vogt-Koyanagi-Harada Syndrome

VKH syndrome is a bilateral, granulomatous panuveitis associated with poliosis, vitiligo, and alopecia with both central nervous system and auditory manifestations [61]. This inflammatory syndrome is considered to be a T-cell-mediated autoimmune disorder against a melanocytic antigen. Typical histopathological features, seen in the early phases of VKH, are a granulomatous T-cell inflammation that primarily involves the choroid, with similar milder inflammatory infiltration in the iris and ciliary body. The retina is preserved except at sites of Dalen-Fuchs nodules, aggregations of proliferating retinal pigment epithelial cells admixed with a few inflammatory cells. Damage of RPE cells is detected by fluorescein angiography as multiple pinpoint areas of leakage at the level of RPE. Disruption of RPE cells is confirmed through electron microscopic examination, by showing Müller cell processes with attachment to Bruch's membrane. Disappearance of choroidal melanocytes, phagocytosis of pigment, and lymphocytic infiltration is also observed [62].

Immunologic studies of ocular VKH syndrome show choroidal infiltration of T lymphocytes with an increased CD4+/CD8+ ratio in two cases [63]. Further investigations of T-lymphocyte subsets in the aqueous humor and peripheral blood indicate an increase in the number of activated memory T cells. Recent studies have centred largely on T-cell immune responses directed against specific candidate antigens in VKH patients. The major candidate autoantigens in VKH are from the tyrosinase family proteins, enzymes involved in melanin formation and specifically expressed in melanocytes. Specific antigenic stimulation, after exposure to specific tyrosinase-derived 30-mer peptides,

has been observed in lymphocytes from affected patients [64, 65]. Furthermore, T-cell clones established from patients with VKH syndrome and stimulated with tyrosinase family peptides demonstrated a predominately proinflammatory, Th1-type T-cell response. Additional experimental support for the uveitogenicity of these proteins comes from experiments with Lewis rats, in which peptide immunization induced ocular and extraocular changes that highly resembled VKH clinical disease manifestations. These important findings suggest that tyrosinase family proteins are the important and clinically relevant target antigens in VKH pathogenesis [65].

Immune responses against other retinal proteins are also reported in patients with VKH. Circulating antibodies from patients with VKH syndrome recognize photoreceptor outer segment, Müller cells and pigmented melanoma cells [66]. Studies of peripheral lymphocyte proliferation indicate that circulating lymphocytes in patients with VKH syndrome respond to S-antigen. These reactivities may reflect a primary anti-retinal immune response or alternatively may reflect a bystander response to antigens liberated during severe posterior pole inflammatory disease.

Summary for the Clinician

- **VKH syndrome is a granulomatous, T-cell-mediated inflammation**
- **HLA-DR4 and HLA-DRw53 have strong associations with VKH syndrome**
- **Antigen specific T lymphocytes against tyrosinase family proteins play a role in the pathogenesis of VKH syndrome**

Complex mechanisms with genetic susceptibility factors in conjunction with specific antigenic exposure in the setting of proinflammatory costimulatory agents result in generation of intraocular inflammatory disease. Studies that document specific mechanisms in both human disease and animal models help define disease pathogenesis and determine potential targets for therapeutic interventions.

References

1. Streilein JW (1995) Unraveling immune privilege. Science 270:1158–1159
2. Ohta K, Wiggert B, Taylor AW, Streilein JW (1999) Effects of experimental ocular inflammation on ocular immune privilege. Invest Ophthalmol Vis Sci 40:2010–2018
3. Willermain F, Caspers-Velu L, Nowak B, Stordeur P, Mosselmans R, Salmon I, Velu T, Bruyns C (2002) Retinal pigment epithelial cells phagocytosis of T lymphocytes: possible implication in the immune privilege of the eye. Br J Ophthalmol 86:1417–1421
4. Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA (1995) Fas ligand-induced apoptosis as a mechanism of immune privilege. Science 270:1189–1192
5. Boyd SR, Young S, Lightman S (2001) Immunopathology of the noninfectious posterior and intermediate uveitides. Surv Ophthalmol 46: 209–233
6. Caspi RR (1999) Immune mechanisms in uveitis. Springer Semin Immunopathol 21:113–124
7. Caspi RR (2002) Th1 and Th2 responses in pathogenesis and regulation of experimental autoimmune uveoretinitis. Int Rev Immunol 21:197–208
8. Pennesi G, Caspi RR (2002) Genetic control of susceptibility in clinical and experimental uveitis. Int Rev Immunol 21:67–88
9. Chan CC, Mochizuki M, Nussenblatt RB, Palestine AG, McAllister C, Gery I, Benezra D (1985) Lymphocyte-T subsets in experimental autoimmune uveitis. Clin Immunol Immunopathol 35:103–110
10. Wacker WB, Donoso LA, Kalsow CM, Yankeelov JA, Organisciak DT (1977) Experimental allergic uveitis – isolation, characterization, and localization of a soluble uveito-pathogenic antigen from bovine retina. J Immunol 119:1949–1958
11. Fox GM, Kuwabara T, Wiggert B, Redmond TM, Hess HH, Chader GJ, Gery I (1987) Experimental autoimmune uveoretinitis (EAU) induced by retinal interphotoreceptor retinoid-binding protein (IRBP): differences between EAU induced by IRBP and by S-antigen. Clin Immunol Immunopathol 43:256–264
12. Adamus G, Chan CC (2002) Experimental autoimmune uveitides: multiple antigens, diverse diseases. Int Rev Immunol 21:209–229
13. Keltner JL, Thirkill CE (1998) Cancer-associated retinopathy vs recoverin-associated retinopathy. Am J Ophthalmol 126:296–302
14. Gery I, Chanaud NP III, Anglade E (1994) Recoverin is highly uveitogenic in Lewis rats. Invest Ophthalmol Vis Sci 35:3342–3345

15. Ham DI, Gentleman S, Chan CC, McDowell JH, Redmond TM, Gery I (2002) RPE65 is highly uveitogenic in rats. Invest Ophthalmol Vis Sci 43:2258–2263
16. Sun B, Rizzo LV, Sun SH, Chan CC, Wiggert B, Wilder RL, Caspi RR (1997) Genetic susceptibility to experimental autoimmune uveitis involves more than a predisposition to generate a T helper-1-like or a T helper-2-like response. J Immunol 159:1004–1011
17. Agnello D, Lankford CS, Bream J, Morinobu A, Gadina M, O'Shea JJ, Frucht DM (2003) Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immunol 23:147–161
18. Egwuagu CE, Mahdi RM, Chan CC, Sztein J, Li W, Smith JA, Chepelinsky AB (1999) Expression of interferon-gamma in the lens exacerbates anterior uveitis and induces retinal degenerative changes in transgenic Lewis rats. Clin Immunol 91:196–205
19. Jones LS, Rizzo LV, Agarwal RK, Tarrant TK, Chan CC, Wiggert B, Caspi RR (1997) IFN-gamma-deficient mice develop experimental autoimmune uveitis in the context of a deviant effector response. J Immunol 158:5997–6005
20. Rizzo LV, Miller-Rivero NE, Chan CC, Wiggert B, Nussenblatt RB, Caspi RR (1994) Interleukin-2 treatment potentiates induction of oral tolerance in a murine model of autoimmunity. J Clin Invest 94:1668–1672
21. Rizzo LV, Morawetz RA, Miller-Rivero NE, Choi R, Wiggert B, Chan CC, Morse HC III, Nussenblatt RB, Caspi RR (1999) IL-4 and IL-10 are both required for the induction of oral tolerance. J Immunol 162:2613–2622
22. Rosenbaum JT, Kievit P, Han YB, Park JM, Planck SR (1998) Interleukin-6 does not mediate endotoxin-induced uveitis in mice: studies in gene deletion animals. Invest Ophthalmol Vis Sci 39:64–69
23. Miyamoto N, Mandai M, Suzuma I, Suzuma K, Kobayashi K, Honda Y (1999) Estrogen protects against cellular infiltration by reducing the expressions of E-selectin and IL-6 in endotoxin-induced uveitis. J Immunol 163:374–379
24. Ohta K, Yamagami S, Taylor AW, Streilein JW (2000) IL-6 antagonizes TGF-beta and abolishes immune privilege in eyes with endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 41:2591–2599
25. Rizzo LV, Xu H, Chan CC, Wiggert B, Caspi RR (1998) IL-10 has a protective role in experimental autoimmune uveoretinitis. Int Immunol 10:807–814
26. Sun B, Sun SH, Chan CC, Caspi RR (2000) Evaluation of in vivo cytokine expression in EAU-susceptible and resistant rats: a role for IL-10 in resistance? Exp Eye Res 70:493–502
27. Tarrant TK, Silver PB, Chan CC, Wiggert B, Caspi RR (1998) Endogenous IL-12 is required for induction and expression of experimental autoimmune uveitis. J Immunol 161:122–127
28. Roberge FG, de Smet MD, Benichou J, Kriete MF, Raber J, Hakimi J (1998) Treatment of uveitis with recombinant human interleukin-13. Br J Ophthalmol 82:1195–1198
29. Magone MT, Whitcup SM (1999) Mechanisms of intraocular inflammation. Chem Immunol 73:90–119
30. Rosenbaum JT, Becker MD, Smith JR (2000) Toward new therapies for ocular inflammation. Arch Soc Esp Oftalmol 75:511–514
31. Whitcup SM, DeBarge LR, Caspi RR, Harning R, Nussenblatt RB, Chan CC (1993) Monoclonal antibodies against ICAM-1 (CD54) and LFA-1 (CD11a/CD18) inhibit experimental autoimmune uveitis. Clin Immunol Immunopathol 67:143–150
32. Xu HP, Forrester JV, Liversidge J, Crane IJ (2003) Leukocyte trafficking in experimental autoimmune uveitis: breakdown of blood-retinal barrier and upregulation of cellular adhesion molecules. Invest Ophthalmol Vis Sci 44:226–234
33. Prendergast RA, Iliff CE, Coskuncan NM, Caspi RR, Sartani G, Tarrant TK, Lutty GA, McLeod DS (1998) T cell traffic and the inflammatory response in experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 39:754–762
34. Shimizu K, Wu GS, Sultana C, Kalra VK, Rao NA (1999) Stimulation of macrophages by retinal proteins: production of reactive nitrogen and oxygen metabolites. Invest Ophthalmol Vis Sci 40:3215–3223
35. Silver PB, Tarrant TK, Chan CC, Wiggert B, Caspi RR (1999) Mice deficient in inducible nitric oxide synthase are susceptible to experimental autoimmune uveoretinitis. Invest Ophthalmol Vis Sci 40:1280–1284
36. Avunduk AM, Avunduk MC, Oztekin E, Baltaci AK (2002) Characterization of T lymphocyte subtypes in endotoxin-induced uveitis and effect of pentoxifylline treatment. Curr Eye Res 24:92–98
37. Shen DF, Buggage RR, Eng HC, Chan CC (2000) Cytokine gene expression in different strains of mice with endotoxin-induced uveitis (EIU). Ocul Immunol Inflamm 8:221–225
38. McMenamin PG, Crewe J, Kijlstra A (1997) Resident and infiltrating cells in the rat iris during the early stages of experimental melanin protein-induced uveitis (EMIU). Ocul Immunol Inflamm 5:223–233

39. Broekhuyse RM, Huitinga I, Kuhlmann ED, Rooijen NV, Winkens HJ (1997) Differential effect of macrophage depletion on two forms of experimental uveitis evoked by pigment epithelial membrane protein (EAPU), and by melanin-protein (EMIU). Exp Eye Res 65:841–848
40. Baggia S, Lyons JL, Angell E, Barkhuizen A, Han YB, Planck SR, Taurog JD, Rosenbaum JT (1997) A novel model of bacterially-induced acute anterior uveitis in rats and the lack of effect from HLA-B*27 expression. J Investig Med 45:295–301
41. Willbanks GA, Rootman DS, Jay V, Wiggert B, Chamberlain J, Inman RD (1997) Experimental autoimmune uveitis in HLA-B*27 transgenic mice. Hum Immunol 53:188–194
42. Baarsma GS, Kijlstra A, Oosterhuis JA, Kruit PJ, Rothova A (1986) Association of birdshot retinochoroidopathy and HLA-A29 antigen. Doc Ophthalmol 61:267–269
43. Szpak Y, Vieville JC, Tabary T, Naud MC, Chopin M, Edelson C, Cohen JH, Dausset J, de Kozak Y, Pla M (2001) Spontaneous retinopathy in HLA-A29 transgenic mice. Proc Natl Acad Sci U S A 98: 2572–2576
44. Zierhut M, Mizuki N, Ohno S, Inoko H, Gul A, Onoe K, Isogai E (2003) Immunology and functional genomics of Behçet's disease. Cell Mol Life Sci 60:1903–1922
45. Verity DH, Wallace GR, Vaughan RW, Stanford MR (2003) Behçet's disease: from Hippocrates to the third millennium. Br J Ophthalmol 87:1175–1183
46. Takeno M, Kariyone A, Yamashita N, Takiguchi M, Mizushima Y, Kaneoka H, Sakane T (1995) Excessive function of peripheral blood neutrophils from patients with Behçet's disease and from HLA-B*51 transgenic mice. Arthritis Rheum 38:426–433
47. Levinson RD, Park MS, Rikkers SM, Reed EF, Smith JR, Martin TM, Rosenbaum JT, Foster CS, Sherman MD, Holland GN (2003) Strong associations between specific HLA-DQ and HLA-DR alleles and the tubulointerstitial nephritis and uveitis syndrome. Invest Ophthalmol Vis Sci 44:653–657
48. Yabuki K, Inoko H, Ohno S (2000) HLA testing in patients with uveitis. Int Ophthalmol Clin 40:19–35
49. Kural-Seyahi E, Fresko I, Seyahi N, Ozyazgan Y, Mat C, Hamuryudan V, Yurdakul S, Yazici H (2003) The long-term mortality and morbidity of Behçet syndrome: a 2-decade outcome survey of 387 patients followed at a dedicated center. Medicine (Baltimore) 82:60–76
50. Eksioglu-Demiralp E, Direskeneli H, Kibaroglu A, Yavuz S, Ergun T, Akoglu T (2001) Neutrophil activation in Behçet's disease. Clin Exp Rheumatol 19:S19–S24
51. Leiba M, Sidi Y, Gur H, Leiba A, Ehrenfeld M (2001) Behçet's disease and thrombophilia. Ann Rheum Dis 60:1081–1085
52. Kaneko S, Suzuki N, Yamashita N, Nagafuchi H, Nakajima T, Wakisaka S, Yamamoto S, Sakane T (1997) Characterization of T cells specific for an epitope of human 60-kD heat shock protein (hsp) in patients with Behçet's disease (BD) in Japan. Clin Exp Immunol 108:204–212
53. Yang P, Ji L, Zhou H, Huang X, Xie C, Jin H, Chen L, Kijlstra A (2001) Disturbed expression of Fas/FasL on CD4(+) and CD8(+)T cells in Behçet's disease, Vogt-Koyanagi-Harada syndrome, and idiopathic anterior uveitis. Ocul Immunol Inflamm 9:185–191
54. Aridogan BC, Yildirim M, Baysal V, Inaloz HS, Baz K, Kaya S (2003) Serum levels of IL-4, IL-10, IL-12, IL-13 and IFN-gamma in Behçet's disease. J Dermatol 30:602–607
55. Raziuddin S, al Dalaan A, Bahabri S, Siraj AK, al Sedairy S (1998) Divergent cytokine production profile in Behçet's disease. Altered Th1/Th2 cell cytokine pattern. J Rheumatol 25:329–333
56. Alpsoy E, Cayirli C, Er H, Yilmaz E (1998) The levels of plasma interleukin-2 and soluble interleukin-2R in Behçet's disease: a marker of disease activity. J Dermatol 25:513–516
57. Frassanito MA, Dammacco R, Cafforio P, Dammacco F (1999) Th1 polarization of the immune response in Behçet's disease: a putative pathogenetic role of interleukin-12. Arthritis Rheum 42:1967–1974
58. Kotter I, Gunaydin I, Zierhut M, Stubiger N (2004) The use of interferon alpha in Behçet disease: Review of the literature. Semin Arthritis Rheum 33:320–335
59. Wechsler B, Bodaghi B, Huong DL, Fardeau C, Amoura Z, Cassoux N, Piette JC, LeHoang P (2000) Efficacy of interferon alfa-2a in severe and refractory uveitis associated with Behçet's disease. Ocul Immunol Inflamm 8:293–301
60. Sfikakis PP, Kaklamanis PH, Elezoglou A, Katsilambros N, Theodossiadis PG, Papaefthimiou S, Markomichelakis N (2004) Infliximab for recurrent, sight-threatening ocular inflammation in Adamantiades-Behçet disease. Ann Intern Med 140:404–406
61. Read RW (2002) Vogt-Koyanagi-Harada disease. Ophthalmol Clin North Am 15:333–341
62. Inomata H, Rao NA (2001) Depigmented atrophic lesions in sunset glow fundi of Vogt-Koyanagi-Harada disease. Am J Ophthalmol 131:607–614
63. Kahn M, Pepose JS, Green WR, Miller J, Foos RY (1993) Immunocytologic findings in a case of Vogt-Koyanagi-Harada syndrome. Ophthalmology 100:1191–1198

64. Gocho K, Kondo I, Yamaki K (2001) Identification of autoreactive T cells in Vogt-Koyanagi-Harada disease. Invest Ophthalmol Vis Sci 42:2004–2009
65. Yamaki K, Gocho K, Hayakawa K, Kondo I, Sakuragi S (2000) Tyrosinase family proteins are antigens specific to Vogt-Koyanagi-Harada disease. J Immunol 165:7323–7329
66. Chan CC, Palestine AG, Nussenblatt RB, Roberge FG, Benezra D (1985) Anti-retinal auto-antibodies in Vogt-Koyanagi-Harada syndrome, Behçet's disease, and sympathetic ophthalmia. Ophthalmology 92:1025–1028

The Tip of the Iceberg: Current Knowledge of Uveitis in Juvenile Arthritis

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Core Messages

- Uveitis is a serious extra-articular manifestation of juvenile chronic arthritis (JCA). It is a particular challenge because of its indigenous onset, high complication rate and risk of being a potentially blinding disorder at a young age
- Screening of children with JCA for uveitis and identifying confounding factors that correlate with intraocular inflammation remains an important task for the reduction of the risk of severe ocular damage
- Whereas gender (girls) and type of arthritis (oligoarthritis) have been previously suggested as risk factors for uveitis, recent prospective studies could not confirm this and indicate the need for refinements in screening efforts
- Genetic factors that play a role in the susceptibility for uveitis in JCA include: HLA-DR5, HLA-DP 2.1 and HLA-B*27, whereas HLA-DR 1 and HLA-DR 4 are negatively associated with intraocular inflammation
- A variety of autoantigens including antinuclear antibodies, anti-histone 3, collagen type II and heat shock protein 60 (hsp60) have been suggested to play a role in JCA-associated uveitis, but still need to be confirmed in independent studies
- Conditions that are often predictive of poor outcome due to uveitis include: gender (boys), early onset of arthritis and short time interval between onset of arthritis and uveitis and the severity of intraocular inflammation at the first presentation
- The role of cytokines in JCA is an active field of interest. TNF-α, INF-γ and their receptors are significantly elevated in synovial tissue of children with JCA. New treatment options directed against these cytokines have been successfully introduced for arthritis; however, their value for uveitis is not yet clear

9.1 Introduction

Uveitis remains a disorder that accounts for approximately 10–15% of blindness. It manifests predominantly in young adults, and 6–10% of all patients are children under the age of 16 years [31, 42]. Uveitis in children remains a particular challenge for physicians because of the often indigenous nature of intraocular inflammation, difficulties in communication with the patient and the high risks of side effects with local and systemic immunomodulating treatment in a developing individual. The spectrum of disorders associated with intraocular inflammation in children includes almost all entities that are known in adults; however, there are noteworthy aspects that differ and need particular attention. By far the most common cause of chronic intraocular inflammation in children is juvenile rheumatoid arthritis (JRA), or juvenile chronic arthritis (JCA) as it is defined (see below) in Europe. Even when JRA/JCA is a heterogeneous disease, uveitis in these children represents a distinctive and enigmatic entity. An

association of uveitis with juvenile arthritis has been suspected since the first report by Ohm [47]; however, it was not firmly established before 1950, when Vesterdal summarized his findings in 34 patients [73]. Since then the association of arthritis and uveitis in the paediatric population has been well established and the clinical and serologic correlates better defined. In addition, our understanding of the pathogenesis and clinical management has improved. This update focuses on uveitis in children with JRA/JCA and discusses the management of its complications.

9.2 Classification of Arthritis

Chronic arthritis in children is a heterogeneous group of disorders that has been repeatedly classified. "Juvenile rheumatoid arthritis" (JRA), which affects up to 120,000 children in the US, has been defined by the American College of Rheumatology (ACR) as chronic peripheral arthritis of at least 6 weeks duration with onset before the age of 16 years, in the absence of any other cause [2]. Three subsets of arthritis have been differentiated: the oligoarticular type accounting for 50–60 % of patients with up to four joints involved, the polyarticular type (20–30 % of patients) affecting five or more joints and systemic disease characterized by fever, rash, lymphadenopathy and splenomegaly.

The European League Against Rheumatism (EULAR) classification proposed the term "juvenile chronic arthritis" that includes systemic JCA (11 %), pauciarticular JCA (50 %), rheuma factor negative polyarticular disease (17 %), juvenile onset seronegative spondyloarthropathies (8 %) [e.g. ankylosing spondylitis (2 %), Reiter's syndrome (1 %)], and psoriatic arthritis (7 %) and excludes rheuma factor-positive polyarthritis (3 %) [27, 69]. For better comparison these two classifications are summarized in Table 9.1.

Recently, the International League against Rheumatism (ILAR) has defined seven subgroups of *juvenile idiopathic* arthritis according to certain clinical and immunological criteria [27]. Since most of the studies regarding uveitis were previously performed in children classified according to either the ACR or the EULAR criteria so far, the ILAR criteria are not further described here.

9.3 Epidemiology

Independent of the different classification systems (ACR or EULAR) the prevalence and incidence of juvenile arthritis seems not to be significantly affected. The prevalence rates of arthritis range from 100/100,000 to 220/100,000 and the overall incidence of chronic inflamma-

Table 9.1. American and European criteria for the classification of juvenile arthritis

Classification	American College of Rheumatology (ACR)	European League Against Rheumatism (EULAR)
Nomenclature	JRA	JCA
Age at onset (years)	<16	<16
Minimal duration of arthritis	6 weeks	3 months
Subtypes	Systemic Polyarticular (Oligoarticular) Pauciarticular	Systemic Polyarticular (Oligoarticular) Pauciarticular
Positive rheumatoid factor	Included as JRA	Excluded as JCA
Negative rheumatoid factor	Included	Included
Spondyloarthropathies	Excluded	Included
Other diseases excluded	Yes	Yes

Table 9.2. Features of JRA/JCA according to disease course during the first 6 months

	Pauciarticular	Polyarticular	Systemic
Frequency of cases	50%	20–30%	10%
Number of joints involved	<4	>4	Variable
Age at onset	Early childhood	Throughout childhood	Throughout childhood
Sex ratio (F:M)	5:1	3:1	1:1
Systemic involvement	None	Moderate	Prominent
Chronic anterior uveitis	20%	5%	Rare
Rheumatoid factor present	Rare	10%	Rare
Antinuclear antibody present	75–85%	40–50%	10%
Articular and overall prognosis	Good to excellent	Fair to good	Poor to good
Ocular prognosis	Variable	Fair	Variable

tory arthritis is estimated to be as high as 25 per 100,000/year [4, 30, 32].

The ranges of prevalence and annual incidence of uveitis in JCA are 8–11/100,000 and 1.5–2/100,000 respectively [6, 21, 32, 35].

It is noteworthy that the rate of severe visual impairment is up to one-third in children with JRA/JCA and chronic uveitis may end with blindness in 6–25% of affected children [11, 13]. It is of further interest that the percentage of children with chronic intraocular inflammation seems to vary according to the subtype of JRA/JCA (Table 9.2).

9.4 Pathogenesis

The exact pathogenesis of intraocular inflammation associated with JRA/JCA is not known. Whereas certain clinical characteristics of children with JCA and uveitis can be identified, the aetiology of the coexistence of ocular and joint inflammation remains obscure [63]. The susceptibility to developing juvenile arthritis as an autoimmune disorder is thought to be influenced by largely unidentified genetic and environmental factors.

The role of *genetic factors* has been intensively investigated and considered to play an important role in the susceptibility for JCA [1]. In addition, there exists a relationship of HLA antigens for the development of uveitis in children with JCA (see Sect. 8 below). HLA-DR5 has been associated with uveitis in JCA children as well as HLA-DP 2.1 [56, 65]. In contrast, HLA-DR 1 and HLA-DR 4 have been negatively associated with intraocular inflammation [32, 40]. It has been suggested that HLA antigens are directly involved in disease susceptibility [65]. Whether inciting agents such as infectious organisms may induce an autoimmune response in a genetically predisposed child is still a matter of debate. Earlier reports on the detection of bacteria could not been confirmed [51, 76].

However, a variety of *autoantigens* has been suggested to play a role in children with JCA-associated uveitis [13, 32]. A high prevalence of anti-nuclear antibodies has been reported but their specificity is still not certain [45]. Furthermore the finding of increased anti-histone 3 antibodies is controversial. Interestingly there is a sequence homology between an 18-amino-acid peptide of retinal S-antigen and a nuclear histone 3 antigen, and the level of anti-histone 3 antibodies seems to be significantly increased in children with active JCA-associated uveitis as compared to other children with intraocular inflammation [44, 48]. Also heat shock proteins have been proposed as a target of a pathologic immune response. Patients with oligoarticular JCA demonstrated a specific T-cell proliferation to (self) heat shock protein 60 (hsp60) and similar results were found in an animal model

of experimental induced arthritis [57]. Whereas all these findings may indicate an abnormality of the immune regulation in children with JCA, their significance remains elusive. Also the assumption of an autoimmune response directed to targets present in the joints and the eye could not be further substantiated. Type II collagen, which is present in the joints and aqueous humour, was one of the candidate antigens. Indeed, type II collagen can be used to induce experimental chronic arthritis in Lewis rats [50]. However, these animals did not demonstrate concomitant uveitis. In addition, in children with arthritis and uveitis the frequency of antibodies directed to type II collagen did not differ from children affected by arthritis alone.

More recently, *the role of cytokines* in JCA has been a broad field of interest. It has been shown that TNF-α, INF-γ and also their receptors are significantly elevated in synovial tissue of children with JCA [25]. As a consequence new treatment options based on these findings have been developed and are already in clinical practice [28, 38, 39].

9.5 Risk Factors for the Development of Uveitis in JRA

Several clinical and laboratory factors have been associated with the development of uveitis in JRA. It has to be kept in mind, however, that retrospective studies investigating the prevalence of uveitis in children with JRA are limited because of differences in the classification systems in the past. Although these limitations need to be borne in mind, several factors including gender, type and onset of arthritis and laboratory findings such as autoantibodies have been proposed as risk factors of uveitis.

A number of studies indicate that *girls* are predominantly affected by uveitis with a prevalence of 77–82 % of all arthritis patients [6, 8, 49]. However, since girls are far more frequently affected by JCA than boys, this observation has to be carefully reevaluated. Indeed, more recent prospective studies were not able to identify gender as an independent risk factor [35].

Similarly, the *type of arthritis* needs also to be critically reevaluated. It has been suggested that children with oligoarthritis are more likely to develop uveitis as compared to children with polyarthritis or systemic onset of JCA. This again has to be carefully reconsidered, since oligoarthritis is the predominant type of arthritis [49]. It also has to be kept in mind that a number of children initially diagnosed with oligoarthritis may subsequently develop polyarthritis during their follow-up. Indeed, when Kotaniemi et al. reclassified children with JCA and uveitis beyond the first 6 months after onset of uveitis, a comparable number of patients with oligoarthritis and polyarthritis was found [35].

Not only the type of arthritis but also the *onset of arthritis* has been proposed as a risk factor for uveitis. In general, uveitis appears significantly more frequent in children with early manifestation of JCA [35]. Young children, aged 2–4 years, are of the highest risk of developing uveitis. With few exceptions, most children develop uveitis within 18–24 months after the onset/diagnosis of arthritis [5, 6, 49]. Subsequently, the risk for intraocular inflammation diminishes on long-term follow-up. Within 4–7 years after onset of arthritis, 90 % of all uveitis manifestations are diagnosed.

Antinuclear antibodies have been repeatedly associated with an increased risk of developing uveitis. ANA positivity was significantly more frequent in patients with uveitis as compared with those that did not have uveitis in a prospective study [35].

9.6 Clinical Features of Uveitis in JCA

The onset of uveitis in children with JCA is usually insidious, bilateral and anterior in type [8]. It is almost characteristic that even severe intraocular inflammation does not lead to extraocular signs and no alarming symptoms are recognized by the parents. Because of this asymptomatic onset there is often a significant delay of adequate diagnosis and treatment. It is not rare that children first seen by an ophthalmologist already present with secondary ocular

complications such as persistent synechiae or cataract formation. In contrast, a more symptomatic, acute onset is more probably seen in HLA-B*27 positive boys at the age of 8–15 years who often develop spondylarthropathy later.

Intraocular inflammation occurs bilaterally in more than 70 % of children and involves the second eye within a few months [34, 67]. Only rarely will the contralateral eye be involved after more than a year has elapsed from the primary manifestation. Most commonly ocular signs are unspecific and present as a nongranulomatous anterior uveitis. However, keratic precipitates can be present as well as Koeppe nodules. Posterior synechiae are frequent findings and of particular importance in these children since they may cause metabolic disturbances leading to early cataract formation. In more severe cases the anterior vitreous humour can also be involved and may contribute to impaired vision.

9.7 Complications of Uveitis in JCA

The overall prognosis and functional outcome in children with JCA may have improved; nevertheless a number of ocular complications can lead to poor prognosis for vision. The most frequent complications include band keratopathy, secondary glaucoma, posterior synechiae, cataracta complicata, and cystoid macular edema (Table 9.3).

Band keratopathy is not necessarily a visually disabling complication in JCA but can be frequently observed. The hydroxylapatite deposits at Bowman's layer are an unspecific manifestation; however, they may even guide the diagnosis of chronic intraocular inflammation associated with JRA/JCA (Fig. 9.1).

Secondary glaucoma has been frequently associated with a poor prognosis in children with uveitis. Up to 27 % of glaucomatous eyes in JCA ended with no light perception [77]. A number of factors may contribute to this unsatisfactory

Table 9.3. Complications: percentage of affected eyes (*nd* not described)

	Smiley 1974	Key 1975	Chylak 1975	Cassidy 1977	Rosenberg 1986	Wolf 1987	Malagon 1992	Cabral 1993	Chalom 1997	Möller 2000	Edelsten 2001
Number of patients	61	45	36	38	35	51	61	49	22	45	163
Number of eyes	nd	85	61	nd	61	89	nd	82	nd	nd	275
Mean follow-up (years)	10	nd	6.3	4.8	<6	10.3	nd	9.4	4	nd	4
Synechiae	75	61	38	37	8	43	33[a]	24	10[a]	14	nd
Band keratopathy	56	49	13	21	5	30	34[a]	17	17[a]	38	nd
Cataract	51	58	22	34	13	46	39[a]	21	14[a]	38	21
Glaucoma	25	22	14	8	3	27	10[a]	9	11[a]	22	14
Phthisis bulbi	11	0	10	5	5	4	2[a]	1	nd	nd	nd

[a] Percentage of affected patients.

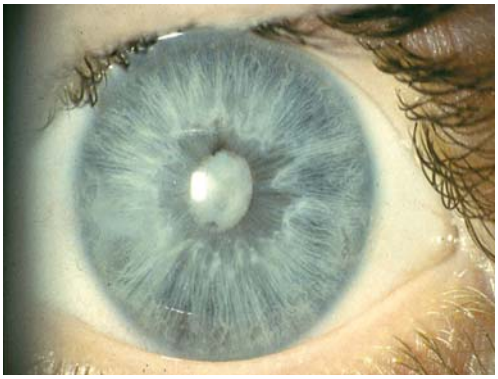

Fig. 9.1. Typical (unspecific) clinical presentation of chronic uveitis in JCA displaying band keratopathy, posterior synechiae and secondary cataract in a 7-year-old girl with oligoarthritis

outcome including difficulties in monitoring IOP in young children, unsatisfactory therapy and a low rate of success in glaucoma surgery. Up to now, no consensus exists about the surgical procedures for secondary glaucoma in children, in particular on the use of drainage implants or conventional filtering surgery with mitomycin C or 5-fluorouracil [18, 72].

Cataract formation is a frequent complication as a result of chronic persistent uveitis, synechiae formation and corticosteroid treatment and significantly contributes to impaired vision. Because these cataracts appear in inflamed eyes with band keratopathy, increased ocular pressure and posterior synechiae, they often offer unique challenges in their surgical treatment. Although cataract extraction is a safe and routinely performed procedure in children, morphological and functional outcome in JCA often remains limited. Several authors have emphasized the difficulties of cataract surgery in patients with JCA associated uveitis [16, 20, 26]. No general consensus exists for optimal care in these eyes; in particular it is not clear whether a pars plana lentectomy or a standard phacoemulsification technique should be preferred. Although implantation of an intraocular lens at cataract surgery has been successfully performed in any kind of uveitis and is a routine procedure in children, strong reservations have been expressed regarding IOL implantation in children with JCA [54]. More recently some reports suggested that in selected patients cataracts caused by juvenile arthritis can undergo standard phacoemulsification and IOL implantation with good results [37, 59]. It has to be stressed, however, that most of these "children" with favourable outcome have already been through their teens, whereas in younger children still no general recommendation for IOL implantation can be given.

9.7.1 Macula Edema

Maculopathy has been infrequently reported in JCA children and mainly considered as a secondary complication following cataract surgery. However, Dana et al. observed macula changes during long-term uveitis in about one-third of their patients [11]. More striking is the finding that the majority of these eyes never underwent intraocular surgery, indicating that macula changes were predominantly due to chronic intraocular inflammation. Since in a number of patients irreversible visual impairment results, more attention has to be paid to prevent this complication.

9.8 Differential Diagnosis

The differential diagnosis includes a broad spectrum of diseases that may present with anterior uveitis (Table 9.4). Chronic anterior uveitis may occur associated with other systemic disorders, such as sarcoidosis, M. Behçet and as an isolated entity. Indeed, some of these diseases associated with uveitis may also manifest with arthritis. Since sarcoidosis and M. Behçet not only involve the anterior segment of the eye but also frequently present as choroiditis or retinal vasculitis, a routine fundus examination in all patients is initially obligatory. Sarcoidosis, keratouveitis due to herpes viruses, lyme borreliosis and trauma are among the main causes of anterior uveitis that have to be ruled out. Even when some ocular findings guide the experienced ophthalmologist in the differential diagnosis,

Table 9.4. Anterior uveitis: differential diagnosis in childhood

Juvenile rheumatoid arthritis	Keratouveitis
Juvenile psoriatric arthritis	*(Herpes simplex virus, Herpes zoster virus)*
Reiter's syndrome	Tuberculosis
Sarcoidosis	Lyme borreliosis
Behçet's disease[a]	Syphilis
Ulcerative colitis	Toxoplasmosis[a]
Lupus erythematodes	Toxocariasis[a]
Kawasaki's disease	
NOMID syndrome	
Vogt-Koyanagi-Harada syndrome[a]	
Fuchs heterochromic cyclitis	
Masquerade syndromes[a]	
(Leukemia, retinoblastoma)	
Trauma	

[a] This type of uveitis predominately manifests as posterior uveitis/panuveitis, but may mislead as anterior uveitis because of "spill over".

close collaboration between the ophthalmologist and paediatrician with appropriate investigations is necessary to establish the diagnosis.

Of particular importance are infectious aetiologies for uveitis in children that include herpes simplex and herpes zoster viruses, *Toxoplasma gondii, Borrelia burgdorferi, Bartonella henselae* and *Toxocara canis*. Depending on the medical history, clinical presentation and manifestation of intraocular inflammation, laboratory investigations are usually necessary to establish the diagnosis. Although serological results are more informative in children as compared to adults, positive antibody findings are often not discriminatory for intraocular infection. Therefore aqueous humour analysis might be beneficial in unclear situations to diagnose an infectious aetiology [12, 70].

A rare, but important systemic disease with close similarities with JCA is the NOMID syndrome (neonatal onset multisystem inflammatory disease syndrome) characterized by arthritis and anterior uveitis [58]. Most of these patients will present with neurologic, cutaneous and articular lesion including papillitis. Therefore particular attention should be paid to any of these signs and symptoms. Interestingly, there is a striking absence of posterior synechiae in this syndrome and consequently only a low risk of cataract formation.

9.9 Prognostic Factors for Uveitis

Attempts to identify risk factors for sight-threatening consequences of uveitis are certainly important. Identification of several characteristics that correlate to the clinical course of chronic uveitis in JCA has led to screening programs and surveillance guidelines for early detection and adequate treatments. Although several attempts to predict the chronic course of uveitis have been made, we have to realize that these criteria remain limited, not yet firmly established and may vary in an individual child. Clinical and general conditions that have been suggested to be predictive of poor visual outcome due to uveitis associated with JCA include: gender, type and onset of arthritis, duration between the onset of arthritis and uveitis and the severity of uveitis at the first presentation (Table 9.5). Furthermore, the presence of certain HLA types, autoantibodies and elevated α_2-globulin has been suggested as predictors [62, 71, 78].

Table 9.5. Proposed risk factors for severe uveitis in children with JCA

Factors	Severe uveitis course	Moderate uveitis course
(Gender)	(Male)	(Female)
Onset of arthritis	<4 years	>4 years
Type of arthritis	Oligoarticular onset, psoriatric arthritis	Systemic arthritis onset
Clinical presentation of uveitis at first visit	Reduced visual acuity, glaucoma, synechiae	Negative
ANA	Negative	Positive

9.9.1 Gender

There are a number of studies indicating that girls might be of greater risk for severe uveitis [6, 49]. However, it has to be kept in mind that girls in general are more frequently affected by JCA and uveitis. Indeed, a prospective study was not able to identify gender as a risk factor [35] and some more recent studies have even shown that boys were significantly more likely to have severe uveitis [3, 7].

9.9.2 Type of Arthritis

The type of arthritis has also been suggested to differentiate children with more severe uveitis. It has been proposed that children with oligoarthritis are particularly endangered, whereas children with polyarthritis or systemic onset of JCA could be considered at "low risk". This has to be carefully reconsidered since children with oligoarthritis represent per se the majority of arthritis patients [49]. In addition, it has to be kept in mind that a number of children presenting initially with oligoarthritis may subsequently develop polyarthritis during follow-up. Indeed, when Kotaniemi et al. did reclassify children with JCA and uveitis following the first 6 months after onset of uveitis they reported a comparable number of patients with oligoarthritis and polyarthritis [35].

9.9.3 Onset of Arthritis

Not only the type of arthritis but also the onset of arthritis may predict severe intraocular inflammation. In general, severe uveitis is more common in children when the onset of arthritis is below the age of 6 years. In addition, several studies found that there was an increased ocular complication rate in children with a short gap between the onset of joint and ocular inflammation at initial presentation [5, 6, 14]. Vice versa, the risk of developing intense uveitis decreases with the interval following onset of JCA [5, 6].

9.9.4 Severity of Uveitis at Onset

Initial ocular examination already provides important prognostic information. Reduced visual acuity at first presentation and glaucomatous optic nerve damage have been found as independent prognostic factors of poor final visual outcome [11]. The presence of posterior synechia is another clinical sign that, if present at initial examination, is an indicator of early cataract progression and often limited functional outcome [77].

Even when it remains difficult to predict the clinical course of uveitis in an individual child, it has been suggested that the overall prognosis and visual outcome in children with arthritis has improved. Whereas earlier reports described blindness in 15–38 % of these children, Table 9.6 demonstrates a decline in recent years

Table 9.6. Visual outcome in children with juvenile chronic arthritis. Visual acuity: percentage of affected eyes (*nd* not described)

	Smiley 1974	Key 1975	Chylak 1975	Cassidy 1977	Rosenberg 1986	Wolf 1987	Malagon 1992	Cabral 1993	Chalom 1997	Edelsten 2001
Number of patients	61	45	36	38	35	51	61	49	22	163
Number of eyes	nd	85	61	nd	61	89	nd	82	nd	275
Normal visual acuity	34	nd	78	55	74[a]	61	69[a]	85	89[a]	nd
Reduced visual acuity	37	nd	8	29	26[a]	17	31[a]	5	11[a]	16[a]
Blindness	29	38	14	16	nd	22	nd	10	0	6
Unilateral	13	nd	nd	5	nd	15	nd	9		nd
Bilateral	16	nd	nd	11	nd	7	nd	1		nd

[a] Percentage of affected patients.

and a more favourable prognosis. However, even when the number of blind children has dropped, we have to keep in mind that there remains a significant risk for severe uveitis [6].

9.10 Correlation of Uveitis and Arthritis Activity

Severity and duration of intraocular inflammation varies widely, but there appears no direct correlation between the activity of ocular and joint manifestations [9, 61]. Interestingly, on long-term follow-up, children with uveitis had more joints involved, more frequent intra-articular steroid injections were necessary to control arthritis and systemic immunosuppressive treatment had to be given more frequently. In addition, at the end of the observation period, systemic laboratory signs such as the blood sedimentation rate were more elevated in children with chronic arthritis and uveitis [36].

9.11 Treatment of Uveitis in JCA

Commonly a "step later approach" has been suggested for the treatment of JCA both for chronic arthritis as well as for concomitant uveitis. Since the activity of both manifestations may differ significantly in an individual child, close communication between paediatricians and ophthalmologists is necessary to provide optimal care [29]. It has to be emphasized that a number of new treatment options, in particular "biologic agents", became available with great promise for clinical benefit in arthritis. However, the impact on uveitis is still uncertain.

9.11.1 Topical Steroid Treatment

Since JCA is not a distinct disease entity and the clinical manifestation of uveitis varies considerably, no standard treatment approach can be recommended.

It cannot be overstressed that children with risk of ocular complications need to be identi-

fied as early as possible. In general, any first manifestation of anterior uveitis will initially undergo topical treatment with potent corticosteroids such as prednisolone acetate, dexamethasone or betamethasone. No general advice on frequency and duration of this first line treatment can be given; however, one of the most frequent reasons for treatment failure is the inadequate use of steroids caused by fear of corticosteroid-induced side effects [66]. In most patients a 2- to 3-day course of intensive topical corticosteroids during daytime is sufficient to control intraocular inflammation. Following appropriate control of inflammation, steroids need to be tapered down under close clinical observation, ensuring that no flare-up occurs after cessation of treatment. Only rarely do first episodes of anterior uveitis not respond to topical treatment and systemic steroid treatment may become necessary. A short course of pulsed intravenous methylprednisolone at a dosage of 10–30 mg/kg body weight per day can be required to control intraocular inflammation [55]. The use of subtenon corticosteroid injections has also been recommended; however, this might be difficult to apply in young children and is not a major advantage since it may also lead to systemic absorption and detectable drug levels in the circulation (for review see [66]).

Any severe anterior inflammation should be carefully monitored for formation of posterior synechiae. Development of cataracts seems to be a particularly characteristic in children following these sequelae. Short acting mydriatics are sufficient to control synechiae formation in most of the patients with moderate inflammation when applied once at bedtime. In more severe manifestations scopolamine twice daily might be necessary. It has to be kept in mind that the pupil should be kept mobile and not constantly dilated, which bears the risk of synechiae formation in a mydriatic state of the pupil. Therefore long-acting agents such as atropine should be avoided. In addition, long-term unilateral cycloplegia may result in amblyopia in young children.

In patients with acute inflammation and recently developed posterior synechiae, dissolution might be achieved using a subconjunctival injection of a cocktail or alternatively a sponge soaked with these enforced mydriatics (Fig. 9.2).

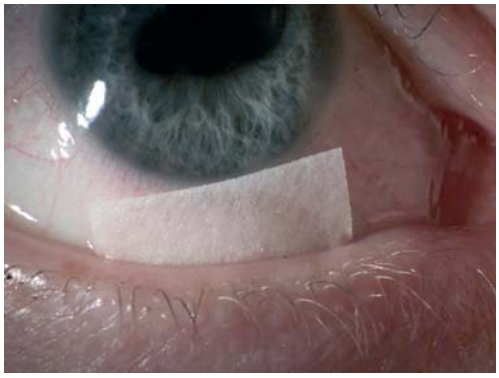

Fig. 9.2. Dissolution of posterior synechiae might be achieved using subconjunctival injection of enforced mydriatics ("lytic cocktail") or alternatively with a soaked sponge

9.11.2 Nonsteroidal Anti-inflammatory Drugs (NSAID)

The role of systemic NSAID to control intraocular inflammation in JCA has not been clearly established. Whereas it has been shown that arthritis will adequately respond in approximately one-third of all patients, this has not been clearly proved for uveitis [32]. However, several reports support the use of NSAID as adjunctive treatment as a steroid sparing agent [11]. Dana et al. reported a significantly better visual outcome in patients on NSAID, but were careful about overinterpreting their value. Since NSAID approved for children are safe and well tolerated by most children, they may merit more attention and a prospective evaluation.

9.11.3 Systemic Immunomodulatory Agents

Since repeated treatment of recurrent uveitis episodes with topical or systemic corticosteroids will result in secondary complications, alternative therapeutic approaches are necessary. Methotrexate (MTX) has become the first choice of treatment in JCA since the placebo controlled double masked trial in children [23]. Given at a dose of 10 mg/m^2 per week, MTX had

a significant treatment effect [23]. Although the efficacy of MTX for many patients with arthritis is well accepted, it has not been proven in any prospective randomized trial to be similarly effective in the treatment and prevention of uveitis. However, in many retrospective studies the more frequent and earlier administrations of MTX are considered a significant factor that reduced the severity of uveitis during long-term follow-up [11, 75]. Methotrexate has distinct advantages over other second line agents that include its once a week dosage, its oral administration, low oncogenic potential and lack of long-term infertility effects.

Weiss and co-workers reported on seven children with JCA and associated uveitis that were refractive to topical steroids and already demonstrated side effects to steroids. In six of these patients MTX was effective in controlling ocular inflammation and could be continued as basic therapy [75].

Similar positive effects were observed by Dana et al., reporting retrospectively on MTX in the treatment of uveitis in JCA [11]. Further evidence on the use of MTX derives from observations in adults with noninfectious uveitis that indicate true disease modification with reduced recurrence frequency and steroid sparing effect in 76% of 160 patients [64]. Adverse effects on long-term treatment with discontinuation of MTX were observed in 18% of all patients, although directly drug related in only 8%.

Only few studies have investigated the effect of cyclosporine A (CsA) in children with JCA and uveitis. In 34 children that were prospectively monitored, 21% were affected by chronic recurrent uveitis [22]. In 8 out of 12 affected eyes treatment with CsA was successful in resolving inflammation and seven eyes had improved visual acuity. However, typical side effects such as renal insufficiency, hirsutism and gum hypertrophy were frequently (50%) observed [74]. In addition, the risk of inducing lymphoma has to be noted.

Recently, the management of juvenile idiopathic arthritis has undergone significant changes and has brought therapeutic benefit regarding the manifestation of arthritis. Since proinflammatory cytokines such as interleukin-1 and TNF-α play an important role in maintaining the chronicity in JCA and mediating tissue destruction, they became a target for therapeutic approaches. In particular TNF-antagonists have emerged as valuable anti-inflammatory agents. Etanercept, a dimerized version of the soluble TNF-receptor II, is approved for the treatment of refractory JCA [60]. Given twice weekly by subcutaneous injection, there was significant suppression of inflammation and prevention of joint destruction [39]. Reiff et al. were able to follow 10 children with Etanercept treatment (7 with JCA) that did not adequately respond to previous medications and observed dissolution of uveitis in 10/16 affected eyes (63%) [60]. More recently the use of Etanercept was evaluated vs. placebo in (adult) uveitis controlled by MTX [19]. In this randomized, double-masked study, Etanercept had no significant efficacy over placebo in preventing relapses of uveitis when patients were tapered down from MTX.

Infliximab is another anti-TNF agent that has been successfully introduced for clinical use. Given as a periodic i.v. infusion it is as effective as Etanercept in adult rheumatoid arthritis, and might be useful in paediatric patients with arthritis as well. Again, little is known about the use of this agent in concurrent uveitis [41].

Smith et al. treated 16 patients with TNF inhibitors and various rheumatoid associated ocular diseases [68]. Six patients (38%) did show improvement of uveitis including four patients with juvenile arthritis. However, it is remarkable that five (adult patients) experienced their first episode of uveitis during this treatment. It seems that at least in some patients there is a risk that some immunomodulatory approaches may even induce or aggravate the onset of uveitis. Interestingly, exacerbation of uveitis following anti-TNF treatment was already reported previously in experimental, endotoxin-induced uveitis [33]. Therefore the role of anti-TNF treatment in uveitis patients has to be carefully monitored before their potential use can be recommended and patients under treatment with these agents need to be monitored by ophthalmologists.

Still, our understanding of the cause and pathogenesis of uveitis in JCA remains rudimentary. Therefore the implementation of more rational, effective treatment is open.

9.12 Recommendations for Ocular Screening in JCA

Patient identification and screening for uveitis remain the most important measures in reducing the risk for severe ocular damage. In particular shortening the interval of high-risk patients for ophthalmic examination may help to prevent irreversible vision loss.

There are unfortunately several factors that may interfere with early detection:

1. The diagnosis of the *arthritis* which typically precedes uveitis is often delayed. Gare and Fasth reported an average of 3 months to the diagnosis of joint problems [21].
2. The severity of uveitis varies greatly at onset. Children typically do not report early symptoms and parents may only notice signs of severe inflammation. This is a particular problem since the youngest children are most severely affected by uveitis and the least reporting subjective symptoms.
3. Despite efforts to diagnose these children early for uveitis, more than half of the patients were not seen within 6 months of onset of arthritis by an ophthalmologist [7]. In fact, recent studies did not show a major difference in the age of the children when diagnosed with uveitis, hence suggesting that the diagnosis is still often delayed [35, 78].

It is unclear how often children with JCA should be seen by an ophthalmologist. Based on the current literature and on the criteria of the American Academy of Pediatrics (1993), the following recommendations could be given:

- All children with JCA should undergo at least one slit lamp examination as soon as the diagnosis of arthritis is made.
- If uveitis is detected, appropriate therapy and follow-up has to be determined by an ophthalmologist.
- If no uveitis is detected initially, girls with JCA should be screened by slit lamp examination every 2 months for the first 6 months after development of arthritis. Boys should also undergo slit-lamp examinations at the same frequency but kept under control for 12 months [7].
- In children with recurrent episodes of uveitis and JCA, a collaborative treatment plan should be set up that reduces the risk of topical/systemic corticosteroid associated side effects in these children.

Summary for the Clinician

- **Uveitis associated with JCA continues to lead to severe ocular damage in a substantial number of children**
- **Delayed diagnosis, due to the silent course of intraocular inflammation and delayed referral to an ophthalmologist, are key factors for serious ocular complications**
- **Patient identification and screening for uveitis are therefore the most important measures to reduce the risk of poor functional outcome**
- **Further benefit for long-term outcome in these patients will result by earlier referral of general ophthalmologists to ocular immunologists who are familiar with systemic immunomodulatory agents. The use of these agents often allows corticosteroids to be spared/avoided that are responsible for significant side effects in particular in young children**
- **"Biologic agents" as new treatment options for arthritis have been successfully introduced; however, their value for uveitis in JCA is not yet clear**

References

1. Alsaeid KM, Haider MZ, al-Awadhi AM, et al. (2003) Role of the human leukocyte antigen DRB1*0307 and DRB1*0308 in susceptibility to juvenile rheumatoid arthritis. Clin Exp Rheumatol 21:399–402
2. American Academy of Pediatrics Section on Rheumatology and Section Ophthalmology (1993) Guidelines for ophthalmologic examinations in children with juvenile rheumatoid arthritis. Pediatrics 92:295–296
3. Berk AT, Kocak N, Unsal E (2001) Uveitis in juvenile arthritis. Ocul Immunol Inflamm 9:243–251

4. Berntson L, Andersson GB, Fasth A, et al. (2003) Incidence of juvenile idiopathic arthritis in the Nordic countries. A population based study with special reference to the validity of the ILAR-EULAR criteria. J Rheumatol 30:2275–2282
5. Cabral DA, Petty RE, Malleson PN, et al. (1994) Visual prognosis in children with chronic anterior uveitis and arthritis. J Rheumatol 21:2370–2375
6. Chalom EC, Goldsmith DP, Koehler MA, et al. (1997) Prevalence and outcome of uveitis in a regional cohort of patients with juvenile rheumatoid arthritis. J Rheumatol 24:2031–2034
7. Chia A, Fraco, Lee V, et al. (2003) Factors related to severe uveitis at diagnosis in children with juvenile idiopathic arthritis in a screening program. Am J Ophthalmol 135:757–762
8. Chylack LT Jr, Bienfang DC, Bellows AR, et al. (1975) Ocular manifestations of juvenile rheumatoid arthritis. Am J Ophthalmol 79:1026–1033
9. Cimaz RG, Fink C (1996) The articular prognosis of pauciarticular onset juvenile arthritis is not influenced by the presence of uveitis. J Rheumatol 23:357–359
10. Collen RJ, Lippe BM, Kaplan SA (1979) Primary ovarian failure, juvenile rheumatoid arthritis and vitiligo. Am J Dis Child 133:598–600
11. Dana MR, Merayo-Lloves J, Schaumberg DA, et al. (1997) Visual outcomes prognosticators in juvenile rheumatoid arthritis-associated uveitis. Ophthalmology 104:236–244
12. de Boer J, Wulffaat N, Rothova A (2003) Visual loss in uveitis of childhood. Br J Ophthalmol 87:879–884
13. Edelsten C, Zaman A, Leak AM, et al. (1996) Antibodies against retinal S-antigen in patients with juvenile chronic arthritis-associated uveitis. Rheumatology 35:101–102
14. Edelsten C, Lee V, Bentley CR, et al. (2002) An evaluation of baseline risk factors predicting severity in juvenile idiopathic arthritis associated uveitis and other chronic anterior uveitis in early childhood. Br J Ophthalmol 86:51–56
15. Fisher M, Nussbaum M, Abrams CA, et al. (1980) Diabetes mellitus, Hashimoto's thyroiditis and juvenile rheumatoid arthritis. Am J Dis Child 134:93–94
16. Flynn HW, Davis JL, Culbertson WW (1988) Pars plana lensectomy and vitrectomy for complicated cataracts in juvenile rheumatoid arthritis. Ophthalmology 95:1114–1119
17. Fiannini EH, Malagon CN, van Kerhove C, et al. (1991) Longitudinal analysis of HLA-associated risks for iridocyclitis in juvenile rheumatoid arthritis. J Rheumatol 18:1394–1397
18. Foster CS, Havlrikova K, Baltatzis S, et al. (2000) Secondary glaucoma in patients with juvenile rheumatoid arthritis-associated iridocyclitis. Acta Ophthalmol Scand 78:576–579
19. Foster CS, Tufail F, Waheed NK, et al. (2003) Efficacy of Etanercept in preventing relapse of uveitis controlled by methotrexate. Arch Ophthalmol 121:437–440
20. Fox GM, Flynn HW Jr, Davis JL, et al. (1992) Causes of reduced visual acuity on long-term follow-up after cataract extraction in patients with uveitis and juvenile rheumatoid arthritis. Am J Ophthalmol 114:708–714
21. Gare BA, Fasth A (1995) The natural history of juvenile chronic arthritis: a population based cohort study. I. Onset and disease process. J Rheumatol 22: 295–307
22. Gerloni V, Cimaz R, Gattinara M, et al. (2001) Efficacy and safety profile cyclosporin A in the treatment of juvenile chronic (idiopathic) arthritis. Results of a 10-year prospective study. Rheumatology (Oxford) 40:907–913
23. Giannini EH, Brewer EJ, Kuzmina N, et al. (1992) Methotrexate in resistant juvenile rheumatoid arthritis. Results of the USA-USSR double-blind, placebo-controlled trial. The Pediatric Rheumatology Collaborative Study Group and the Cooperative Children's Study Group. N Engl J Med 326:1043–1049
24. Godfrey WA, Lindsley CB, Cuppage FE (1981) Localization of IgM in plasma cells in the iris of a patient with iridocyclitis and juvenile rheumatoid arthritis. Arthritis Rheum 24:1195–1198
25. Grom AA, Murray KJ, Luyrink L, et al. (1996) Patterns of expression of tumor necrosis factor alpha, tumor necrosis factor beta and their receptors in the synovia of patients with juvenile rheumatoid arthritis and juvenile spondyloarthropathy. Arthritis Rheum 39:1703–1710
26. Häberle H, Velhagen KH, Pleyer U (1998) Pseudophakia in children with juvenile arthritis. Ophthalmologe 95:823–827
27. Hofer M, Southwood TR (2002) Classification of childhood arthritis. Best Pract Res Clin Rheumatol 16:379–396
28. Horneff G, Burgos-Vargas R (2002) TNF-alpha antagonists for the treatment of juvenile-onset spondylarthropathies. Clin Exp Rheumatol 20:137–142
29. Jabs DA, Rosenbaum JT, Foster CS, et al. (2000) Guidelines for the use of immunosuppressive drugs in patients with ocular inflammatory disorders: recommendations of an expert panel. Am J Ophthalmol 130:492–513
30. Kaipiainen-Seppänen O, Savolainen A (1996) Incidence of chronic juvenile rheumatic diseases in Finland during 1980–1990. Clin Exp Rheumatol 14:441–444
31. Kanski JJ, Shun-Shin A (1984) Systematic uveitis syndromes in childhood: an analysis of 340 cases. Ophthalmology 91:1247–1252

32. Kanski JJ, Petty RE (1996) Chronic childhood arthritis and uveitis. In: Pepose JS, Holland GN, Wilhelmus KR (eds) Ocular infection and immunity, Mosby-Yearbook, St. Louis
33. Kasner L, Chan CC, Whitcup SM (1993) The paradoxical effect of tumor necrosis factor alpha (TNF-alpha) in endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 34:2911–2917
34. Körner-Stiefbold U, Sauvain MJ, Gerber N, et al. (1993) Eye complications in chronic juvenile arthritis. Klin Monatsbl Augenheilkd 202:269–280
35. Kotaniemi K, Kautiainen H, Karma A, et al. (2001) Occurrence of uveitis in recently diagnosed juvenile chronic arthritis: a prospective study. Ophthalmology 108:2071–2075
36. Kotaniemi K, Kotaniemi A, Savolainen A (2002) Uveitis as a marker of active arthritis in 372 patients with juvenile idiopathic seronegative oligoarthritis or polyarthritis. Clin Exp Rheumatol 20:109–112
37. Lam LA, Lowder CY, Baerveldt G, et al. (2003) Surgical management of cataracts in children with juvenile rheumatoid arthritis-associated uveitis. Am J Ophthalmol 135:772–778
38. Lovell DJ, Giannini EH, Reiff A, et al. (2000) Etanercept in children with polyarticular juvenile rheumatoid arthritis. N Engl J Med 342:763–769
39. Lovell DJ, Giannini EH, Reiff A, et al. (2003) Long-term efficacy and safety of Etanercept in children with polyarticular-course juvenile rheumatoid arthritis. Arthritis Rheum 48:218–226
40. Malagon C, Van Kerckhove C, Giannini EH, et al. (1992) The iridocyclitis of early onset pauciarticular juvenile rheumatoid arthritis: outcome in immunogenetically characterized patients. J Rheumatol 19:160–163
41. Mangge H, Heinzle B, Grubbauer HM, et al. (2003) Therapeutic experience with infliximab in a patient with polyarticular juvenile idiopathic arthritis and uveitis. Rheumatol Int 23:258–261
42. McCannel CA, Holland GN, Helm CJ, et al. (1996) Causes of uveitis in the general practice of ophthalmology. UCLA Community-based uveitis study group. Am J Ophthalmol 121:35–46
43. Möller DE, Urban A, Kraft H, et al. (2000) Eye complications in 458 children with rheumatoid arthritis. Klin Monatsbl Augenheilkd 217:15–22
44. Monestier M, Losman JA, Fasy TM, et al. (1990) Antihistone antibodies in antinuclear antibody-positive juvenile arthritis. Arthritis Rheum 33:1836–1841
45. Neuteboom GH, Hertzberger-ten Cate R, de Jong J, et al. (1992) Antibodies to a 15 kD nuclear antigen in patients with juvenile chronic arthritis and uveitis. Invest Ophthalmol Vis Sci 33:1657–1660
46. Nomenclature and classification of arthritis in children. European League Against Rheumatism (EULAR) Bulletin 4; Basel, 1977, National Zeitung AG
47. Ohm J (1910) Hornhauttrübung bei einem neunjährigen Mädchen und ihre Behandlung mit subkonjunktivalen Sodkaliumeinspritzungen. Klin Monatsbl Augenheilkd 48:243–246
48. Ostensen M, Fredriksen K, Kass E, et al. (1989) Identification of antihistone antibodies in subsets of juvenile chronic arthritis. Ann Rheum Dis 48:114–117
49. Petty RE (1987) Current knowledge of the etiology and pathogenesis of chronic uveitis accompanying juvenile rheumatoid arthritis. Rheum Dis Clin North Am 13:19–36
50. Petty RE (1993) Is there a useful model for the study of childhood uveitis and arthritis? Clin Exp Rheumatol 11:7–8
51. Petty RE, Hunt DW, Mathers DM, et al. (1994) Experimental arthritis and uveitis in rats associated with *Mycobacterium butyricum*. J Rheumatol 21:1491–1496
52. Petty RE, Smith JR, Rosenbaum JT (2003) Arthritis and uveitis in children. A pediatric rheumatology perspective. Am J Ophthalmol 135:879–884
53. Pleyer U, Liekfeld A, Baatz H, et al. (1999) Pharmakologische Modulation immunmediierter Reaktionen. Klin Monatsbl Augenheilkd 213:160–170
54. Pleyer U, Velhagen KH, Scherer M, et al. (2000) Intraokularlinsen – Implantation bei Uveitis, assoziiert mit kinderrheumatologischen Erkrankungen. 13. Kongreß der DGII. Biermann-Verlag, Köln, pp 29–36
55. Pleyer U (2001) Therapie intraokularer Entzündung: anteriore und posteriore Uveitis – therapeutischer Stufenplan. In: Kampik A, Grehn W (eds) Therapie okulärer Erkrankungen. Thieme, Stuttgart, pp 324–343
56. Ploski R, Vinje O, Ronningen KS, et al. (1993) HLA class II alleles and heterogeneity of juvenile rheumatoid arthritis. DRB1 0101 may define a novel subset of disease. Arthritis Rheum 36:465–472
57. Prakken AB, van Eden W, Rijkers GT, et al. (1996) Autoreactivity to human heat-shock protein 60 predicts disease remission in oligoarticular juvenile rheumatoid arthritis. Arthritis Rheum 39: 1826–1832
58. Prieur A (2000) Chronic infantile neurological, cutaneous and articular/neonatal onset multisystem inflammatory disease syndrome. Arch Ophthalmol 118:1386–1392
59. Probst LE, Holland EJ (1996) Intraocular lens implantation in patients with juvenile rheumatoid arthritis. Am J Ophthalmol 122:161–170

60. Reiff A (2003) Long-term outcome of etanercept therapy in children with treatment refractory uveitis. Arthritis Rheum 48:2079–2080
61. Rosenberg AM, Oen KG (1986) The relationship between ocular and articular disease activity in children with juvenile rheumatoid arthritis and associated uveitis. Arthritis Rheum 29:797–800
62. Rosenberg AM, Hauta SA, Prokopchuk PA, et al. (1996) Studies on associations of antinuclear antibodies with antibodies to an uveitogenic peptide of retinal S-antigen in children with uveitis. J Rheumatol 23:370–373
63. Sabates R, Smith T, Apple D (1979) Ocular histopathology in juvenile rheumatoid arthritis. Ann Ophthalmol 11:733–737
64. Samson CM, Waheed N, Baltatzis S, et al. (2001) Methotrexate therapy for chronic noninfectious uveitis: analysis of a case series of 160 patients. Ophthalmology 108:1134–1139
65. Scholz S, Albert ED (1993) Immunogenetic aspects of juvenile chronic arthritis. Clin Exp Rheumatol 11:37–41
66. Sherif Z, Pleyer U (2002) Corticosteroids in ophthalmology: past – present – future. Ophthalmologica 216:305–315
67. Smiley WK (1974) The eye in juvenile rheumatoid arthritis. Trans Ophthalmol Soc U K 94:817–829
68. Smith JR, Levinson RD, Holland GN, et al. (2001) Differential efficacy of tumor necrosis factor inhibition in the management of inflammatory eye disease and associated rheumatic disease. Arthritis Rheum 45:252–257
69. Symmons DP, Jones M, Osborne J, et al. (1996) Pediatric rheumatology in the United Kingdom: data from the Rheumatology Group National Register. J Rheumatol 23:1975–1980
70. Torun N, Liekfeld A, Hartmann C, et al. (2002) Okuläre Toxoplasmose-Antikörper in Kammerwasser und Serum. Ophthalmologe 99:109–112
71. Uusitalo RJ, Stjernschartz J, Mahlborg K, et al. (1985) Serum antibody levels to S-antigen in children with chronic uveitis. Br J Ophthalmol 69:212–216
72. Välimäki J, Airaksinen J, Tuulonen A (1997) Molteno implantation for secondary glaucoma in juvenile rheumatoid arthritis. Arch Ophthalmol 115:1253–1256
73. Vesterdal E, Sury B (1950) Iridocyclitis and band-shaped corneal opacity in juvenile rheumatoid arthritis. Acta Ophthalmol(Copenh) 28:321–335
74. Vitale AT, Rodriguez A, Foster CS (1996) Low-dose cyclosporine A therapy in treating chronic, noninfectious uveitis. Ophthalmology 133:365–373
75. Weiss AH, Wallace CA, Sherry DD (1998) Methotrexate for resistant chronic uveitis in children with juvenile rheumatoid arthritis. J Pediatr 133: 266–268
76. Wirostko E, Johnson L, Wirostko W (1989) Juvenile rheumatoid arthritis inflammatory eye disease. Parasitization of ocular leukocytes by mollicute-like organisms. J Rheumatol 16:1446–1455
77. Wolf MD, Lichter PR, Ragsdale CG (1987) Prognostic factors in the uveitis of juvenile rheumatoid arthritis. Ophthalmology 94:1242–1248
78. Zulian F, Martini G, Falcini F, et al. (2002) Early predictors of severe course of uveitis in oligoarticular juvenile idiopathic arthritis. J Rheumatol 29:2446–2453

Herpes Viruses in Ocular Inflammation

10

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Core Messages

- Herpes viruses are highly adapted opportunistic agents, which have evolved several means of evading the immune system and of establishing latency
- The status of the host's immune system largely defines the outcome of herpetic ocular infections and their complications
- One of the major characteristics of herpetic ocular infections is the dual occurrence of a replicative lytic reaction followed or accompanied by subsequent ocular inflammation
- The prevalence of seropositivity for herpes viruses increases with age and varies geographically
- Intraocular herpetic infections can be usually divided into two major entities: anterior uveitis and viral retinopathies
- New clinical entities including CMV-induced anterior uveitis in immunocompetent patients and non-necrotizing herpetic retinopathies have been recently reported
- The diagnosis of herpetic intraocular inflammation is initially based on the analysis of clinical features. Confirmation of intraocular viral inflammation with or without viral replication relies on molecular techniques such as PCR applied to ocular fluids
- The other method to confirm viral disease is to ascertain intraocular antibody production against different herpes viruses. However, the amount of ocular fluids is a limiting factor in performing diagnostic tests in patients with suspected viral intraocular inflammation
- Systemic antiviral drugs should be proposed rapidly in order to control viral replication before the use of corticosteroids
- All anti-herpes virus drugs available today are virostatic. Therefore, relapses may occur especially in the absence of antiviral prophylaxis

10.1 Introduction

Herpes viruses represent a major aetiology of ocular inflammation which should always be taken into consideration in typical clinical presentations. Primary infection by members of this family is usually efficiently controlled by the immune system. However, the immune system does not completely clear the virus. Herpes viruses have developed a love-hate relationship with their hosts, which they have maintained for over 30×10^6 years. These highly adapted opportunistic agents have evolved several means of evading the immune system and of establishing latency. Herpes virus genomes persist in a latent form at specific sites in the infected host. The status of the host's immune system largely defines the outcome of viral infections and their complications. The majority of the population is seropositive for at least four (varicella, cytomegalovirus, herpes simplex type I, Epstein-Barr virus) of the eight human herpes viruses. Initial infection with these herpes viruses is usually clinically inapparent. And the virus(es) become latent without causing any pathologic condition during the subject's lifetime. In a

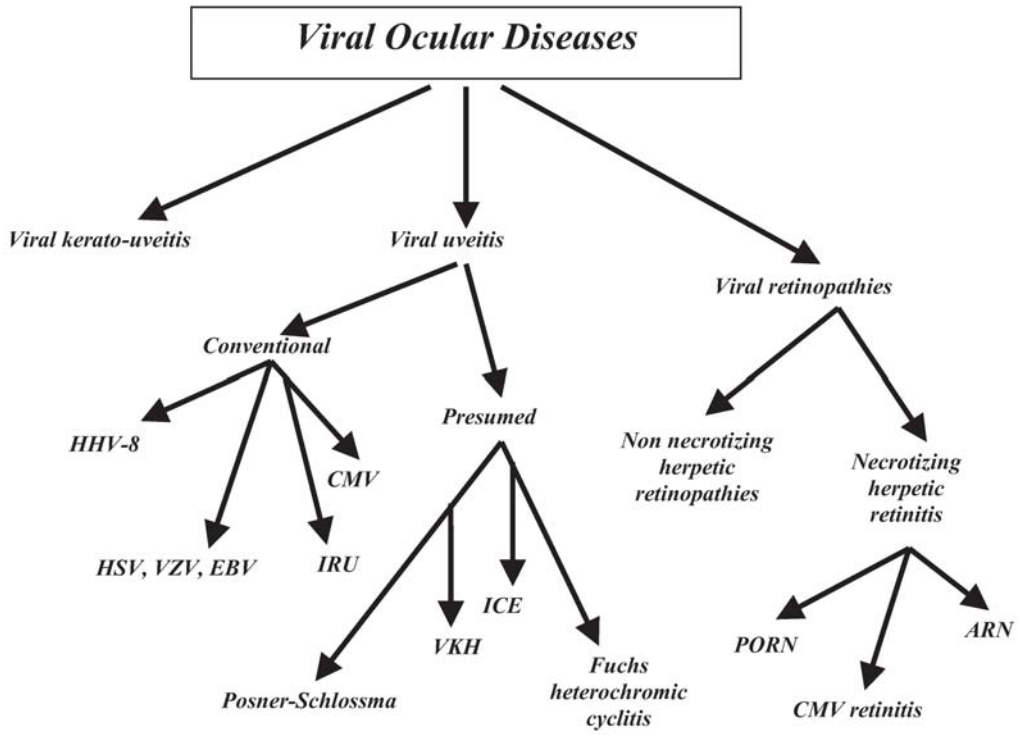

Fig. 10.1. Different patterns of ocular herpetic infection (*IRU*: Immune recovery uveitis, VKH: Vogt-Koyanagi-Harada disease, *ICE*: Irido-corneo-endothelial syndrome, *ARN*: Acute retinal necrosis syndrome, *PORN*: Progressive outer retinal necrosis syndrome)

small percentage of people, cytopathogenicity with subsequent inflammation may occur after the acute phase of viral disease or upon recurrences. This may induce uveitis or retinitis with subsequent complications in the absence of adequate therapeutic strategy. Any delay may worsen the final prognosis and highlights the importance of an energetic diagnostic approach based on thorough clinical and virological examination. Diagnostic confirmation is a major issue and allows specific therapeutic strategies based on antivirals and corticosteroids. Therefore, it could be possible to prevent major relapses. The use of molecular biological techniques is particularly informative. They serve not only to characterize the previously well-known subgroup of presumed viral uveitis, but also to define the role of these agents or emerging viruses in atypical forms of autoimmune uveitis resistant to conventional therapy. The detection of viral DNA by PCR from patients with uveitis is a rapid, sensitive and accurate procedure. Therefore, aqueous humor analysis could be performed when uveitis is unresponsive to anti-inflammatory molecules, in order to exclude a viral condition and modify dramatically the therapeutic management. Several new viral entities have been recently identified, such as cytomegalovirus-associated chronic anterior uveitis and non-necrotizing herpetic retinopathies in immunocompetent hosts. They have been included in the previously well described group of viral ocular diseases (Fig. 10.1). Systemic antiviral drugs should be proposed rapidly in order to control viral replication *before* the use of corticosteroids. Maintenance therapy based on low dose antivirals can reduce the rate of recurrence and should be considered.

10.2 Fundamental Virology

Eight human herpesviruses have already been characterized. All of these viral agents can induce ocular inflammation. The family includes herpes simplex virus (HSV) type 1 and type 2, varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes virus 6 (HHV-6), herpes virus 7 (HHV-7) and herpes virus 8 (HHV-8), also known as an agent of Kaposi's sarcoma, (KSHV). HSV and VZV are alpha herpesviruses that infect a wide variety of cell types, where they replicate rapidly (within 24 h) and can cross species barriers. CMV, HHV-6 and HHV-7 are beta herpesviruses that grow slowly in a limited number of cell types and are very species specific. EBV and HHV-8 infect mainly lymphocytes, grow slowly and are species specific. Morphologically, all herpes viruses are enveloped particles 150–200 nm in diameter, composed of a icosaedric nucleocapsid with 20 equilateral triangle faces, containing a double strand DNA [54, 70]. Herpes viruses have the next to largest genomes in the world of viruses, just after pox viruses. Due to the presence of repeat sequences, the DNA can adopt four isomeric forms. Between the capsid and the envelope is an amorphous protein called tegument composed mainly of phosphoproteins, which interfere with host-cell protein synthesis after viral penetration into host cells. Herpes virus genomes have been sequenced and identification of open reading frames, transcriptional regulatory sequences and proteins has disclosed a close relationship between the different members of this family. Glycoproteins integrated into the viral envelope allow serological identification. Herpes viruses become latent in their infected hosts, therefore developing a love-hate relationship, which has allowed them to evolve with their hosts for millions of years. These viruses are found in mammals, reptiles, fish and birds. Their role in the evolution of species is under debate.

Human CMV (HCMV) represents the prototype virus of the beta subgroup. Its double-stranded DNA genome of more than 238 kbp encodes more than 200 viral proteins. Many of these proteins functionally mimic host proteins. This opportunist agent has a strict species specificity, which is indicative of a long pathogen-host coevolution [48]. In fully permissive cells, viral replication occurs according to a cascade of three consecutive phases: immediate early (IE), early (E) and late (L). Viral DNA synthesis occurs at the end of the E phase. Proteins produced during the IE and E phases play an immunoregulatory role during viral replication. HCMV usually replicates in tissues that have a less stringent immune surveillance, but also infects hematopoietic stem cells and their derivatives. Viral latency is established in various infected tissues and cell types, notably monocytes and endothelial cells. During latency, HCMV expresses a limited number of viral genes in order to minimize exposure to the immune system. To achieve permanent coexistence with its host, HCMV has developed different strategies to evade immune control. Virus-host interactions are particularly original, involving modulation of different metabolic pathways of the cell, on the one hand, and by avoiding immune detection, on the other. By downregulating cell surface expression of MHC class I molecules, it avoids the presentation of viral peptides to cytotoxic T cells. It can also modulate the production of soluble mediators of immunity and sequestrate chemokines within infected cells, thereby protecting infected cells from attraction by killer leukocytes.

10.3 Epidemiology

Herpes viruses can be transmitted to a susceptible individual following close contact with the secretions, skin, or mucous membranes of an infected person shedding virus. Infections with herpes viruses are very common in the general population, but in most cases they do not cause clinically apparent disease. Ocular herpes simplex virus type 2 infection seems to spread via an oculogenital route in the majority of cases. The prevalence of seropositivity increases with age and varies geographically. In the United States, positive serology against HSV-1 is generally detected in 50 % of high-status and 80 % of

low-status socioeconomic persons by the age of 30 [50] and prevalence increases with age. VZV seroconversion has been reported in the United States in nearly 90 % of the population by the age of 60 years [25]. The global incidence and the prevalence of uveitis are, respectively, 17–24/100,000 and 35–200/100,000 habitants. Based on the International Uveitis Study Group classification of uveitis and various scientific reports, herpetic uveitis represents the second aetiology of anterior uveitis (25.9 %: HSV uveitis). VZV-related uveitis may develop after herpes zoster ophthalmicus (HZO), herpes zoster sine herpete (reactivation of VZV without cutaneous vesicules), but also in conjunction with chickenpox. Anterior uveitis is more common after HZO than herpes zoster sine herpete. Acyclovir treatment during HZO significantly decreases the risk of ocular complications and especially uveitis [30].

Herpes virus infections involving the posterior segment of the eye are less common and include CMV retinitis or progressive outer retinal necrosis in immunocompromised hosts and acute retinal necrosis or non-necrotizing viral retinopathies mainly in immunocompetent patients. The prevalence of herpetic retinitis has been found to be closely sex independent. The disease has been reported worldwide.

10.4 Pathophysiology

10.4.1 Experimental Viral Uveitis

Viral eye diseases are common and are associated with different well-known forms of uveitis. Different animal and cellular models have been used to study herpetic ocular infections [71, 16]. However, experimental models and clinical observations support the notion of an infectious, especially a viral, aetiology in different autoimmune conditions. One of the major characteristics of herpetic ocular infections is the dual occurrence of a replicative lytic reaction followed or accompanied by subsequent ocular inflammation. This duality is undoubtedly the pathophysiologic basis of viral uveitis and retinitis in experimental models, but also in human diseases. Progress made in the study of both entities has led to improved therapeutic strategies. Animal models including rabbits, mice and guinea pigs have been developed to study herpetic ocular infections. Rabbits were used to induce CMV chorioretinitis [17]. Intracamerular injection of herpes viruses induced severe anterior uveitis in the injected eye and a moderate contralateral chorioretinitis after a period of 7–10 days. The absence of posterior segment involvement in the injected eye remains unexplained. Intravitreal injection of an active viral preparation induces a total primary uveitis due to viral cytopathic effects, starting less than 1 week after injection. Intraocular reintroduction of the virus, whatever its replicative potential may be, triggers secondary uveitis with an acute onset, associated with an immunopathologic reaction mediated by virus-specific T-lymphocytes. Based on interesting studies, viral propagation after intravitreal injection seems to occur along optic fibres, across chiasma and to the contralateral retina. However, the route of propagation from the anterior chamber remains unknown.

It is interesting to note that primary viral infections affecting the lip will exhibit a pattern of virus gene expression consistent with HSV-1 latency (latency-associated transcripts without HSV-1 antigens) in parts of the nervous system that have direct or indirect connections with ocular tissues. Therefore, reactivation from latency will induce corneal, uveal or retinal infections [43, 44]. This situation is analogous to another viral agent, the lymphochoriomeningitis virus, which proliferates in the uvea after intracamerular injection without causing any disease. In a second phase, a severe antiviral response will occur with negligible cytopathic effects. It is possible to transfer ocular inflammation via activated lymphocytes. Intraocular presence of a viral antigen may thus induce chronic relapsing episodes of uveitis.

10.4.2 Experimental Models to Study Viral Retinitis

Animal models have been developed to define the main characteristics of viral retinitis [4, 33, 42, 51, 66]. Human cytomegalovirus has a different pattern of replication than that of HSV and VZV.

Pertinent permissive cell cultures, such as retinal glial cells or retinal pigment epithelial cells, have been used to study HCMV replication (Fig. 10.2). Virus replicates slowly and progressively [11, 16]. In the era of highly active antiretroviral therapy, intraocular inflammation, like cystoid macular edema, vitritis or papillitis, has been reported in patients presenting with completely cicatricial retinitis. It was therefore of interest to analyse different pathways involving antiviral immune responses that might potentially play a role in these situations. The model of retinal pigment epithelial cells was used to analyse virus-host interactions. The occurrence of HCMV retinitis in the late phase of immunosuppression may be related to perturbation of cytokine production and secretion, associated with the progressive loss of the CD3+CD4+ cell subset. The effects of different cytokines such as IFN-γ, IFN-β, IL-1β, TGF-β and TNF-α, which are present in the eye under different immunopathological conditions, were studied to discover which of them play a role during HCMV replication in retinal pigment epithelial cells. IFN-γ and IL-1β appeared to be the major antiviral cytokines in this in vitro system. The antiviral effect of IFN-γ could be reversed by adding the amino acid L-tryptophan to the culture medium. Tryptophan is an essential amino acid for HCMV replication. IFN-γ stimulates an enzyme, indoleamine 2, 3-dioxygenase (IDO), which is responsible for the conversion of tryptophan and its derivatives to kynurenine. Tryptophan is an essential amino acid for HCMV replication. Cytokines such as IFN-γ may drive the virus underground before viral proteins (the targets of the immune system) are expressed, thereby creating viral reservoirs that escape immune surveillance [8]. A lack of IFN-γ in the final stage of immunosuppression could play a role in the pathogenesis of HCMV retinitis. Immune reconstitution may be associated with the infiltration of retina with leukocytes that recognize resident cells expressing latent viral antigens. Immune activation induces immune recovery uveitis in the absence of active viral replication.

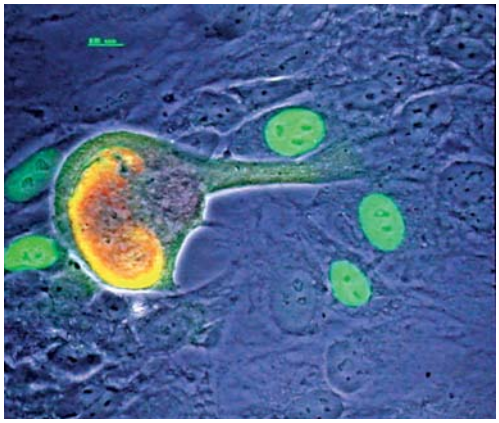

Fig. 10.2. Confocal microscopy disclosing cytomegalic effects in CMV-infected human retinal pigment epithelial cells. Indirect immunofluorescence (*green* immediate early antigens, *red* late antigens)

10.5 Clinical Features

Intraocular herpetic infections can be divided into two major entities, anterior uveitis and viral retinopathies. Clinical manifestations have been relatively well defined. However, it is still difficult to present strong clinical and virological correlations without molecular analysis. The disease is usually unilateral with an acute onset.

10.5.1 Anterior Uveitis

10.5.1.1 Herpes Simplex Virus (HSV) Infection

There is no direct correlation between herpetic keratitis and uveitis. Patients with stromal keratitis may present concurrent anterior uveitis.

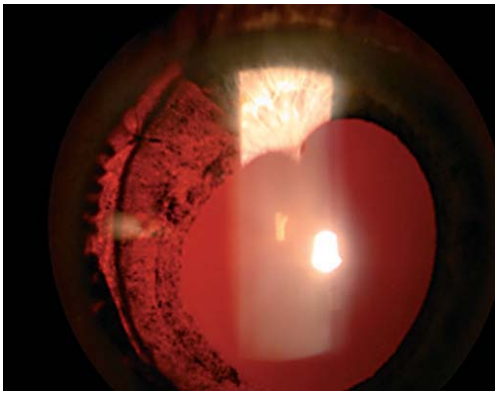

Fig. 10.3. Iris atrophy associated with HSV-1 infection

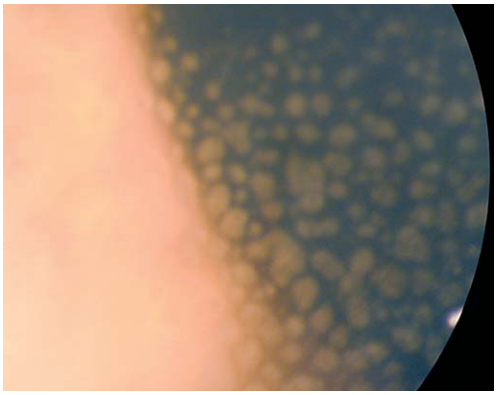

Fig. 10.4. "Leopard" aspect of keratic precipitates in a patient with VZV-associated anterior uveitis

However, anterior uveitis may occur with no active corneal inflammation. Recurrent episodes can damage the eye and spread to the posterior segment, involving the vitreous and the retina. Despite virological confirmation, mostly by polymerase chain reaction, it is still not known if uveitis is due to a viral cytopathic effect or an immunopathologic phenomenon [24]. Redness, photophobia, pain and visual impairment are frequently observed. Intraocular inflammation is usually acute, unilateral, and granulomatous with posterior synechiae and sectoral iris atrophy (Fig. 10.3). Iris masses have been reported. Secondary glaucoma due to trabeculitis is a major sign of herpetic uveitis. Glaucoma may be temporary, but chronic forms without concomitant active infection or inflammation are also possible. Mild to severe cell and flare reaction in the aqueous humor is common. Hypopion can be observed in severe forms, but hyphema is also possible.

Van der Lelij et al. have reported molecular diagnosis of herpetic infection in a series of 31 patients presenting with anterior uveitis and sectoral iris atrophy [61]. Anterior chamber paracentesis (ACP) was performed in 24 patients and secondary glaucoma was present in 90 % of cases. The final visual prognosis was satisfactory in the majority of cases. ACP was positive in 23 cases (HSV: 20 cases and VZV: 3 cases). The mean age at onset of HSV-associated uveitis was lower than that of VZV-associated uveitis.

10.5.1.2 Varicella-Zoster Virus (VZV)

Uveitis is much more common after herpes zoster ophthalmicus. Nearly two-thirds of patients with herpes zoster involving the ophthalmic division of the trigeminal nerve may present ocular involvement, especially without acyclovir prophylaxis. Uveitis is less frequent after an episode of varicella. Ocular inflammation is often delayed relative to the onset of cutaneous lesions. An interval of 2–4 weeks may separate both diseases. Uveitis seems to be more severe when it is delayed and associated viral retinitis should be eliminated, especially in immunocompromised or elderly patients. Grey or brown keratic precipitates are localized in the inferior part of the cornea, but may be more diffuse with a "leopard pattern" (Fig. 10.4). Ischaemia of the anterior segment is classically associated with VZV uveitis. The presence of sector and patchy atrophy may be related to virus-induced ischaemia. Anterior uveitis is often acute, unilateral, granulomatous, and associated with posterior synechiae and glaucoma.

10.5.1.3 Cytomegalovirus (CMV) Uveitis

CMV involvement of the anterior segment in immunocompetent patients was reported very recently [47]. This entity differs from HSV or VZV uveitis. Chronicity is a major characteris-

tic. Ocular inflammation lasts for a long time and seems to be mild to moderate. Small size KPs are brown and scattered in the lower part of the cornea. This disease occurs more frequently in elderly patients. Sectorial iris atrophy is not a common finding and posterior synechiae are rarely observed. Secondary glaucoma seems to be a consistent complication and becomes resistant to medical anti-glaucomatous therapy in the absence of specific anti-CMV therapy. It is important to note that the posterior segment of the eye is usually not involved.

10.5.1.4 Epstein-Barr Virus (EBV) Uveitis

Benign and transitory uveitis has been described during the course of EBV-associated infectious mononucleosis. Different forms of anterior uveitis, multifocal choroiditis and panuveitis have been attributed to EBV infection [58]. However, it is difficult to isolate a distinct entity to characterize EBV infections of the eye. This is probably due to the risk of blood contamination associated with the presence of EBV in mononuclear cells, which may be found after anterior chamber paracentesis. Uveitis may be chronic, uni-or bilateral, granulomatous, and associated with diffuse iris atrophy and severe glaucoma.

10.5.2 Viral Retinopathies

10.5.2.1 Necrotizing Herpetic Retinopathies

Necrotizing viral retinopathies have been described and largely characterized by electron microscopic studies, immunocytochemistry [13], viral culture from intraocular specimens, serologic analysis of serum and/or intraocular fluids [15, 63] and, finally, by the polymerase chain reaction [3, 22, 59]. They include acute retinal necrosis syndrome, progressive outer retinal necrosis syndrome and CMV retinitis.

10.5.2.1.1 Acute Retinal Necrosis (ARN)

In 1971 Urayama and associates reported the first six cases of presumed viral retinal necrosis and called it *unilateral acute uveitis with retinal periarteritis and detachment* [60]. The disease is known in Japan as *Kirisawa uveitis*. Bilateral forms were described later by Western authors. Fisher et al. used the term "acute retinal necrosis" for the first time in 1982 [18]. Later studies confirmed the role of VZV and HSV in the pathogenesis of ARN. In 1994, Holland and the American Uveitis Society unified the spectrum of presumed herpetic retinopathies and defined it as necrotizing herpetic retinopathies [32]. The prevalence of ARN is nearly equal in both sexes. The majority of cases occur in the 5th–7th decades of life. Viral retinitis may occur congenitally, in newborns or in young children. It is still not known why the disease was not reported before the 1970s. Both healthy and immunocompromised patients may develop ARN during either primary or recurrent herpes virus infection. However, clinical presentation may vary in severity.

The ARN syndrome is characterized by acute primarily peripheral, necrotizing retinitis, retinal arteriolitis and mild to severe vitritis. The disease is usually unilateral and is known to be caused by VZV, HSV, but rarely CMV. Recently, studies from the United States and Japan pointed out the high frequency of VZV or HSV-2 infection in cases of acute retinal necrosis [22, 36].

The main clinical characteristics of ARN syndrome were defined by the American Uveitis Society and include: focal, well-demarcated areas of retinal necrosis located in the peripheral retina (Fig. 10.5), rapid, circumferential progression of necrosis, evidence of occlusive vasculopathy and a prominent inflammatory reaction in the vitreous and anterior chamber [32]. Mild optic disk edema may be present early in the course of the disease and increases progressively. A granulomatous anterior uveitis with secondary glaucoma may be associated. Retinitis progresses rapidly in the absence of treatment, but classically spreads to the posterior pole. An exudative retinal detachment occasionally develops if inflammation is important

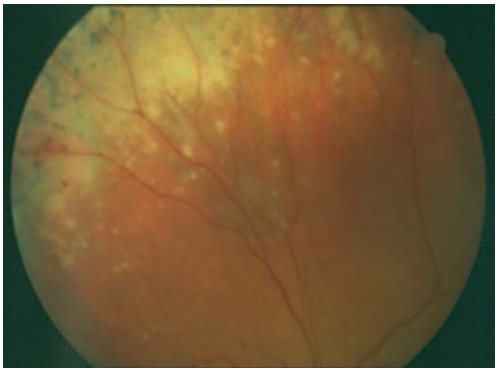

Fig. 10.5. Acute retinal necrosis syndrome in an immunocompetent patient with white retinal infiltrates and few haemorrhages

and rhegmatogenous retinal detachment occurs frequently in the absence of antiviral, and sometimes anti-inflammatory, therapy. Occasionally, retinitis remains limited defining low-spreading forms of retinal necrosis.

10.5.2.1.2 Progressive Outer Retinal Necrosis

This variant of herpetic retinopathy was first described by Forster and associates [19]. It usually occurs in immunocompromised patients and is characterized by a minimal non-granulomatous anterior uveitis without vitritis, associated with a fulgurant necrotizing retinitis starting at the posterior pole and spreading toward the peripheral retina. Foci of lesions become rapidly confluent and involve the entire retina. Unlike ARN syndrome, retinal vasculitis and optic neuritis are less common, but retinitis is often bilateral. The disease is quite severe and visual prognosis is usually poor due to resistance to antivirals and occurrence of retinal detachment

10.5.2.1.3 Cytomegalovirus Retinitis

CMV is one of the most puzzling members of the Herpesviridae family. Ocular involvement has been reported during congenital infection and in patients with acquired immunodeficiency syndrome (AIDS) [34]. Primary infection during pregnancy is the major cause of intrauterine infection in developed countries, with a mortality rate of 20 % and a risk of retinitis in 15 % of children. Before the use of highly active antiretroviral therapy (HAART) in AIDS patients, CMV disease was the major cause of blindness. CMV retinitis was observed late during the disease and the risk was significant when the level of CD4 lymphocytes dropped below 50–100/mm^3. In a small percentage of patients, CMV retinitis may be the first clinical manifestation of AIDS. CMV retinitis is probably secondary to the passage of the virus across the blood retinal barrier, when local defense mechanisms are almost completely abolished. Viral progression into the retina seems to occur in a polarized manner. The internal blood-retinal barrier is initially disrupted after primary replication in endothelial cells, allowing viral particles to reach retinal glial cells. CMV then spreads towards the retinal pigment epithelium. The retinal site of HCMV latency is still under debate: retinal pigment epithelium or glial cells are two putative candidates. CMV retinitis occurs initially in the peripheral retina. Visual complaints remain rare for a long period of time. Systematic fundus examination should be performed every 3 months if CD4 lymphocytes counts are below 50/mm^3. Active CMV retinitis is usually diagnosed on ophthalmoscopic signs, such as white fluffy areas of necrotizing retinitis associated with haemorrhages and vascular sheathing. Early CMV retinitis may begin with a small, white retinal infiltrate. The lesion may masquerade as a cotton-wool spot present in HIV-related microvasculopathy. Fundus examination must be controlled in order to confirm a putative CMV retinitis. Two distinct subtypes of CMV retinitis have been described. The fulminant or edematous variant is the classic appearance of disease (Fig. 10.6). Dense, white confluent opacifications of the retina without any central atrophic lesion occur usually along vessels associated with retinal haemorrhages and inflammatory vascular sheathing. The indolent or granular variant of disease associates granular foci of retinal necrosis with a central atrophic zone, fewer haemorrhages and less vascular sheathing. The border of retinal necrosis is usually irregular in both variants, surrounded by

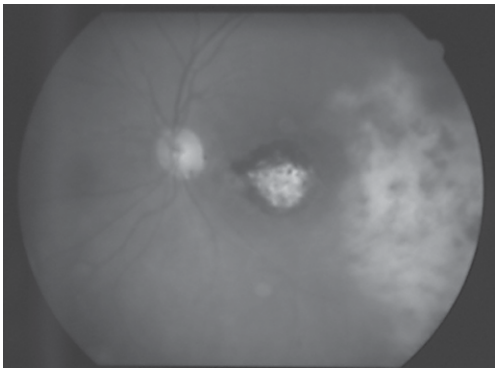

Fig. 10.6. PCR-proven CMV retinitis in a patient with a previous history of macular toxoplasmosis

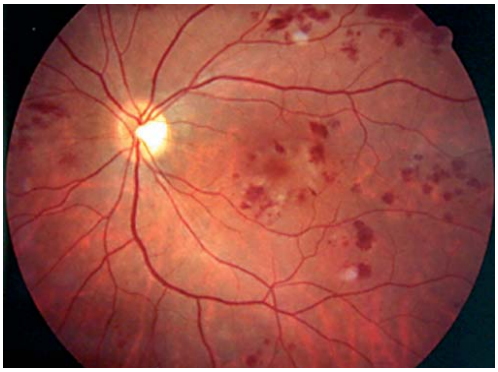

Fig. 10.7. Atypical aspect of VZV-associated retinitis with diffuse retinal hemorrhages 2 weeks after an episode of varicella

satellite infiltrates. The optic disk is rarely infiltrated initially, but papillitis may be observed when retinitis progresses toward the posterior pole. Mild vitritis is associated with minor anterior segment inflammation. Despite slow progression of retinitis, destruction of the entire retina occurs within 3–6 months in the absence of anti-CMV therapy. Cicatricial lesions are atrophic retina with vessel rarefaction. Fluorescein angiography may be helpful in complex cases, when other differential diagnoses such as retinochoroidal toxoplasmosis, candida endophthalmitis, syphilitic retinitis, herpes simplex and herpes zoster retinitis are suspected.

The administration of HAART to AIDS patients has dramatically changed the course of CMV disease by improving the function of the immune system and increasing survival [37, 55]. If CD4 cell counts increase after HAART, a beneficial effect on viral recurrences may be observed and anti-CMV maintenance therapy can be discontinued [69]. In patients presenting with previous areas of CMV retinitis before immune restitution under HAART, episodes of posterior uveitis, vitritis, retinal vasculitis, papillitis and macular edema have been reported [12, 38]. The pathophysiology of disease, known as immune recovery uveitis, remains controversial [57]. However, personal data and results reported recently seem to exclude viral replication and highlight an immune reaction due to restoration of lymphocytes recognizing chronically infected retinal cells expressing CMV antigens at their surface.

10.5.2.2 Non-Necrotizing Herpetic Retinopathies (NNHR)

This entity was reported recently [10]. Molecular analysis applied to ocular fluids confirmed the presence of herpes virus DNA in patients presenting with different forms of chronic and atypical posterior uveitis, such as Behçet's disease, retinal vasculitis and birdshot retinochoroidopathy. Non-necrotizing retinopathies associated with haemorrhages have been described (Fig. 10.7). The disease is usually bilateral. It is important to emphasize that all patients with NNHR are corticoresistant or corticodependent at a high level. The initiation of specific antiviral therapy improved ocular inflammation. Immunosuppressors were discontinued and steroids were significantly tapered. Evolution under therapy is close to that observed in herpetic keratouveitis and steroids cannot be interrupted.

10.5.3 Differential Diagnosis

Despite the AUS criteria, diagnosis of ARN may be difficult. Clinical presentations range from focal to extensive retinal necrosis and the clinical course ranges from mild to fulminating. Moreover, in some patients the clinical findings can be atypical and may not be clear enough to

make a definitive diagnosis or to promptly initiate an antiviral agent. The delay in diagnosis can lead to loss of vision that could otherwise be prevented. In addition, similar necrotizing retinitis may also occur from nonviral infectious agents, including *Toxoplasma gondii,* bacteria, or fungi [21]. Other disease entities (namely, retinal vasculitis, intraocular tumours, Behçet's disease and sarcoidosis) can also present with retinitis that mimics ARN. Thus, specific and sensitive laboratory tests are necessary to confirm a diagnosis of ARN or the nonviral retinopathies that present with features simulating ARN. Toxoplasmic retinochoroiditis is the most frequent disease simulating necrotizing viral retinopathies. When either a viral infection or ocular toxoplasmosis is suspected, aqueous humor analysis by PCR and the use of the Goldmann-Witmer coefficient appear to be the best methods for a final diagnostic confirmation. Results may also be obtained within 72 h. Both antiparasitic and antiviral regimens may be required until a final diagnosis is obtained.

10.5.4 Putative Viral-Associated Uveitis

10.5.4.1 Irido-corneo-endothelial Syndrome (ICE)

ICE syndrome associates corneal involvement, glaucoma and iris lesions. Endothelial abnormalities have been shown by specular microscopy. The presence of HSV-DNA has been confirmed at the level of the corneal endothelium [35], but also in the aqueous humor [27].

10.5.4.2 Posner-Schlossman Syndrome

Posner-Schlossman syndrome is strictly unilateral associating a granulomatous anterior uveitis with central KPs and secondary glaucoma. Posterior synechiae are absent. Intraocular synthesis of anti-CMV antibodies was previously reported by Bloch-Michel and associates [7]. The disease is probably multifactorial and different entities must be considered. Antiviral therapy based on acyclovir is not effective, but anti-CMV drugs may prevent recurrences and control ocular hypertension.

10.5.4.3 Fuchs Heterochromic Cyclitis (FHC)

Fuchs' heterochromic iridocyclitis is frequently diagnosed in young patients. The disease is usually unilateral even though bilateral cases have been rarely reported. Pain and redness are absent. Small stellate keratic precipitates are uniformly scattered over the endothelium. Different infectious conditions such as toxoplasmosis have been described during FHC, but the putative role of a pathogen is still under debate. Recently, Barequet et al. have analysed the aqueous humor and the anterior capsule of the lens obtained during cataract surgery in a patient with FHC. HSV DNA was identified in the ocular fluid but not in the lens capsule [5]. More specific data are needed before drawing further conclusions about the pathophysiology of the disease.

10.5.4.4 Vogt-Koyanagi-Harada Syndrome (VKH)

VKH is a multisystemic disorder involving eyes, ears, skin and meninges. It appears to be concentrated in certain racial and ethnic groups. The pathophysiology of VKH remains unclear. A specific antigen-driven immune response may occur in this disorder. However, the concept that VKH is a viral-induced disease has been attractive. EBV seems to be associated with VKH but molecular data are still needed before further conclusions are drawn [6]. Interestingly, atypical forms of VKH may occur in hepatitis C virus-infected patients treated with alpha interferon.

10.6 Diagnosis

The diagnosis of herpetic intraocular inflammation is initially based on the analysis of clinical features. Laboratory tests can help the clinician confirm the disease. However, more than

75 % of the population in developed countries is seropositive for herpes viruses (HSV-1, VZV and/or CMV). Therefore, routinely used serologic testing is of little interest unless it shows the presence of IgM and eventual seroconversion. Confirmation of intraocular viral inflammation with or without viral replication relies on molecular techniques such as PCR [9, 64]. In fact, it is quite difficult to isolate the viral agent from ocular fluids in cell culture. This can be done using ocular tissues but its success rate remains quite low. The other method to confirm disease is to ascertain intraocular antibody production against different herpes viruses. The amount of ocular fluids is a limiting factor to performing diagnostic tests in patients with suspected viral intraocular inflammation.

10.6.1 Obtention of Ocular Fluids

10.6.1.1 Anterior Chamber Paracentesis (ACP)

ACP seems to be safe, but it should be considered a surgical procedure and be performed by an experienced ophthalmologist. It can be performed at the slit lamp or under a microscope in the operating room after instillation of topical antibiotic and anaesthetic drops. Up to 0.2 ml can be obtained. In a retrospective study of 361 patients, Van der Lelij and Rothova reported no serious complications, such as cataract, keratitis or endophthalmitis after paracentesis [62]. Hyphema was reported in seven cases. The same degree of safety was reported in another series [65].

10.6.1.2 Vitreous Tap/Vitrectomy

Vitreous fluid analysis has to be considered in both inflammatory conditions that do not respond to treatment and in masquerade syndromes. Diagnostic vitrectomy can be associated with therapeutic vitrectomy, especially with a hazy vitreous. This procedure is more complicated than ACP, but provides a larger amount of material for analysis. It is mostly performed in the operating room using a surgical microscope with subtenon or peribulbar anesthesia. During three-port vitrectomy, 0.5–1 ml of undiluted vitreous can be aspirated initially. Retinal detachment remains a possible complication of vitreous biopsy or vitrectomy, but the incidence is particularly low. Viruses are difficult to culture, especially when the amount of the inoculum is limited, as with ocular fluids. Moreover, standard shell vial culture is frequently negative after antiviral treatment. The sensitivity of PCR for detection of virus is superior to culture and to evaluation of specific intraocular antibody production.

10.6.2 Herpes Viruses

10.6.2.1 Herpetic Uveitis

Clinical presentations are usually well defined. Among herpetic eye diseases, unilateral anterior uveitis with sectoral iris atrophy in the absence of keratitis is a distinct entity mostly related to HSV and less frequently to VZV [61]. PCR has been used to identify viral genomes in the aqueous humor (AH) of patients with previously well-known uveitis entities. Posner-Schlossman syndrome and Fuchs heterochromic cyclitis are two different examples but there is also a report of the iridocorneoendothelial syndrome [5, 27]. However, there have been many cases of Posner-Schlossman syndrome, Fuchs cyclitis and iridocorneoendothelial syndrome where HSV DNA could not be found in the AH. These results are suggestive, but further information is required before drawing any conclusions.

10.6.2.2 Necrotizing Herpetic Retinopathies (NHR)

Acute retinal necrosis (ARN), progressive outer retinal necrosis (PORN) and cytomegalovirus retinitis are well-defined entities. Diagnosis is quite unequivocal when the clinical presentation is typical, but fundus examination can be difficult in the presence of cloudy media. Cun-

ningham et al. reported the first two cases of AIDS-associated herpetic retinitis, confirmed by PCR and restriction analysis of the vitreous biopsy [14]. The amount of herpes virus DNA, detectable by PCR techniques in ocular fluids of patients without ocular inflammation in a control study published by Pendergast et al., appears to be quite low [53]. Therefore, the authors suggest that a positive result obtained in a patient presenting with vitreoretinal inflammation should be regarded as significant. In 1991, Fox et al. confirmed by PCR the presence of CMV genome in the AH, vitreous and subretinal fluid of patients with a clinical diagnosis of CMV retinitis [20]. Ganatra et al. used PCR to confirm the diagnosis of ARN in 30 eyes of 28 patients [22]. Viral genome was identified in 96.5% of cases. Moreover, viral identification showed that VZV and HSV-1 retinitis are more common in patients older than 25 years, whereas HSV-2 retinitis occurs in younger patients. HSV-2-associated ARN is highly prevalent in Japan [36]. Serologic tests may be less specific than PCR in discriminating HSV-1 and HSV-2 retinitis. Results of other series show the importance of PCR in the diagnosis of atypical viral retinitis [40]. PCR can be the first step for molecular epidemiology of viral retinitis. About 25% of VZV strains in Japan carry a mutation lacking the *Pst*I recognition site. Kumano et al. have detected a VZV genome with a *Pst*I site from the AH of a Japanese patient with ARN [41]. There is a putative polymorphism among VZV strains in patients with ARN. More recently, Mochizuki et al. showed that there is no clinical difference related to the presence of the *Pst*I recognition site [49]. Viral amplification and subsequent sequencing are promising in defining correlations based on molecular virology and polymorphism. VZV seems to be the principal agent of atypical necrotizing herpetic retinopathies [23]. Despite aggressive antiviral therapy, the clinical course of NHR is variable and complications can lead to blindness. Viral analysis of ocular fluids allows the determination of risk factors. Abe et al. estimated the number of VZV copies in the AH and vitreous of patients with ARN by PCR and semi-nested PCR. The number of virus copies seems to be higher in elderly or immunocompromised patients [1]. Severity of retinal necrosis may be due to the implication of different viral strains [2]. Antiviral treatment is less efficient in these cases and the visual outcome remains poor. The same group has shown that different VZV strains participate in ARN. Analysis of the R1 variable region of VZV may help determine the viral strains associated with more fulminant types of ARN. Multiple viral infections may occur in immunodeficient patients, mimicking leukemic infiltration of the eye [45]. Viral identification may show point mutations in the UL97 gene of CMV amplified after vitreous biopsy in patients with ganciclovir-resistant retinitis. Results can be compared with extraocular CMV isolates [46]. In 1999, Smith et al. interestingly showed that CMV retinitis activity can be evaluated by the level of CMV DNA obtained by quantitative PCR applied to ocular fluids [56].

Prospective studies to evaluate PCR results, clinical evolution, and treatment are needed to corroborate the real value of PCR in the diagnostic and therapeutic management of viral retinitis.

PCR may often be negative due to prior antiviral treatment. Thus, alternative techniques have been developed, among which analysis of the coefficient of immunological burden is most frequently applied. Theoretically, one should use both methods in association [15].

10.6.3 Search for Antiviral Antibodies

This type of analysis was the method of choice in numerous institutes until the advent of techniques of molecular biology. Serology by immunoenzymatic (ELISA) and radioimmunological techniques allows the assessment of antibodies in 40–50 µl of aqueous humor or vitreous. However, the presence of intraocular specific antibodies does not necessarily correspond to a local synthesis, but may also reflect passive antibody passage across the blood-ocular barrier ruptured by inflammation. In order to confirm local synthesis, a coefficient of immune burden must be evaluated by comparing ocular and serum antibody levels. This is the Witmer-Gold-

mann C coefficient also used for the diagnosis of ocular toxoplasmosis. This remains a laborious technique. Several groups use a variant of the C coefficient by calculating the ratio of antibody for a given virus in aqueous humor and serum. This ratio is then compared to that of another virus to which the patient is immune, but which is not responsible for the ocular disease. The measles virus is regularly used for this purpose.

10.7 Treatment

The therapeutic strategy of viral ocular inflammatory disorders includes associations of antiviral, anti-inflammatory and anti-glaucomatous medications. All anti-herpes virus drugs available today are virostatic and cannot sterilize retinal cells. Relapses may occur especially in the absence of antiviral prophylaxis.

10.7.1 Anterior Uveitis

No codified treatment has been used on clinical trials. Topical antiviral therapy is of little benefit during viral anterior uveitis and keratouveitis. Acyclovir and valacyclovir are active on herpes simplex and varicella zoster virus but inactive on other herpes viruses. Oral administration of antivirals is proposed in the majority of cases. In immunocompetent patients, treatment of herpes zoster ophthalmicus in the first 3 days is able to reduce the occurrence of keratouveitis and uveitis from 50 % to 29 % [30]. Intravenous acyclovir (10 mg/kg per day) may be proposed occasionally in severe forms of anterior uveitis and must be proposed to all immunocompromised hosts. It is important to respect a 48-h period of antiviral therapy before proposing topical corticosteroids during herpetic uveitis. Anti-inflammatory therapy should be started with high dose topical dexamethasone for a period of 8–10 days followed by a gradual tapering to be evaluated on an individual basis when inflammation is stabilized. Subconjunctival injections of steroids may be initially proposed in severe forms of anterior segment inflammation. Interestingly, oral prednisone is not necessary in the majority of cases. Previous studies have shown the importance of long-term topical low-dose corticosteroids associated with low-dose oral acyclovir. Improvement of uveitis may be monitored clinically but also by laser flare photometry. The duration of antiviral prophylaxis remains controversial, but it must be continued as long as steroids are used. Interesting results reported in patients with herpetic keratouveitis must be taken into consideration [29]. Cycloplegics should be given to all patients at the acute phase. Secondary glaucoma is usually controlled with topical medications, but surgical management may be necessary in the absence of significant efficacy. Analogues of prostaglandins should be avoided. Cases of herpetic uveitis in humans or experimental models have been reported when these drugs were used [39, 67].

CMV-associated anterior uveitis is resistant to acyclovir. Infection can be controlled with intravenous ganciclovir or foscarnet [47]. Valganciclovir may be proposed in order to avoid intravenous administration. Our personal data show that 1 month of antiviral therapy is necessary to control anterior segment inflammation and glaucoma. Topical steroids are progressively tapered. Further clinical monitoring is mandatory and consecutive paracentesis may be necessary when relapses occur. Cycloplegics and anti-glaucomatous medications are necessary.

Treatment is highly controversial in patients with EBV-associated uveitis. Most antivirals have no effect on EBV and ocular disease is mostly self-limiting. Local and systemic steroids have been proposed, but their use may present a risk of chronic inflammation with sight-threatening complications. Cidofovir may be proposed but cases of anterior segment inflammation and nephrotoxicity limit its use. Alpha interferon has antiviral and immunomodulatory effects and may be proposed in severe cases. Patients should be monitored for hematotoxicity and hepatotoxicity.

10.7.2 Viral Retinopathies

Necrotizing retinopathies represent a diagnostic and therapeutic challenge. In such cases, ARN is usually suspected, since it carries a poor visual outcome and a high rate of complications, such as rhegmatogenous retinal detachment.

10.7.2.1 Acute Retinal Necrosis

Antivirals represent the major tools in the treatment of viral retinopathies. Any delay in the initiation of antiviral therapy may be dangerous for the visual outcome of the patient. Despite a few reports on the efficacy of oral valacyclovir, intravenous administration of acyclovir remains the classical approach. It is effective against HSV and VZV. Close monitoring of the retina is necessary in order to confirm antiviral efficacy. Lesions must be stabilized after a mean period of 48 h. In resistant cases, more aggressive antiviral therapy should be initiated based on intravenous foscarnet or ganciclovir. Associations are recommended and intravitreal injections of ganciclovir seem to be efficient, especially in immunocompromised patients. Intravenous antiviral therapy must be proposed for a period of 14–21 days, after which 4 g daily acyclovir or 3 g daily valacyclovir is necessary for an additional period of 1–3 months before regular tapering. The total duration of antiviral therapy is controversial but long-term therapy is the only possible way to avoid further relapses, especially in patients with monophthalmus status. After antiviral therapy, infection of the fellow eye is reduced from 70 % to 13 % in the first year [52].

Anti-inflammatory molecules are still under discussion in ARN syndrome. Vitritis and retinal vasculitis are due to secondary inflammation and not to cytopathic effects. The use of antivirals without the management of secondary inflammation may control viral inflammation with irreversible inflammatory macular or optic nerve damages. However, ophthalmologists should be aware of a possible activation of virus by corticosteroids leading to increased viral replication and further ocular complications. Corticosteroids should not be administered in the absence of antivirals. Steroids have been used in different atypical cases of ARN syndrome when the diagnosis was unknown for a few days or weeks, inducing major complications leading to blindness despite further aggressive antiviral therapy. This highlights the importance of a rapid confirmation of a viral agent and initiation of antiviral therapy. Steroids may be initiated at 0.5–1 mg/kg/day with progressive tapering. In some cases, intravenous pulses of methylprednisolone are administered during the first 3 days, then relayed with high dose oral prednisone. Steroids are not indicated in immunocompromised patients. The effects of anticoagulants and aspirin on occlusive vasculopathy and vasculitis remain controversial. Their influence on the course of the disease is not clear. Anti-inflammatory treatment of anterior segment uveitis is usually effective.

Retinal detachment may occur in patients with viral retinitis [68]. Rapidly initiated antiviral and anti-inflammatory strategies may avoid retinal detachment in the majority of cases. Retinal detachment occurs in more than 75 % of untreated cases within a period of 12 weeks from the onset of retinitis. Scleral buckling, pars plana vitrectomy and long-term internal tamponade with silicone oil allow good anatomic success but visual function remains impaired after macular detachment or optic neuropathy. Retinal detachment prophylaxis has been evaluated [28]. Laser photocoagulation may be efficient in the presence of peripheral retinal tears. Anti-inflammatory therapy before photocoagulation is important for reducing vitreoretinal tractions.

10.7.2.2 CMV Retinitis

More than other viral retinopathies in immunocompromised patients, the therapeutic management of patients with CMV retinitis requires close collaboration between ophthalmologists and infectious disease physicians. Multiple anti-CMV strategies have been proposed since the beginning of AIDS.

In AIDS patients, the immune status should be improved with the introduction of highly active antiretroviral therapy (HAART), combining reverse transcriptase inhibitors and antiprotease medication. In an average period of 3 months, the CD4+ cell count is increased and the level of HIV replication is significantly reduced. The prevalence of all opportunistic infections, especially CMV retinitis, has dramatically decreased since the introduction of HAART. Before immune reconstitution, CMV retinitis should be treated with systemic or local anti-CMV medications [34].

Ganciclovir (DHPG), valganciclovir, foscarnet and cidofovir are the major anti-CMV molecules available. Treatment is based on an induction course of 3–4 weeks' duration. Ganciclovir or foscarnet is administered at, respectively, 5–10 mg/kg per 12 h and 90–120 mg/kg per 12 h. Fundus examination is performed to confirm viral control before proposing maintenance therapy at 5–10 mg/kg/day and 90–120 mg/kg/day, respectively. The total duration of maintenance therapy is not clearly defined. However, it should be followed until immune reconstitution, which may take more than 3 months. For any relapse, a new induction course is started until complete cicatrization. Ganciclovir, as the first anti-CMV drug, was introduced in 1984. Hematotoxicity is the main side effect and neutropenia may be controlled using granulocyte colony-stimulating factor (G-CSF). Oral ganciclovir was proposed for maintenance therapy before the introduction of valganciclovir. The biodisponibility of the latter is excellent and allows better compliance, giving a possibility for induction or maintenance regimens. Foscarnet requires intravenous administration and nephrotoxicity is its main side effect.

Because of their inconvenience when administered intravenously, cidofovir was proposed during the late 1990s. The drug was given IV weekly during the induction phase and every 2 weeks during the maintenance therapy. However, nephrotoxicity is still a major side effect, requiring probenecid and hydration. Anterior uveitis (Fig. 10.8) and hypotony remain the main problems observed today in patients with immune reconstitution and controlled CMV disease but presenting complications such as phtysis bulbi.

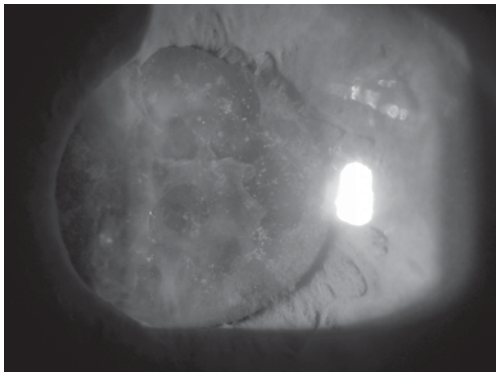

Fig. 10.8. Non-granulomatous anterior segment inflammation in an HIV-infected patient treated with cidofovir for CMV retinitis

Local anti-CMV therapy has been developed in patients who cannot tolerate prolonged intravenous therapy. However, local therapy will not control systemic CMV disease. Intravitreal injections of ganciclovir may be associated with oral valganciclovir. Intravitreal injections of ganciclovir may be performed twice and once weekly for the induction and maintenance phases, respectively. Intravitreal administration of cidofovir has major side effects such as hypotony and uveitis and is no longer recommended.

The ganciclovir intraocular implant was a sustained-release device providing continuous high intraocular levels of the drug. Relapses were significantly inhibited and progression of retinitis occurred after 221 days with the implant and 71 days with intravenous ganciclovir. The mean duration of drug delivery is 6 months. Due to the dramatic decrease of CMV retinitis in patients on HAART, since 2003 the implant has no longer been manufactured.

Another promising strategy based on antisense molecules has been used since just after the introduction of HAART. Fomivirsen is an antisense oligonucleotide that inhibits viral replication. Its use has been limited by the decrease incidence of CMV retinitis and the development of more convenient antiviral strategies.

In the pre-HAART era, visual morbidity was substantial [31]. However, ocular complications

have been reported in the HAART era despite healed CMV retinitis [26].

Retinal detachment is a possible complication of CMV retinitis. In the pre-HAART era, the incidence was estimated at between 18% and 29%. Multiple or single holes, as well as microholes, were observed in areas of retinal necrosis leading to complex retinal detachment. Recurrences seem to occur after exclusive external surgical procedures. Vitrectomy, localized scleral buckling, endophotocoagulation and silicone oil tamponade allow good anatomic results. Poor functional outcome is related to an optic nerve vascular disease or the size of retinal necrosis.

10.7.2.3 Immune Recovery Uveitis

Inflammation plays a major role in the development of IRU. Systemic or periocular injections of steroids are the main therapeutic strategies to reduce ocular inflammation (Fig. 10.9) but must be administered under clinical control of HIV load and CD4+ cell counts.

10.7.2.4 Non-Necrotizing Herpetic Retinopathies

Atypical forms of posterior uveitis may be associated with viral replication. High dose steroids and conventional immunosuppressors fail to control ocular inflammation. After viral confirmation, the use of intravenous acyclovir or oral valacyclovir reduces inflammation and allows the discontinuation of immunosuppressors. However, low-dose oral prednisone is necessary in the majority of cases. Alpha interferon may be proposed in severe cases.

Summary for the Clinician

- **The implication of herpes viruses in ocular inflammatory diseases has been recently characterized by the use of molecular techniques applied to ocular fluids**
- **Herpes viruses are highly adapted opportunist agents that use several ways to evade immune regulation and to establish latency**
- **The status of the host's immune system largely defines the outcome of viral infections and their complications**

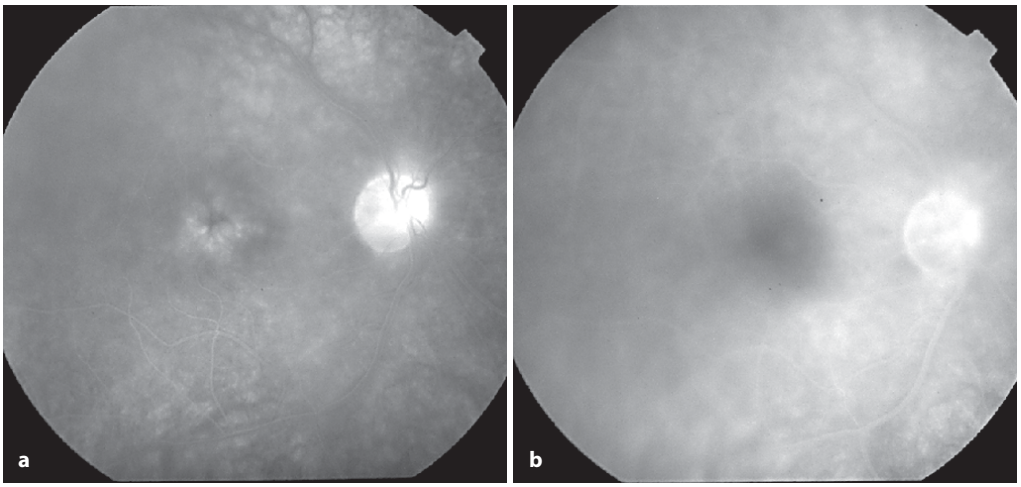

Fig. 10.9. a, b Fluorescein angiography showing cystoid macular oedema during immune recovery uveitis (**a**). Improvement is seen 1 month after periocular subtenon injections of triamcinolone acetonide (**b**)

- **Emerging entities have been reported and the spectrum of viral-induced diseases involving the eye has been enlarged**
- **Systemic specific antiviral drugs should be proposed rapidly in order to control viral replication before the use of corticosteroids**

References

1. Abe T, Sato M, Tamai M (1998) Correlation of varicella-zoster virus copies and final visual acuities of acute retinal necrosis syndrome. Graefes Arch Clin Exp Ophthalmol 236:747–752
2. Abe T, Sato M, Tamai M (2000) Variable R1 region in varicella zoster virus in fulminant type of acute retinal necrosis syndrome. Br J Ophthalmol 84:193–198
3. Abe T, Tsuchida K, Tamai M (1996) A comparative study of the polymerase chain reaction and local antibody production in acute retinal necrosis syndrome and cytomegalovirus retinitis. Graefes Arch Clin Exp Ophthalmol 234:419–424
4. Atherton S, Pesicka G, Streilein J (1987) Retinitis and deviant immune responses following intravitreal inoculation of HSV-1. Invest Ophthalmol Vis Sci 28:859–866
5. Barequet IS, Li Q, Wang Y, O'Brien TP, Hooks JJ, Stark WJ (2000) Herpes simplex virus DNA identification from aqueous fluid in Fuchs heterochromic iridocyclitis. Am J Ophthalmol 129: 672–673
6. Bassili S, Peyman G, Gebhardt B, Daun M, Ganibang J (1996) Detection of Epstein-Barr virus DNA by polymerase chain reaction in the vitreous of a patient with Vogt-Koyanagi-Harada syndrome. Retina 16:160–161
7. Bloch-Michel E, Dussaix E, Cerquetti P, Patarin D (1987) Possible role of cytomegalovirus in the etiology of the Posner-Schlossmann syndrome. Int Ophthalmol 11:95–96
8. Bodaghi B, Goureau O, Topilko A, Zipeto D, Laurent L, Virelizier J, Michelson S (1999) Pathogenesis of human cytomegalovirus retinitis: replication in retinal pigment epithelial cells is regulated by IFN-induced indoleamine 2, 3-dioxygenase but not inducible NO-synthase. J Immunol 162:957–964
9. Bodaghi B, LeHoang P (2002) Testing ocular fluids in uveitis. Ophthalmol Clin North Am 15:271–279
10. Bodaghi B, Rozenberg F, Cassoux N, Fardeau C, LeHoang P (2003) Non-necrotizing herpetic retinopathies masquerading as severe posterior uveitis. Ophthalmology 110:1737–1743
11. Bodaghi B, Slobbe-van Drunen M, Topilko A, et al. (1999) Entry of human cytomegalovirus into retinal pigment epithelial and endothelial cells by endocytosis. Invest Ophthalmol Vis Sci 40:2598–2607
12. Cassoux N, Lumbroso L, Bodaghi B, Zazoun L, Katlama C, LeHoang P (1999) Cystoid macular edema and cytomegalovirus retinitis in patients with HIV disease treated with highly active antiretroviral therapy. Br J Ophthalmol 83:47–49
13. Culbertson WW, Blumenkranz MS, Haines H, Gass DM, Mitchell KB, Norton EW (1982) The acute retinal necrosis syndrome, part 2: Histopathology and etiology. Ophthalmology 89:1317–1325
14. Cunningham ET Jr, Short GA, Irvine AR, Duker JS, Margolis TP (1996) Acquired immunodeficiency syndrome-associated herpes simplex virus retinitis. Clinical description and use of a polymerase chain reaction-based assay as a diagnostic tool. Arch Ophthalmol 114:834–840
15. De Boer JH, Verhagen C, Bruinenberg M, et al. (1996) Serologic and polymerase chain reaction analysis of intraocular fluids in the diagnosis of infectious uveitis. Am J Ophthalmol 121:650–658
16. Detrick B, Rhame J, Wang Y, Nagineni C, Hooks J (1996) Cytomegalovirus replication in human retinal pigment epithelial cells. Altered expression of viral early proteins. Invest Ophthalmol Vis Sci 37:814–825
17. Dunkel E, De Freitas D, Siegel M, Zau Q, Whitley R, Schaffer P (1993) A rabbit model for human cytomegalovirus induced chorioretinal disease. J Infect Dis 168:336–344
18. Fisher JP, Lewis ML, Blumenkranz M, et al. (1982) The acute retinal necrosis syndrome, part 1: Clinical manifestations. Ophthalmology 89:1309–1316
19. Forster DJ, Dugel PU, Frangieh GT, Liggett PE, Rao NA (1990) Rapidly progressive outer retinal necrosis in the acquired immunodeficiency syndrome. Am J Ophthalmol 110:341–348
20. Fox GM, Crouse CA, Chuang EL, Pflugfelder SC, Cleary TJ, Nelson SJ, Atherton SS (1991) Detection of herpesvirus DNA in vitreous and aqueous specimens by the polymerase chain reaction. Arch Ophthalmol 109:266–271
21. Freilich JM, Ryan EA, Lou PL, Kroll AJ, Brockhurst RJ, Harooni M (1996) Acute retinal necrosis syndrome: findings, management, and differential diagnosis. Int Ophthalmol Clin 36:141–146
22. Ganatra JB, Chandler D, Santos C, Kuppermann B, Margolis TP (2000) Viral causes of the acute retinal necrosis syndrome. Am J Ophthalmol 129:166–172
23. Garweg J, Bohnke M (1997) Varicella-zoster virus is strongly associated with atypical necrotizing herpetic retinopathies. Clin Infect Dis 24:603–608

24. Gaynor BD, Margolis TP, Cunningham ET Jr (2000) Advances in diagnosis and management of herpetic uveitis. Int Ophthalmol Clin 40:85–109
25. Gershon A, Steinberg S (1981) Antibody response to varicella-zoster virus and the role of antibody in host defense. Am J Med Sci 282:12–17
26. Goldberg DE, Wang H, Azen SP, Freeman WR (2003) Long term visual outcome of patients with cytomegalovirus retinitis treated with highly active antiretroviral therapy. Br J Ophthalmol 87: 853–855
27. Groh M, Seitz B, Schumacher S, Naumann G (1999) Detection of herpes simplex virus in aqueous humor in iridocorneal endothelial (ICE) syndrome. Cornea 18:359–360
28. Han DP, Lewis H, Williams GA, Mieler WF, Abrams GW, Aaberg TM (1987) Laser photocoagulation in the acute retinal necrosis syndrome. Arch Ophthalmol 105:1051–1054
29. Herpetic EDSG (1998) Acyclovir for the prevention of recurrent herpes simplex virus eye disease. N Engl J Med 339:300–306
30. Hoang-Xuan T, Buchi E, Herbort C (1992) Oral acyclovir for herpes zoster ophthalmicus. Ophthalmology 99:1062–1071
31. Holbrook JT, Jabs DA, Weinberg DV, Lewis RA, Davis MD, Friedberg D (2003) Visual loss in patients with cytomegalovirus retinitis and acquired immunodeficiency syndrome before widespread availability of highly active antiretroviral therapy. Arch Ophthalmol 121:99–107
32. Holland G (1994) Executive committee of the American Uveitis Society. Standard diagnostic criteria for the acute retinal necrosis syndrome. Am J Ophthalmol 117:663–667
33. Holland G, Fang E, Glasgow B, et al. (1990) Necrotizing retinopathy after inoculation of murine cytomegalovirus in immunosuppressed adult mice. Invest Ophthalmol Vis Sci 31:2326–2334
34. Holland G, Tufail A, Jordan M (1996) Cytomegalovirus diseases. In: Pepose J, Holland G, Wilhelmus K (eds) Ocular infection and immunity. Mosby, St. Louis, pp 1088–1128
35. Hooks J, Kupfer C (1995) Herpes simplex virus in iridocorneal endothelial syndrome. Arch Ophthalmol 113:1226–1228
36. Itoh N, Matsumura N, Ogi A, Nishide T, Imai Y, Kanai H, Ohno S (2000) High prevalence of herpes simplex virus type 2 in acute retinal necrosis syndrome associated with herpes simplex virus in Japan. Am J Ophthalmol 129:404–405
37. Jabs DA, Van Natta ML, Kempen JH, Reed Pavan P, Lim JI, Murphy RL, Hubbard LD (2002) Characteristics of patients with cytomegalovirus retinitis in the era of highly active antiretroviral therapy. Am J Ophthalmol 133:48–61
38. Karavellas MP, Azen SP, MacDonald JC, et al. (2001) Immune recovery vitritis and uveitis in AIDS: clinical predictors, sequelae, and treatment outcomes. Retina 21:1–9
39. Kaufman HE, Varnell ED, Thompson HW (1999) Latanoprost increases the severity and recurrence of herpetic keratitis in the rabbit. Am J Ophthalmol 127:531–536
40. Knox CM, Chandler D, Short GA, Margolis TP (1998) Polymerase chain reaction-based assays of vitreous samples for the diagnosis of viral retinitis. Use in diagnostic dilemmas. Ophthalmology 105:37–44; discussion 44–45
41. Kumano Y, Manabe J, Hamamoto M, Kawano Y, Minagawa H, Fukumaki Y, Inomata H (1995) Detection of varicella-zoster virus genome having a PstI site in the ocular sample from a patient with acute retinal necrosis. Ophthalmic Res 27:310–316
42. Labetoulle M, Kucera P, Ugolini G, Lafay F, Frau E, Offret H, Flamand A (2000) Neuronal pathways for the propagation of herpes simplex virus type 1 from one retina to the other in a murine model. J Gen Virol 81:1201–1210
43. Labetoulle M, Kucera P, Ugolini G, Lafay F, Frau E, Offret H, Flamand A (2000) Neuronal propagation of HSV1 from the oral mucosa to the eye. Invest Ophthalmol Vis Sci 41:2600–2606
44. Labetoulle M, Maillet S, Efstathiou S, Dezelee S, Frau E, Lafay F (2003) HSV1 latency sites after inoculation in the lip: assessment of their localization and connections to the eye. Invest Ophthalmol Vis Sci 44:217–225
45. Levinson RD, Hooks JJ, Wang Y, Chiu MT, Kellaway J, Chan CC (1998) Triple viral retinitis diagnosed by polymerase chain reaction of the vitreous biopsy in a patient with Richter syndrome. Am J Ophthalmol 126:732–733
46. Liu W, Kuppermann BD, Martin DF, Wolitz RA, Margolis TP (1998) Mutations in the cytomegalovirus UL97 gene associated with ganciclovir-resistant retinitis. J Infect Dis 177:1176–1181
47. Markomichelakis NN, Canakis C, Zafirakis P, Marakis T, Mallias I, Theodossiadis G (2002) Cytomegalovirus as a cause of anterior uveitis with sectoral iris atrophy. Ophthalmology 109:879–882
48. Mocarski E (1993) Cytomegalovirus biology and replication. In: Roizman B, Lopez C (eds) The human herpesviruses. Raven Press, New York, pp 173–225
49. Mochizuki K, Matsushita H, Hiramatsu Y, Yanagida K (1998) Detection of varicella-zoster virus genome in the vitreous humor from two patients with acute retinal necrosis; lacking or having a PstI cleavage site. Jpn J Ophthalmol 42:208–212
50. Nahmias A (1990) Sero-epidemiological and sociological patterns of herpes simplex virus infection in the world. Scand J Infect Dis (Suppl) 69:19–36

51. Oh J (1976) Primary and secondary herpes simplex uveitis in rabbits. Surv Ophthalmol 21:178–184
52. Palay DA, Sternberg P Jr, Davis J, et al. (1991) Decrease in the risk of bilateral acute retinal necrosis by acyclovir therapy. Am J Ophthalmol 112: 250–255
53. Pendergast SD, Werner J, Drevon A, Wiedbrauk DL (2000) Absence of herpesvirus DNA by polymerase chain reaction in ocular fluids obtained from immunocompetent patients. Retina 20:389–393
54. Roizman B, Sears A (1996) Herpes simplex viruses and their replication. In: Fields B, Knipe D, Howley P (eds) Virology. Raven Publishers, Philadelphia, pp 2231–2295
55. See RF, Rao NA (2002) Cytomegalovirus retinitis in the era of combined highly active antiretroviral therapy. Ophthalmol Clin North Am 15:529–536, viii
56. Smith IL, Macdonald JC, Freeman WR, Shapiro AM, Spector SA (1999) Cytomegalovirus (CMV) retinitis activity is accurately reflected by the presence and level of CMV DNA in aqueous humor and vitreous. J Infect Dis 179:1249–1253
57. Song MK, Azen SP, Buley A, et al. (2003) Effect of anti-cytomegalovirus therapy on the incidence of immune recovery uveitis in AIDS patients with healed cytomegalovirus retinitis. Am J Ophthalmol 136:696–702
58. Spaide R, Sugin S, Yannuzzi L, DeRosa J (1991) Epstein-Barr virus in multifocal choroiditis and panuveitis. Am J Ophthalmol 112:410–413
59. Tran TH, Rozenberg F, Cassoux N, Rao NA, LeHoang P, Bodaghi B (2003) Polymerase chain reaction analysis of aqueous humor samples in necrotising retinitis. Br J Ophthalmol 87:79–83
60. Urayama A, Yamada N, Sasaki T, et al. (1971) Unilateral acute uveitis with retinal periarteritis and detachment. Jpn J Ophthalmol 25:607–629
61. Van der Lelij A, Ooijman FM, Kijlstra A, Rothova A (2000) Anterior uveitis with sectoral iris atrophy in the absence of keratitis: a distinct clinical entity among herpetic eye diseases. Ophthalmology 107:1164–1170
62. Van der Lelij A, Rothova A (1997) Diagnostic anterior chamber paracentesis in uveitis: a safe procedure? Br J Ophthalmol 81:976–979
63. Van Gelder R, Willig J, Holland G, Kaplan H (2001) Herpes simplex virus type 2 as a cause of acute retinal necrosis syndrome in young patients. Ophthalmology 108:869–876
64. Van Gelder RN (2001) Applications of the polymerase chain reaction to diagnosis of ophthalmic disease. Surv Ophthalmol 46:248–258
65. Verbraak FD, Galema M, van den Horn GH, Bruinenberg M, Luyendijk L, Danner SA, Kijlstra A (1996) Serological and polymerase chain reaction-based analysis of aqueous humor samples in patients with AIDS and necrotizing retinitis. Aids 10:1091–1099
66. Von Szily A (1924) Experimentelle endogene Infektionsübertragung von Bulbus zu Bulbus. Klin Monatsbl Augenheikl 72:593–602
67. Wand M, Gilbert CM, Liesegang TJ (1999) Latanoprost and herpes simplex keratitis. Am J Ophthalmol 127:602–604
68. Weinberg D, Lyon A (1997) Repair of retinal detachments due to herpes varicella-zoster virus retinitis in patients with acquired immune deficiency syndrome. Ophthalmology 104:279
69. Whitcup SM, Fortin E, Lindblad AS, et al. (1999) Discontinuation of anticytomegalovirus therapy in patients with HIV infection and cytomegalovirus retinitis. JAMA 282:1633–1637
70. Whitley R (1996) Herpes simplex viruses. In: Fields B, Knipe D, Howley P (eds) Virology. Raven Publishers, Philadelphia, pp 2297–2342
71. Yamamoto Y, Hill J (1986) HSV-1 recovery from ocular tissues after viral inoculation into the superior cervical ganglia. Invest Ophthalmol Vis Sci 27:1447–1452

Cytomegalovirus and the Eye

Marc D. de Smet

Core Messages

- Cytomegalovirus (CMV) retinitis is less common than prior to the introduction of highly active anti-retroviral therapy (HAART) in AIDS patients. However, it remains an important AIDS defining infection
- Choice of therapy and length of use is dependent on the response to HAART therapy, and time since its introduction
- Elimination of maintenance therapy is possible when the CD4 count is above 100 cells/µl with a sustained increase and the patient is on HAART for at least 6 months
- Some patients will develop recurrent disease despite high CD4 counts
- CMV may be present in other immunosuppressive states and require sustained therapy

11.1 Introduction

In immunosuppressed patients, or those having specifically lost their cellular immunity against the cytomegalovirus (CMV) virus, a retinal infection can develop. In the 1980s and early 1990s, CMV retinitis was a common cause of blindness in AIDS patients. Its incidence has dramatically decreased in the last few years in large part due to the introduction of highly active anti-retroviral therapy (HAART). HAART has also led to a new syndrome of HAART associated ocular inflammation which might be related to the presence of CMV antigens in the eye.

CMV is not only found in AIDS patients, but can be found in any immunosuppressed patients. Usually witnessed while the patient is under active immunosuppression, we have recently become aware that even a history of past immunosuppresion may be compatible with the late occurrence of active CMV retinitis, without any overt signs of immunodeficiency.

11.2 Populations at Risk

Screening, treatment, and follow-up protocols are dependent on the origin and nature of the disease. CMV retinitis is seen in two particular settings as an AIDS related infection, or as a result of immunosuppression. Both populations present certain characteristics of incidence and risk.

11.2.1 HIV Associated Immunosuppression

CMV was among the most common opportunistic infections in patients with AIDS. Prior to HAART, CMV retinitis accounted for 75–85 % of cytomegalovirus disease in patients with AIDS, with an estimated lifetime risk of CMV retinitis of 30 % [1]. Since the introduction of HAART, the incidence of CMV retinitis has dropped by 80 %, and has stabilized at about 15 % of the population at risk, mainly as an AIDS defining infection.

The only reported systemic factor significantly associated with CMV retinitis is a low CD4+ T-cell count. The prevalence rate of CMV

retinitis in AIDS patients with a CD4+ T-cell count <50 cells/μl is estimated at 30 % and patients with a CD4+ T-cell count >100 cells/μl are felt to be at very low risk [2]. In the setting of HAART therapy, screening and/or treatment should continue for 3–6 months after a rise in CD4 count, as the recovery of specific immunity lags behind the recovery of CD4+ T-cell counts. Thereafter when the rise in CD4+ cells is sustained with a low HIV mRNA load, it is possible to consider withdrawal of anti-viral treatment [3, 4].

Withdrawal may be unsuccessful or risky in the following three settings. (1) Some patients have a persistent deficiency in specific anti-CMV CD4+ cells. These patients are at risk for persistent and recurrent ocular disease despite high CD4+ T-cell counts [5]. Frequent follow-up will be required. (2) Some patients will fail HAART therapy due to drug intolerance or the development of viral resistance. In these patients, if they were retinitis free for some time, the risk of retinitis recurrence is low but has been reported [6]. Systemic anti-CMV titres may be of some help in follow-up [7]. (3) In some patients, HAART treatment is insufficient to maintain an increased immunologic response. These patients are at high risk of persistent ocular disease. While ocular disease can be controlled by local means, survival will be significantly improved by systemic anti-CMV therapy [8].

Summary for the Clinician

- **CD4+ counts below 50 cells/μl indicate a high risk for CMV**
- **CD4+ counts above 100 cells/μl indicate a low risk for CMV**

11.2.2 Immunosuppression in Non-HIV Settings

Patients being immunosuppressed for organ transplantation are at a high risk of CMV infection. Initially, these patients are carefully followed for the development of systemic CMV antigenemia, and are given when needed prophylactic treatment with antivirals. In patients with increasing CMV titres, particularly those at high risk for CMV infection, retinitis may develop in spite of these measures. Usually symptomatic, they may require prolonged treatment [9]. CMV may manifest itself at a later time, even 1–2 years after stopping immunosuppressives [10]. When CMV develops, its clinical course and manifestations are indistinguishable from early disease. It is not associated with traditional risk factors. A female preponderance has been suggested due to a hormonal predisposition. Clinical awareness and adequate patient instructions are most commonly used to detect this late CMV manifestation. In some centers, an extended antigenemic surveillance program has allowed detection of late disease [10, 11].

Another group at increased risk are patients with autoimmune disease, particularly collagen vascular diseases requiring intensive immunosuppression. CMV may develop with any immunosuppressive, including cyclosporine or tacrolymus, but is particularly associated with the use of cyclophosphamide, which has a generalized action on T and B cells. Patients who are lymphopenic, with low CD4 and or CD8 counts, are at particular risk. While there is often a temporal relationship to recent cyclophosphamide use, this is not always the case as patients may develop an active retinitis even months later [12]. These patients usually present with a generalized reduction of their cellular immunity sometimes related to the use of other medications such as antiviral agents.

Summary for the Clinician

- **Stop or reduce immunosuppression to recover specific immunity to CMV**
- **In post-transplant patients, and collagen vascular disease, late CMV may occur months after stopping severe immunosuppresive therapy.**

11.3 Appearance and Course

CMV retinitis has a fairly characteristic appearance which varies slightly depending on its location in the eye. Initially, infected retinal tissue is transparent, but as viral replication increases within infected cells, the translucency is lost and is replaced by a white lesion. This gives CMV its characteristic appearance: a white granular retinal infiltrate, often in the form of a brushfire border (Fig. 11.1). High levels of viral replication ultimately lead to cell death, leaving in its wake a zone of diaphenous thinned atrophic retina. The retinal pigment epithelium (RPE) is also affected, resulting in a granular, speckled RPE. Limited areas of fibrous proliferation, even calcification are occasionally seen within the area of atrophy. Invariably, the retinal vessels are attenuated in the atrophic areas, as the oxygen requirement in these areas is limited (Fig. 11.2).

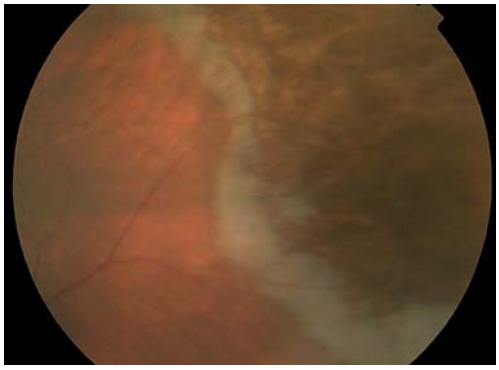

Fig. 11.1. This image depicts a typical active CMV lesion which consists of a peripheral atrophic retina, followed by a white active lesion, more or less confluent. Small satellite lesions are often found in normal retina ahead of the active frond

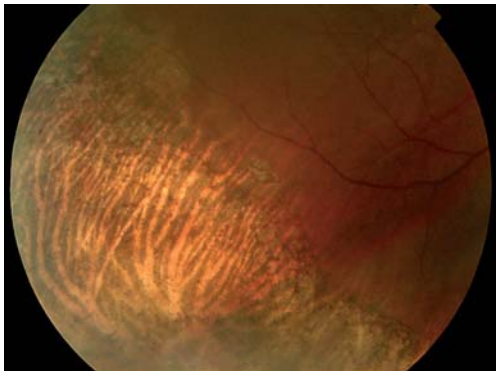

Fig. 11.2. Following treatment, there is a sharp demarcation between normal and abnormal retina.

11.3.1 Manifestation Prior to Initial Treatment

Often asymptomatic, CMV retinitis may be associated with a complaint of increasing floaters. If the disease is present centrally, it will lead to a permanent noticeable decrease in vision. Most CMV lesions do not start in the fovea, but are initially found somewhere in the peripheral retina, or along the arcades. In AIDS patiens not receiving HAART, untreated CMV retinitis will progress at 750 µm/week [13]. In other immunosuppressed patients, the progression rate is less, and is related to the level of immunocompetence. Bilaterality at presentation is common in AIDS patients, with one eye being more severely affected. In young children, the first eye is often blind at presentation, unless these children were part of a well established screening program. The extent of initial disease appears to have some prognostic implications with regards to response to treatment and risk of recurrence [14].

Initially, CMV lesions present as small white infiltrates, which are often difficult to distinguish from a cotton wool spot (Fig. 11.3) [15]. However, with time the CMV lesion will grow centripetally, leaving in its wake atrophic retina. When lesions develop close to, or within, the arcades, they are often associated with retinal haemorrhages, while in peripheral lesions these haemorrhages are absent (Fig. 11.4). Satellite or skip lesions at the leading edge of the active infiltrate are a common feature. This leading edge varies in diameter but can reach 500–700 µm. Retinal edema, characteristic of zoster infection, is absent.

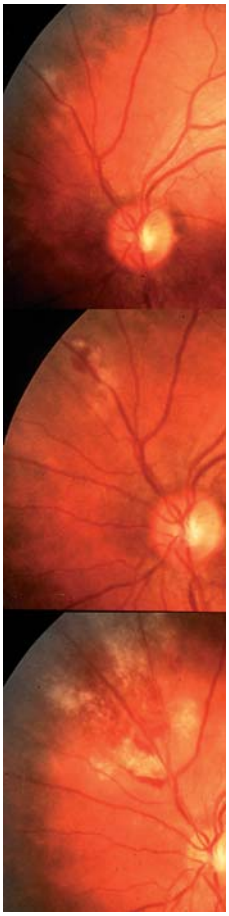

Fig. 11.3. Initial small CMV lesions can be difficult to distinguish from cotton wool spots or small areas of atrophy. However, without treatment, they increase in size over a few weeks. Photographic documentation facilitates follow-up as in this case which demonstrates progression of a CMV lesion over a 3-week period

11.3.2 Manifestations Under Therapy

Treatment will lead to a progressive decrease in the diameter of the leading edge, though extension in the initial 2 weeks is to be expected as viral replication in already infected retina leads to opacification of the retinal tissue. A progression of 750 µm or more is not incompatible with an adequate response. Response to treatment will be visible only after 1–2 weeks of treatment. Once the acute infection resolved, a sharp edge will appear between healthy and atrophic retina.

Recurrences in AIDS patients under maintenance therapy, or in the setting of a partially functional immune system, may show little evidence of activity. The leading edge creeps progressively closer to the fovea (Fig. 11.5). Diagnosis of such progression necessitates a sharp clinical acumen, or preferably a close comparison of the current clinical picture to fundus photos taken some time before. If photos cannot be taken, the chart should document the relationship between the leading edge of the lesion and neighbouring landmarks such as vessels.

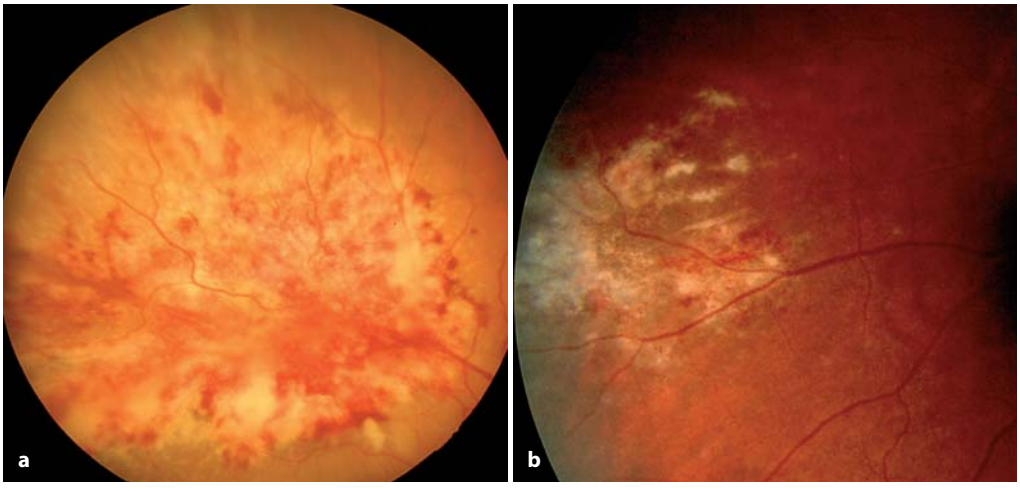

Fig. 11.4. a, b Central CMV retinitis (**a**) is often associated with more hemorrhage than peripheral lesions (**b**)

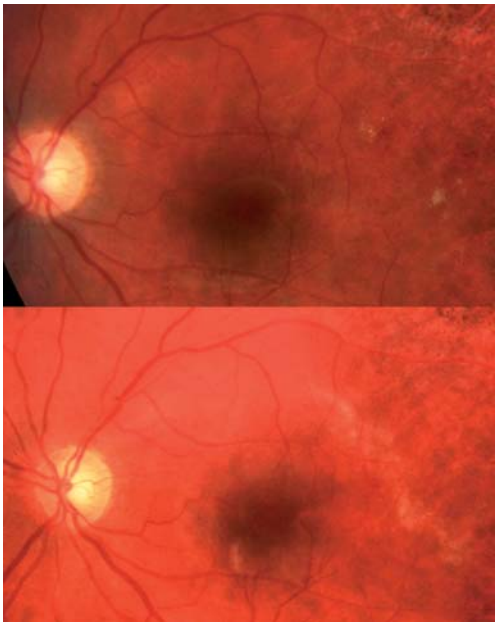

Fig. 11.5. Anti-viral therapy can allow progression to occur without any significant border activity. A thin zone of reactivation associated with a larger atrophic zone is the only sign of ongoing activity in these patients. Photographs were taken 1 month apart

Summary for the Clinician

- **In treated patients, CMV retinitis may remain active as a "brush fire" border**
- **The long-term follow-up is provided by photographic documentation with a panoramic set of photos of the whole retina**

11.3.3 Differential Diagnosis

In its classical presentation, CMV retinitis is easy to diagnose. However, it may simulate (or be masked by) a number of ocular conditions from which it can be distinguished by its presentation, time course, or response to treatment.

When CMV retinitis starts adjacent to a blood vessel, it can either cause a vascular occlusion with the typical arcuate haemorrhages seen along the course of an occluded branch retinal vein (BRVO), or it may present as a frosted angiitis. In the latter case, the vessels show extensive sheathing extending far into the periphery [16]. There may be little associated retinitis, but in the course of the subsequent week, along the edge of the major vessels, the typical white leading edge of retinitis will appear. In BRVO, there is usually a distinguishable focus of retinitis close to the site of occlusion. Here on fluorescein angiogram, the central area of necrosis will be surrounded by a zone of hyperfluorescence, and may help differentiate the lesion from a simple vein occlusion.

Patients may also present with a zone of active retinitis adjacent to the optic nerve. Differentiating this lesion from an optic neuritis may be difficult. The optic nerve head often will become hyperemic, but vision and visual fields are usually preserved until the optic nerve becomes infected with CMV [17]. Rapid initiation of therapy is required. Optic neuritis may be a manifestation of other viral infections such as zoster retinitis where rapid initiation of therapy is also required [18, 19]. Often these patients have a more significant visual impairment or have other manifestations of disease.

There is usually little difficulty in differentiating CMV retinitis from other ocular infections. Zoster retinitis presents as a confluent peripheral retinitis with considerable retinal edema. In immunocompetent patients, there is usually severe vitreous inflammation, an unusual manifestation in AIDS patients. A more common diagnostic problem is the differentiation of toxoplasmosis from CMV, which in AIDS patients can present with a similar white granular lesion. This lesion is usually static, and will show no response to antiviral treatment. Note that some patients will present with several concurrent infections, which all require treatment if vision is to be preserved [19]. Luckily such cases are rare. Diagnostic tests can be of some help in such cases.

Summary for the Clinician

- **Toxoplasmosis may mimic a CMV lesion in AIDS patients**
- **CMV may be associated with BRVO, optic neuritis**
- **Early CMV lesions can mimic a cotton wool spot**

11.3.4 Investigations

It will generally not be necessary to confirm the diagnosis with diagnostic tests. The most useful approach is to combine the measurement of ocular antibody production with PCR for CMV genome [20, 21]. Ocular antibody production against CMV is compared to serum levels. A Goldman-Witmer coefficient above 3 is indicative of active antibody production. Several subtypes should be analysed simultaneously (IgM, IgG and IgA) as any one of them could be elevated. While PCR is most likely to be positive in most CMV infections [22], if there is little inflammation present in the anterior chamber, it may be negative. The highest sensitivity is obtained by combining both techniques.

Viral CMV loads in serum do not appear to correlate with ocular disease progression, though its presence indicates the presence of active disease [23]. Subtyping can be of use if the lesions does not seem to respond well to treatment, or if drug resistance is common in your patient population. Subtyping to aimed at identifying ganciclovir resistant strains which may indicate an increased risk for retinitis progression [24].

11.4 Therapeutic Agents

11.4.1 Ganciclovir

Ganciclovir is the antiviral drug most widely studied for the treatment of CMV retinitis and is effective when administered by intravenous, intravitreal, or oral routes, or when released in the pars plana from a slow release implant. The systemic route is associated with neutropenia, which limits its use. IV it is associated with local infections in a substantial number of patients (20 % of patients in one study), while the oral route has a low bioavailability, limiting it to maintenance therapy. Oral ganciclovir can lead to gastric intolerance, its main advantage at present is its ability to prolong survival in patients unresponsive to HAART, and in whom the eye is protected by local therapy [25]. Intravitreal injections can lead to local control in a high number of patients with a low risk of endophthalmitis provided adequate antisepsic measures are taken at the time of injection [26, 27]. As initial therapy, it should be given twice a week (standard dose of 200 μg). Maintenance is given on a weekly basis. By increasing the dose to 2 mg, it is possible to delay repeat injections to once every 2 weeks (Table 11.1) [28].

Table 11.1. Dosing requirements of anti-CMV drugs

Drug name	Dose	Frequency	Major side effect
Ganciclovir [47]	5 mg/kg IV 5 mg/kg IV	I-2×/day M-1×/day	Myelosuppression
or [28]	200–2000 μg IO in 0.1 ml	Weekly	
or [30]	Intraocular implant	Every 8 months	
or [48]	1,500 mg p.o.	M-3×/day	
Valganciclovir [31]	900 mg p.o. 900 mg p.o.	I-2×/day M-1×/day	Myelosuppression
Foscarnet [13]	90 mg/kg IV 60 mg/kg IV	I-3×/daily M-3×/day	Nephrotoxicity
Cidofovir [49]	5 mg/kg IV 3–5 mg/kg IV	I-1×/week ×2 M–Q 2 weeks	Nephrotoxicity Hypotony, AC uveitis
Fomivirsen [41]	330 μg IO 330 μg IO	I-1×/week M-1×/month	Uveitis increased IOP

The intravitreal implant capable of releasing drug for up to 8 months has been very popular in North America. It is associated with a high local response rate even in cases of systemic resistance to ganciclovir [29, 30].

11.4.2 Valganciclovir

Valganciclovir is a monovalyl ester prodrug of ganciclovir that when administered orally is rapidly hydrolysed to the active compound ganciclovir. The absolute bioavailability of ganciclovir from valganciclovir is 60 %. With an oral valganciclovir dose of 900 mg, given twice daily the same systemic levels of ganciclovir are achieved as with IV administered ganciclovir. In a randomized controlled clinical trial, oral valganciclovir was found to be as effective as intravenous ganciclovir for induction and maintenance therapy without having the inconvenience of IV ganciclovir. The side effects due to valganciclovir are identical to those of ganciclovir [31]. It is now the drug of choice for first line therapy.

11.4.3 Foscarnet

Foscarnet is the second most common treatment for CMV retinitis. This agent is usually given intravenously. It is not metabolized intracellularly as is ganciclovir. Thus, dosing must be given fairly frequently to ensure steady state intraocular concentrations [13]. It is not myelotoxic, but requires extensive hydration to prevent renal toxicity. As with ganciclovir, prolonged use can lead to the development of resistant strains, at a similar incidence rate [32]. When effectivity drops, a switch to an alternate treatment is needed, or one can consider the combined use of foscarnet and ganciclovir. These act synergistically [33, 34]. Unfortunately, this is a temporizing measure rather than a long-term solution, as the patient will suffer from the toxic effects of both drugs. One might consider using IV foscarnet combined with intraocular ganciclovir in this situation, if no other alternative is available. An intravitreal formulation also exists but requires frequent administrations to maintain effectivity [35].

11.4.4 Other Agents

Among the other drugs that can be used to treat CMV retinitis, one should mention cidofovir, and fomivirsen. Cidofovir is an acyclic nucleoside phosphonate which does not require a virus encoded phosphorylation step as does ganciclovir [36]. It is a very potent anti-CMV compound which can be given on a less frequent basis than ganciclovir or foscarnet. It is effective in cases of retinitis relapsing on either medication [37]. While the incidence of anterior uveitis is higher when given concomitantly with HAART, the induced anterior segment inflammation is easily controlled with drops [38]. Toxicity, renal and ocular, resolve with discontinuation of therapy. While it can be used by intravitreal injection, its use can lead to severe hypotony and uveitis [39].

Fomivirsen is a phosphorothioate oligonucleotide which specifically binds to the mRNA encoding for the immediate – early activation proteins of the CMV virus [40]. In two randomized clinical studies, it was shown to inhibit CMV retinitis by intravitreal injections. In its maintenance regimen, it can be given once a month [41–43].

11.5 Screening and Treatment Algorithms

CMV retinitis develops in patients who are immunosuppressed. Since all antivirals to date are virostatic, unless one can restore the patient's own immunity against CMV, antiviral treatment will have to be prolonged, possibly lifelong. If immune recovery is possible or can be induced, treatment can be discontinued and follow-up tailored to the observed response.

11.5.1 CMV in HIV Infected Patients

A distinction should be made between patients receiving HAART treatment and those who do not. Patients diagnosed with HIV infection and with low CD4+ counts <100 cells/μl should be screened and instructed regarding the signs and symptoms of CMV retinitis. Patients with CD4+ counts under <50 cells/μl should be screened roughly every 3–6 months. Above 100 CD4+ cells/μl frequent screening is unnecessary. The same algorithm holds for patients on HAART treatment, expect that for the first 6 months after achieving a CD4+ count equal or above 100 cells μl, the same protocol holds as for patients without HAART and a low CD4+ count.

Treatment choices will depend on available medications, and observed response. The preferred choice is presently oral valganciclovir. If it is not tolerated, placement of an intraocular implant would be the next choice. Other options are kept for more recalcitrant cases, or when the first two named options are unavailable. Follow-up is weekly until response is seen. Once the lesion is quiescent, follow-up can be on a monthly to 3 monthly basis. HAART status is also important in the choice of therapy. If a patient is HAART naive, treatment will likely be required for 6–12 months, after which it can be stopped. If CMV has occurred while on HAART, or after stopping HAART, the patient should be followed as a non-HAART patient.

11.5.2 CMV in Other Immunosuppressive States

Well-established algorithms do not exist for these patients. In general, an effort is made to reduce or stop the immunosuppressant causing the drop in immunity. As the lymphocyte counts improve, CMV retinitis will resolve. Local treatment with intravitreal agents (ganciclovir or fomivirsen) is the preferred approach, as it minimizes the potential immunosuppression which can be caused by systemic ganciclovir or valganciclovir.

If CMV retinitis develops as a late manifestation, months after stopping immunosuppressive agents, a ganciclovir implant, cidofovir, or fomivirsen are the preferred approaches.

11.6 Management of Complications

In addition to vision loss, CMV retinitis can lead to a number of complications ranging from cataract formation to the development of a retinal detachment. With the introduction of HAART, about 15% of patients will develop uveitis as the immune system recovers.

11.6.1 Immune Recovery

With the introduction of HAART, a number of patients with quiescent CMV retinitis started to develop signs of significant intraocular inflammation. This inflammatory response can be controlled in most patients with topical corticosteroids and is transient. However, in some patients it leads to the development of recalcitrant cystoid macular edema, the formation of epiretinal membranes, cataract or glaucoma [44]. The process is most likely due to an enhanced humoral immune response to ocular antigens [45]. The possibility that active viral replication is present has been suggested though not proven.

11.6.2 Retinal Detachment

Vision loss in CMV infection is either due to the retinitis itself, or as a result of retinal detachment. In the pre-HAART era, the risk of detachment was about 33% per affected eye per year. Lesion size and anterior location of the lesion were both associated with an increased risk, particularly at the time of posterior hyaloid separation. Introduction of HAART therapy has dramatically reduced this risk by about 60% [46]. This reduction is most significant in patients that do not develop immune recovery uveitis, as

this variable doubles the risk of detachment in HAART treated patients (but nevertheless represents a significant reduction in incidence as compared to non-HAART patients). The incidence of retinal detachment in patients receiving an implant does not appear to be higher than in patients receiving only systemic therapy.

Treatment options are dependent on the location of the detachment and response to HAART treatment. More conservative approaches such as placing a laser barrier around the detachment are often successful if the detachment is of limited size. More extensive detachments will require a pars plana vitrectomy. In non-HAART patients the use of silicone oil is required in most cases. For HAART patients, it can often be avoided provided there is no significant proliferative vitreoretinopathy present.

References

1. Jabs DA, van Natta ML, Kempen JH, et al. (2002) Characteristics of patients with cytomegalovirus retinitis in the era of highly active antiretroviral therapy. Am J Ophthalmol 133:48–61
2. Jabs DA, Enger C, Bartlett JG (1989) Cytomegalovirus retinitis and acquired immunodeficiency syndrome. Arch Ophthalmol 107:75–80
3. van den Horn GJ, Meenken C, Danner SA, et al. (1998) Effects of protease inhibitors on the course of CMV retinitis in relation to CD4+ lymphocyte responses in HIV+ patients. Br J Ophthalmol 82:988–990
4. Lin DY, Warren JF, Lazzeroni LC, et al. (2002) Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy in HIV-infected patients. Natural history and clinical predictors. Retina 22:268–277
5. Lilleri D, Piccinini G, Baldanti F, et al. (2003) Multiple relapses of human cytomegalovirus retinitis during HAART in an AIDS patient with reconstitution of CD4+ T cell count in the absence of HCMV-specific CD4+ T cell response. J Clin Virol 26:95–100
6. Torriani FJ, Freeman WR, Macdonald JC, et al. (2000) CMV retinitis recurs after stopping treatment in virological and immunological failures of potent antiretroviral therapy. AIDS 14:1041–1049
7. Salmon-Ceron D, Mazeron MC, Chaput S, et al. (2000) Plasma cytomegalovirus DNA, pp65 antigenemia and a low CD4 cell count remain risk factors for cytomegalovirus disease in patients receiving highly active antiretroviral therapy. AIDS 14:1041–1049
8. Kempen JH, Jabs DA, Wilson LA, et al. (2003) Mortality risk for patients with cytomegalovirus retinitis and acquired immune deficiency syndrome. Clin Infect Dis 37:1365–1373
9. Fishburne BC, Micrani AA, Davis JL (1998) Cytomegalovirus retinitis after cardiac transplantation. Am J Ophthalmol 125:104–106
10. Shibolet O, Ilan Y, Kalish Y, et al. (2003) Late cytomegalovirus disease following liver transplantation. Transpl Int 16:861–865
11. Machado CM, Menezes RX, Macedo MC (2001) Extended antigenemia surveillance and late cytomegalovirus infection after allogeneic BMT. Bone Marrow Transplant 28:1053
12. Agrawal A, Dick AD, Olson JA (2003) Visual symptoms in patients on cyclophosphamide may herald sight threatening disease. Br J Ophthalmol 87:122–123
13. Palestine AG, Polis MA, de Smet MD, et al. (1991) A randomized, controlled trial of foscarnet in the treatment of cytomegalovirus retinitis in patients with AIDS. Ann Intern Med 115:665–673
14. Holbrook JT, Davis MD, Hubbard LD, et al. (2000) Risk factors for advancement of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome. Arch Ophthalmol 118:1196–1204
15. de Smet MD, Nussenblatt RB (1991) Ocular manifestations of AIDS. J Am Med Assoc 266:3019–3022
16. Spaide RF, Vitale AT, Toth IR, et al. (1992) Frosted branch angiitis with cytomegalovirus retinitis. Am J Ophthalmol 113:522–528
17. Grossniklaus HE, Frank KE, Tomsak RL (1987) Cytomegalovirus retinitis and optic neuritis in acquired immune deficiency syndrome. Ophthalmology 94:1601–1604
18. Meenken C, van den Horn GJ, van der Meer JTM, et al. (1998) Optic neuritis heralding varicella zoster virus retinitis in a patient with AIDS. Ann Neurol 43:534–536
19. de Smet MD, De Fen S, Pepose JS, et al. (2003) Microdissection combined with the polymerase chain reaction to identify potentiating viral coinfection in patients with HIV/AIDS with ocular infection. Can J Ophthalmol 38:207–213
20. de Boer JH, Verhagen C, Bruinenberg M, et al. (1996) Serologic and polymerase chain reaction analysis of intraocular fluids in the diagnosis of infectious uveitis. Am J Ophthalmol 121:650–658
21. Doornenbal P, Baarsma GS, Quint WGV, et al. (1996) Diagnostic assays in cytomegalovirus retinitis: detection of herpes virus by simultaneous application of the polymerase chain reaction and local antibody analysis on ocular fluid. Br J Ophthalmol 80:235–240

22. Ando Y, Terao K, Narita M, et al. (2002) Quantitative analyses of cytomegalovirus genome in aqueous humor of patients with cytomegalovirus retinitis. Jpn J Ophthalmol 46:254–260
23. Scholz M, Doerr HW, Cinatl J (2003) Human cytomegalovirus retinitis: pathogenicity, immune evasion and persistence. Trends Microbiol 11:171–178
24. Jabs DA, Martin BK, Forman MS, et al. (2003) Cytomegalovirus resistance to ganciclovir and clinical outcomes of patients with cytomegalovirus retinitis. Am J Ophthalmol 135:26–34
25. Martin DF, Kuppermann BD, Wolitz RA, et al. (1999) Oral ganciclovir for patients with cytomegalovirus retinitis treated with a ganciclovir implant. N Engl J Med 340:1063–1070
26. Cochereau-Massin I, LeHoang P, Lautie-Frau M, et al. (1991) Efficacy and tolerance of intravitreal ganciclovir in cytomegalovirus retinitis in acquired immune deficiency syndrome. Ophthalmology 98:1348–1353
27. Baudouin C, Gastaud P (1992) A modified procedure for intravitreal injections of ganciclovir in the treatment of cytomegalovirus retinitis. Ophthalmology 99:1183
28. Young SH, Morlet N, Heery S, et al. (1992) High dose intravitreal ganciclovir in the treatment of cytomegalovirus retinitis. Med J Aust 157:370–373
29. Martin DF, Dunn JP, Davis JL, et al. (1999) Use of the ganciclovir implant for the treatment of cytomegalovirus retinitis in the era of potent antiretroviral therapy: recommendations of the International AIDS Society – USA Panel. Am J Ophthalmol 127:329–339
30. Martin DF, Parks DJ, Mellow SD, et al. (1994) Treatment of cytomegalovirus retinitis with an intraocular sustained-release ganciclovir implant. Arch Ophthalmol 112:1531–1539
31. Martin DF, Sierra-Madero J, Walmsley S, et al. (2002) A controlled trial of valganciclovir as induction therapy for cytomegalovirus retinitis. N Engl J Med 346:1119–1126
32. Weinberg A, Jabs DA, Chou S, et al. (2003) Mutations conferring foscarnet resistance in a cohort of patients with acquired immunodeficiency syndrome and cytomegalovirus retinitis. J Infect Dis 187:777–784
33. Cytomegalovirus Retreatment Trial (1996) Combination foscarnet and ganciclovir therapy vs monotherapy for the treatment of relapsed cytomegalovirus retinitis in patients with AIDS. Arch Ophthalmol 114:23–33
34. Butler KM, de Smet MD, Husson RN, et al. (1992) Treatment of aggressive CMV retinitis with ganciclovir in combination with foscarnet in a child with human immunodeficiency virus infection. J Pediatr 120:483–485
35. Berthe P, Baudouin C, Garraffo R, et al. (1994) Toxicologic and pharmacokinetic analysis of intravitreal injections of foscarnet, either alone or in combination with ganciclovir. Invest Ophthalmol Vis Sci 35:1038–1045
36. De Clercq E (2003) Clinical potential of the acyclic nucleoside phosphonates cidofovir, adefovir, and tenofovir in treatment of DNA virus and retrovirus infections. Clin Microbiol Rev 16:569–596
37. Lalezari JP, Holland GN, Kramer F, et al. (1998) Randomized, controlled study of the safety and efficacy of intravenous cidofovir for the treatment of relapsing cytomegalovirus retinitis in patients with AIDS. J Acquir Immune Defic Syndr Hum Retrovirol 17:339–344
38. Berenguer J, Mallolas J, Spanish Cidofovir Study Group (2000) Intravenous cidofovir for compassionate use in AIDS patients with cytomegalovirus retinitis. Clin Infect Dis 30:182–184
39. Banker AS, Arevalo JF, Munguia D, et al. (1997) Intraocular pressure and aqueous humor dynamics in patients with AIDS treated with intravitreal cidofovir (HPMPC) for cytomegalovirus retinitis. Am J Ophthalmol 124:168–180
40. de Smet MD, Meenken C, van den Horn GJ (1999) Fomivirsen: a phosphorothioate oligonucleotide for the treatment of cytomegalovirus retinitis. Ocul Immunol Infl 7:189–198
41. Vitravene Study Group (2002) Randomized dose-comparison studies of intravitreous fomivirsen for treatment of cytomegalovirus retinitis that has reactivated or is persistently active despite other therapies in patients with AIDS. Am J Ophthalmol 133:475–483
42. Vitravene Study Group (2002) A randomized controlled clinical trial of intravitreous fomivirsen for treatment of newly diagnosed peripheral cytomegalovirus retinitis in patients with AIDS. Am J Ophthalmol 133:467–474
43. Vitravene Study Group (2002) Safety of intravitreous fomivirsen for treatment of cytomegalovirus retinitis in patients with AIDS. Am J Ophthalmol 133:484–498
44. Goldberg DE, Wang H, Azen SP, et al. (2003) Long term visual outcome of patients with cytomegalovirus retinitis treated with highly active antiretroviral therapy. Br J Ophthalmol 87:853–855
45. Stone SF, Price P, Tay-Kearney ML, et al. (2002) Cytomegalovirus (CMV) retinitis immune restoration disease occurs during highly active antiretroviral therapy – induced restoration of CMV-specific immune responses within a predominant Th2 cytokine environment. J Infect Dis 185:1813–1817

46. Kempen JH, Jabs DA, Dunn JP, et al. (2001) Retinal detachment risk in cytomegalovirus retinitis related to the acquired immunodeficiency syndrome. Arch Ophthalmol 119:33–40
47. Holland GN, Buhles WC, Mastre B, et al. (1989) A controlled retrospective study of ganciclovir treatment for cytomegalovirus retinopathy. Arch Ophthalmol 107:1759–1766
48. Drew WL, Ives D, Lalezari JP, et al. (1995) Oral ganciclovir as maintenance treatment for cytomegalovirus retinitis in patients with AIDS. N Engl J Med 333:615–620
49. Studies of Ocular Complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group (2000) Long-term follow-up of patients with AIDS treated with parenteral cidofovir for cytomegalovirus retinitis: the HPMOC peripheral cytomegalovirus retinitis trial. AIDS 14:1571–1581

Behçet's Disease

12

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Core Messages

- Behçet's disease (BD) is a recurrent systemic vasculitis, possibly affecting nearly all organ systems
- The diagnosis of BD is based on criteria of the International Study Group for Behçet's Disease
- Prevalence of BD ranges from 0.4 (USA) to 370 (Turkey) per 100,000
- Genetic (association with HLA-B*51 in up to 77 %) and immunopathological parameters (T cells, neutrophils) seem to be important in the pathogenesis of BD
- Frequent manifestations of BD are oral and genital ulcers, ocular symptoms and skin lesions
- Ocular involvement is characterized by a posterior uveitis with occlusive retinal vasculitis, running a progressive, chronic-relapsing course
- Visual prognosis of ocular BD is poor; therefore early and aggressive immunosuppression is often necessary
- New immunomodulating substances such as IFN-α and probably also TNF-α antagonists seem to improve the long-term visual prognosis, because they are effective even if other immunosuppressive treatment fails

12.1 Introduction

Behçet's disease (BD) is a recurrent systemic disorder, characterized by an immune-mediated occlusive vasculitis. For better clinical definition various nomenclatures have been suggested, which seem to be very helpful in most but not all patients. Four major symptoms characterize BD: oral aphthous ulcers, ocular lesions, skin lesions and genital ulcerations. Inflammation of other locations may also be found in these "complete Behçet's patients".

The prevalence is up to 370/100,000 in Turkey, resulting in a severe problem for the medical system, because despite adequate treatment the prognosis of at least the ocular involvement is often unfavourable.

Although the cause and pathogenesis of BD are still mysterious, important factors have been identified which influence the beginnings of this disease. The past few years have shown that besides genetic factors like HLA-B*51, environmental factors and immune mechanisms also seem to play a role in the pathogenesis, but probably also in the maintenance, of BD. Besides CD4 positive T cells, natural killer (NK) cells and neutrophils are being given more and more attention in BD research. The neutrophil hyperactivity induces oxygen overproduction with increasing endothelial cytotoxicity. Also γδ-T cells are stimulated and may play a role in the maintenance of BD. Besides their discovery in inflamed tissue, a significant γδ-T cell response to mycobacterial 65-kDa heat shock protein (hsp) peptides and their homologous peptides derived from the human 60-kDa has been detected in BD patients, indicating the possible role of microbial infections in BD.

Recent developments for the treatment of BD have been extraordinarily successful, resulting in complete remission and reduced recurrence. This also seems to change the prognosis for ocular BD, which, besides CNS BD, is the most challenging problem.

12.2 Definition and Epidemiology of BD

12.2.1 Definition

BD is a multisystem vasculitis [123, 130]; thus, almost any organ system can be affected. The diagnosis of BD is based on the criteria of the International Study Group from 1990 (Table 12.1) [22]. As these were developed as a classification and not as diagnostic criteria and especially early stages of the disease often do not allow diagnosis [83], older sets of criteria, most commonly those by O'Duffy [109] and Dilsen [26], and in Asia those of the Japanese [100], (Table 12.2) are still in use.

12.2.2 History

Probably the first description of the symptoms of BD was in the fifth century BC by Hippocrates in his third book of epidemiology [62, 156]. Since that time and especially during the nineteenth century, isolated symptoms of BD have been reported [28]. In 1937, Hulusi Behçet, a Turkish dermatologist, described three patients with findings of oral and genital ulcers and recurrent iritis [9, 156]. The disease is therefore commonly known by his name, despite the fact that in 1930 the Greek ophthalmologist Benedictos Adamantiades presented at the Medical Society of Athens the case of a 20-year-old man who was suffering from recurrent iritis with hypopyon resulting in blindness, associated with phlebitis, mouth ulcers, genital ulcers, and knee arthritis. One year later he published this case in the *Annales d Óculistique* [1]. The name "Behçet's disease" therefore led to a long discussion between Turkish and Greek scientists. One explanation for the use of "Behçet's disease" might be the wider distribution of the paper by H. Behçet in the medical literature [156]. However, the combination of "Adamantiades-Behçet's disease" is also acceptable.

12.2.3 Epidemiology

BD is a rare disorder with wide distribution but varying prevalence around the world. It is most common in the countries of the eastern Mediterranean and in the eastern rim of Asia. The disease is predominantly reported between the 30° and 45° north latitudes in Asian and European populations and these geographic areas correspond to the old silk route [156] (Fig. 12.1), supporting the hypothesis that the disease was carried with the immigration of the old nomadic tribes [111, 163].

Table 12.1. Criteria of the International Study Group 1990

Recurrent oral aphthous ulcers	Small or large aphthous or herpetiform ulcerations, recurring at least 3 times in a 12-month period
Plus two of the following:	
Recurrent genital ulcerations	Aphthous ulcerations or scarring
Eye lesions	Anterior uveitis, posterior uveitis or cells in vitreous on slit lamp examination or retinal vasculitis observed by an ophthalmologist
Skin lesions	Erythema nodosum, pseudofolliculitis, or papulopustulous lesions or acneiform papules in postadolescent patients without steroid treatment
Positive pathergy testing	Intracutaneous needle stick with 21 G on forearm (inside), read by a physician after 24–48 h

Table 12.2. Dilsen, Mason and Barnes, O'Duffy and Japanese criteria

Dilsen criteria [26]	Mason and Barnes criteria [91]	O'Duffy criteria [109]	Japan criteria [100]
Positive pathergy test (specific)			
Main criteria	**Main criteria (major)**	**Main symptoms (major)**	**Main criteria (major)**
Recurrent oral ulcerations	Oral ulcerations	Aphthous stomatitis	Recurrent oral ulcerations
Genital ulcerations	Genital ulcerations	Genital ulcerations	Skin lesions
Eye (anterior or posterior)	Eye lesions	Uveitis	
Skin (erythema nodosum or other)	Uveitis + hypopyon	Dermal vasculitis	Uveitis or retinitis
Thrombophlebitis (superficial or deep)	Corneal ulcerations	Arthritis	Genital ulcerations
	Retrobulbar neuritis		
	Skin lesions		
	Pustules		
	Ulcerations		
	Erythema nodosum		
	Erythema multiforme		
Minor criteria	**Minor criteria**	**Minor symptoms**	**Minor criteria**
Clinical:			
Peripheral arthritis	Gastrointestinal lesions	Central nervous system	Arthritis
Neuropsychiatric	Thrombophlebitis	Colitis	Gastrointestinal symptoms
Gastrointestinal	Cardiovascular lesions	Phlebitis	Epididymitis
Pleuropulmonal	CNS lesions	Large vessel arteritis	Vascular symptoms
Arterial	Family history		Neuropsychiatric symptoms
Orchiepididymitis			
Other:			
History			
Skin hypersensitivity			
Positive family history			

Table 12.2. (Continued)

Dilsen criteria [26]	Mason and Barnes criteria [91]	O'Duffy criteria [109]	Japan criteria [100]
Diagnosis *Definitive:* – Pathergy (+), one major or minor criterion – Pathergy (+)(–), two major criteria or one major and one minor criterion – Three major or two major and two minor criteria *Incomplete:* – Pathergy (+/–), one major or one minor criterion	**Diagnosis** Three major criteria or two major and two minor criteria	**Diagnosis** Oral or genital ulcerations *plus* Two other major symptoms	**Types** *Complete:* Four major criteria *Incomplete:* Three major criteria *or* uveitis plus one major criterion *Suspect:* Two major criteria *Possible:* One major criterion

12.2.3.1 Prevalence

The highest prevalence (Table 12.3) of the disease has been reported in Turks living in the northeastern part of Turkey, with 370 patients per 100,000 inhabitants, while the overall prevalence in Asia is 10- to 150-fold lower and in Europe and the USA more than 150-fold lower [111, 163].

12.2.3.2 Incidence

The exact incidence of the disease is unknown [156]. In Japan, a country with well-organized registration of patients with BD, 0.9 new cases per 100,000 inhabitants were diagnosed in the year 1984 [104]. In 1990, 0.8 new patients per 100,000 inhabitants were registered, indicating that a plateau had been reached after a rapid increase in incidence since 1972 [163].

The annual incidence in Iran is approximately 0.57 per 100,000 patients in a population of 60 million [23, 156] and in Taiwan 14 patients per year presented for the first time in one of the six major medical centres from 1984 to 1988 [20, 163].

12.2.3.3 Age

The age of onset varies in many studies, because most authors consider the onset of the disease to be the age at which the patient fulfilled the diagnostic criteria of the disease, others the onset of the first symptom [156]. The mean age of onset is 25–35 years worldwide, with a range of 2 months to 72 years [156, 160], an observation which is independent of the origin of the patients or their gender [163]. An average age of onset of 19.9 years was recorded in Israel, 25.6 years in Turkey, 25.9 years in Europe, 26.5 years in Arab countries, 28.3 years in America, and 31.7 years in the countries of east Asia [163].

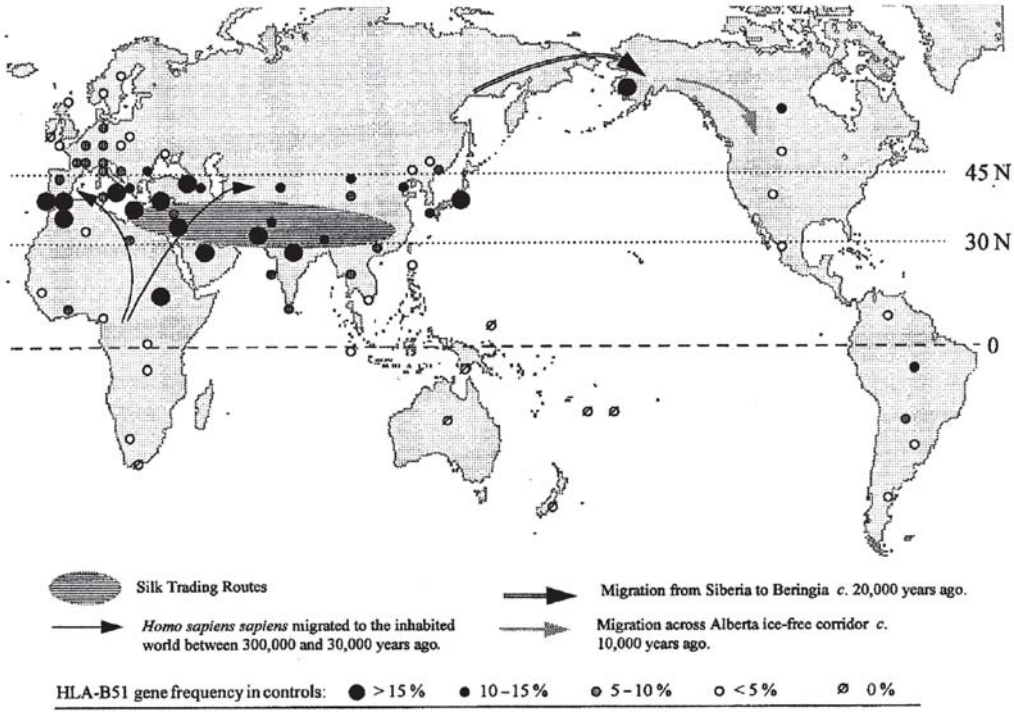

Fig. 12.1. Prevalence of HLA-B*51. (From Verity et al. [146])

Table 12.3. Distribution of Behçet's disease. Studies comparing epidemiological features of the disease over time have found throughout an increasing prevalence during the last 40 years, which may be due to the chronic character of the disease [163] or may be also related to a better awareness of the illness, and in some countries to migration [156]

Population	Year	Prevalence per 100,000 inhabitants
Asia		
Turkish (northeastern Anatolia) [163]	1987	370
Iranian [23]	1996	16.7
Kuwait [163]	1986	2.1
Japanese [104]	1991	13.5
Chinese [163]	1998	14.0
Europe		
Germany [160]	1994	0.55
Italian [161]	1988	2.5
Northern Spain [161]	1998	7.5
Greek [114]	1984	6
America		
U.S. American [16]	1975	0.4

12.2.3.4 Sex Distribution

Old Japanese and Turkish reports have shown a preponderance of males to females [103, 156, 163]. But in the last 20 years the male-to-female ratio has decreased and is currently reaching equality [48, 104, 126, 163]. This more even distribution of BD between the sexes has also been observed in Germany [161] and in Israel [16, 78]. Other data have shown an androtropism in Egypt (male: female = 5:1), Saudi Arabia (3.4:1), and Italy (2.4:1), whereas women predominate in some northern European countries (e.g. Sweden: 0.67:1) and in the USA (0.2:1) [163].

A real increase in female patients, which is associated with a trend towards a mild clinical course of BD, was achieved in Japanese studies [104, 163], whereas the complete type is more frequent in males [156].

Although the disease is believed to have a worse overall prognosis in males than in females [114] in the Mediterranean and Arab countries, no such difference has been noted in western European and American countries [156].

12.2.3.5 Heredity

In up to 18% of patients [163], Behçet's disease affects more than one member of the same family [29, 144, 156]. Therefore, familial occurrence is one of the major epidemiological features of the disease [163] but is more frequent in families of Arab, Israeli, Turkish or Asian origin (2.0–18.2%) than in European patients (0–4.5%) [163].

Summary for the Clinician

- **Behçet's disease is a rare, at least genetically determined, systemic disorder**
- **Up to now the exact aetiology has remained unknown**
- **The disease occurs around the 2nd and 3rd decades of life, with both genders being equally affected**
- **Besides the diagnostic criteria of the International Study Group, some other sets of criteria are still in use**

12.3 Genetics of BD

BD is not a genetic disease with Mendelian inheritance. The disease is sporadic in most families; however, a familial aggregation of BD has long been noted and an increased disease risk has been observed among first-degree relatives. Sibling recurrence rate has been found to be 4.2% in Turkish patients. Analysis of multicase families suggests a complex genetic inheritance model [45]. The association of BD with HLA-B5, which has been known since 1973, provides the strongest evidence supporting the involvement of genetic factors in its pathogenesis. At that time, HLA-B5 was called HL-A. In the following decade, it became clear that this association exists not only in Japan, but also all over the world, with the exception of the USA (Fig. 12.1, Table 12.4) [146]. In the different ethnic groups a comparison with healthy controls results in a p value of less than 0.0001, which hints at a strong correlation between BD and HLA-B5. As this association is comparably strong in the different ethnic groups, it is an attractive hypothesis to assume that BD was spread to Asian and Eurasian populations from Japan to the Middle East by nomadic or Turkish tribes along the Silk Route together with the antigen HLA-B5. HLA-B5 is present in 40–80% of all BD cases [98], while in the respective healthy population its prevalence lies between 8% (northern Europe)

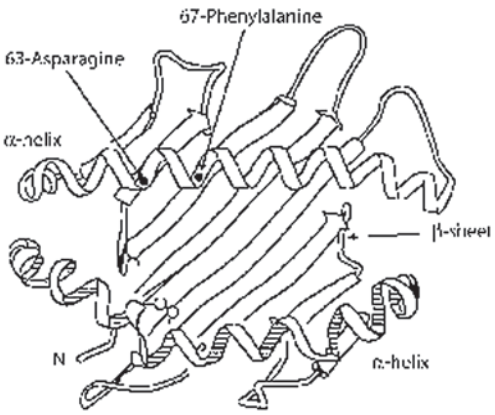

Fig. 12.2. HLA-B*51 molecule

Table 12.4. Prevalence of HLA-B*51 in patient groups, healthy controls and general population, and relative risk for BD. Adapted from Verity et al. [146] and Zouboulis et al. [162] (modified) (*empty fields* no data available)

Country	Patients (*n*)	HLA-B*51+ (%)	Controls (*n*)	HLA-B*51+ (%)	RR	*P* value	Population HLA-B*51 (%)
Asia							
Japan	91	52	140	20	7.9	<0.00005	18–22.3
Korea	113	51	112	16	4.0–6.8	<0.001	10–13
Taiwan	51	51	128	11	8.5		2.6–7.7
China	120	56	100	12	9.3		2.5–8.8
India	31	32	400	30	1.1		6.4–16
Iraq	52	62	175	29	3.9		2.3
Iran		53		33	2.3		
Turkey	520	77	1106	26	9.2		24
Saudi Arabia	85	72		26	9.0		26
Jordan	68	74	43	23	9.2		
Lebanon	100	54	100	34			61
Israel	126	75	790	21	11.5		
Africa							
Egypt	84	58	200	7	20.1	<0.0001	
Tunisia	55	62	80	24	5.2		
Morocco [59]	86	30.2	111	15.3	2.4	<0.015	15.8
Europe							
Russia	19	37	150	15	3.2		4.9
Great Britain	107	25	2032	9	3.3		1.9–4.4
Ireland [60]	24	25	96	3	6.3	<0.002	0
Germany	75	36	1415	14	3.5		6.1
Switzerland	8	38		17	3.0		9.5
Portugal	318	53	135	24	3.6		17
Spain	100	42	452	21	2.7		6.1–24.5[a]
France	105	51	591	13	6.7		6.5
Italy	57	75	304	22	10.9		17.4
Greece	170	79	670	28	9.7	<0.001	15.1
America							
USA	32	13	523	10	1.3		1.5–4.5%
Mexico	10	70	105	31	5.1		

[a] Southern Spain.

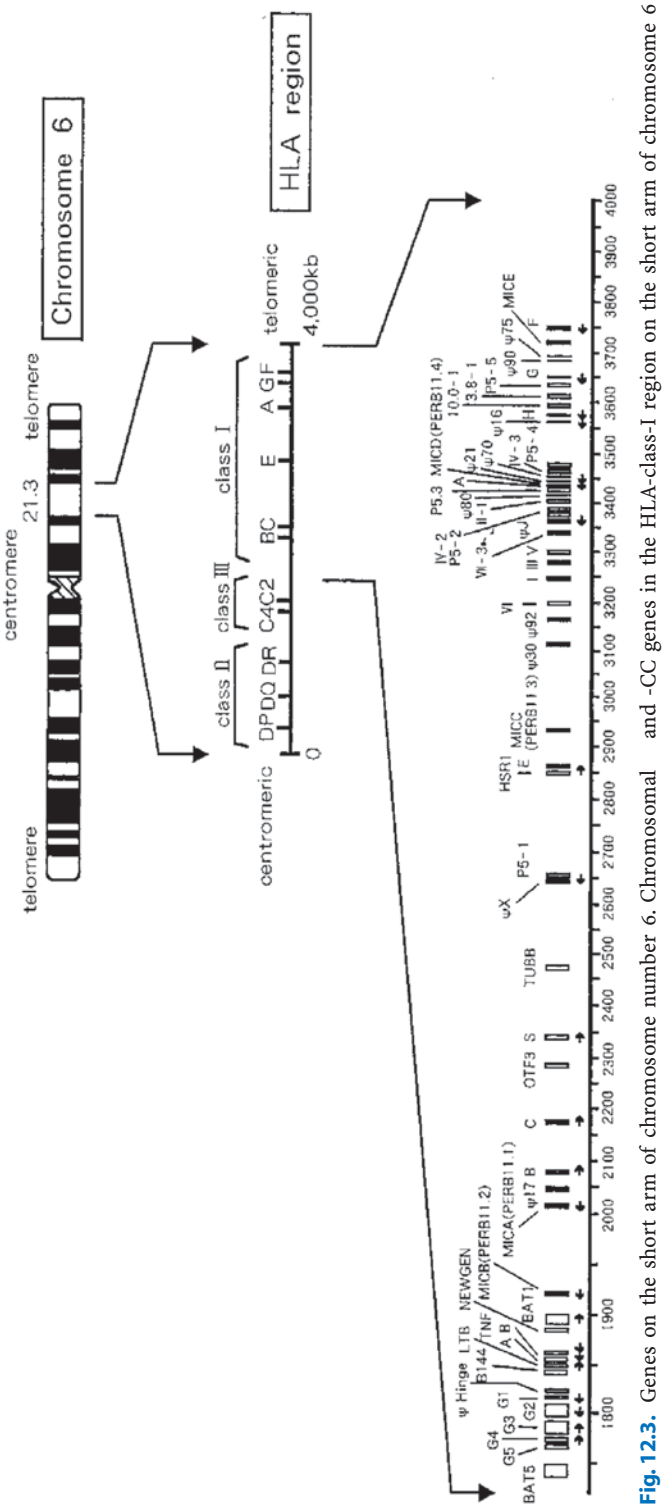

Fig. 12.3. Genes on the short arm of chromosome number 6. Chromosomal mapping of Y109 YAC clone. Seven cosmid clones, pM677, pM213-5, pM117, pM125, pMN201, pM30, pM56, covering the 237-kb segment around the HLA-B and -CC genes in the HLA-class-I region on the short arm of chromosome 6 (6p21.3), were examined by DNA sequencing. (From Mizuki et al. [98])

and 24% (Turkey, Middle East) [146]. In the presence of HLA-B*51 the relative risk for BD lies between 1.4 in Portugal [146] and 17.1 in Israel [17]. In Germany the respective relative risk is estimated to be 2.6 [159]. Meanwhile it is known that HLA-B5 consists of two split antigens, HLA-B*51 and HLA-B*52. BD is obviously associated with HLA-B*51, but not with HLA-B*52 (only one publication from Israel describes an association with both split antigens) [117]. The regional prevalence of BD corresponds to that of HLA-B*51 (Fig. 12.1); in countries where HLA-B*51 does not occur, BD does not exist (Fig. 12.1).

Two amino acids have been localized differing between HLA-B*51 and HLA-B*52 (63-asparagine, 67-phenylalanine). Both amino acids form a part of the B-pocket in the HLA-antigen-binding groove [98].

The peptide binding groove of HLA-class-I antigens has six pockets (A, B, C, D, E, F). The largest is the B-pocket, which is built under participation of the amino acid residues in positions 63 and 67. The peptides which bind to HLA-molecules depend among other things on the amino acids which form the B-pocket. There are different peptide-binding motifs for each HLA allele [119]. Thus one may assume that the two amino acids which are specific for HLA-B*51, asparagine in position 63 and phenylalanine in position 67, contribute to the pathogenesis of BD. Although HLA-B*51 (B*5101-B*5103) and B*52 (B*5201 allele) differ only in two positions in the α2-domain of their amino acid sequences, their peptide-binding motifs differ considerably, because these two positions form the B-pocket [38, 98, 119] (Fig. 12.2).

As the contribution of HLA-B*51 to the pathogenesis of BD is less than 20%, the other factors which are relevant in the pathogenesis of BD remain to be proven. Repeatedly, the contribution of other genes in the pathogenesis of BD has been discussed. Polymorphisms of still unknown significance are for example the alleles E469 and R241 of the intercellular adhesion molecule ICAM-1 in Jordanians and Palestinians or Italians. The results of these studies are contradictory in part [13]. The allele ENOS-Asp298 of endothelial nitric oxide synthase was significantly more common in BD patients than in healthy controls [125]. Examinations of the MEFV mutations in areas endemic for familiar Mediterranean fever (FMF) hint at an increased polymorphism of certain MEFV genes in BD [88]. An association of both diseases (BD and FMF) is known [10]. IL-1 single nucleotide polymorphisms IL-1A-889C and IL-1B+5887T were increased in BD patients in a recent study [65]. However, most examinations focus on chromosome number 6, which also contains the genes for the major histocompatibility complex (MHC) and thus for the HLA-class-I molecules (Fig. 12.3). HLA-Cw*14 seemed to be more prevalent in patients with BD, but is in linkage disequilibrium with HLA-B*51 [97]. For TAP2 (transporters associated with antigen processing) and LMP7 (proteasome subunit), also genes on chromosome 6 close to the MHC, which are coding for molecules responsible for the transportation and preparation of peptide ligands, no significant associations could be shown [56]. TNF-gene loci, which lie centromeric to the HLA-B-locus, were not associated with BD or the TNF B* *Nco*1 RFLP (restriction length fragment polymorphism) was in linkage disequilibrium with HLA-B*51 [147]. The TNF promoter polymorphism –1031C was associated with BD in the absence of HLA-B*51 [3]. MICA genes, like HLA-class-I genes, are located on chromosome 6 code for a molecule which has similarities with HLA-class I antigens, but is not associated with β2-microglobulin. It does not bind peptides and its expression is increased upon cellular stress. MICA antigens are able to directly activate γδ T cells and NK cells through the NKG2D receptor and DAP10. An intracellular variant A6 and the MICA 009 allele seemed to be associated with BD, but finally a linkage disequilibrium with HLA-B*51 was proven [116].

The direct association of HLA-B*51 with BD meanwhile has been supported by microsatellite analysis: eight polymorphic microsatellite markers within 1,100 kb of the HLA-B gene were analysed. This analysis revealed a linkage disequilibrium of HLA-B*51 with MICA and MICB; the strongest association was found for HLA-B*51 itself in all three populations examined (Japanese: p=0.000000000017; Greek: 0.00000032; Italians: 0.00047) [99]. It is a mat-

ter of debate whether the presence of HLA-B*51 is associated with a more severe course of the disease or specific manifestations [47, 103]. Triggering factors such as bacteria or virus may have agretopes with a high affinity for the HLA-B*51 molecule. With today's refined molecular biological methods, so far 29 suballeles of HLA-B*51 have been described (HLA-B*5101 to HLA-B*5129) [120]. Of these, especially HLA-B*5101 and HLA-B*5108 seem to be associated with BD.

In our own study comparing German and Turkish patients, we found similar associations, but additionally HLA-B*5107 seemed to be negatively associated with the disease [75]. Similar observations were also made in a Spanish study [41]. HLA-B*5101 and HLA-B*5108 share the amino acid substitutions in positions 63 and 67, which differ between HLA-B*51x and HLA-B*5201. These substitutions are identical in all HLA-B*51x suballeles except HLA-B*5107 and HLA-B*5122. HLA-B*5107 shares serine (Ser) in position 67 with HLA-*B5201; HLA-B*5122 has a cysteine (Cys) in this position. As antigen binding takes place in this region, it may well be possible that the antigenic peptides bound by HLA-B*5107 and HLA-B*5122 differ from those bound by the other HLA-B*51x suballeles. However, it should be noted that the amino acid substitutions in positions 63 and 67 (aspartate and phenylalanine), which separate the associated split antigen HLA-B*5101 from the non-associated HLA-B*5201, also exist in HLA-B*08, most alleles of HLA-B*35, B*53, B*5901, B*78 and some rare alleles such as B*0708, B*1522, B*1529, B*4008 and B*4406. Their possible role in HLA-B*51 negative patients remains to be shown.

In conclusion the critical region containing the susceptibility antigens for BD lies between the HLA-B locus and the TNF locus and the locus for MICA (MHC-Class-I-chain related A), or possibly at the telomeric end of chromosome 6pi [46]. The only association which has been unanimously proven in all populations where BD exists is that for HLA-B*51 and its suballeles.

12.4 Immunology

Behçet's disease seems to be, at least in parts, an immune mediated disease, and various cells participate in the immunopathology. Recently an extended review has been published regarding the variety of parameters [158].

12.4.1 T Cells

T-cell-mediated immune responses play a major role in the pathogenesis of BD [158]. Analysis of peripheral blood T-cell receptor V and J segment gene usages revealed oligoclonal T-cell responses in CD4+ and CD8+ cells correlating with activity of clinical BD patients [34]. This suggests an antigen-driven immune response. Recently also an oligoclonal T-cell response in the anterior chamber has been described [67].

The role of T-cell subsets remains unclear at the moment, but a reduced CD4+/CD8+ T-cell ratio has been reported, due to a decrease in CD4+ T cells and an increase in CD8+ T cells [122]. A significantly reduced CD4+CD45RA+ (helper-inducer) T-cell population was found in the peripheral blood in patients with active BD, despite similar CD4+CD29+ (helper-inducer) T-cell frequency in active and inactive BD [60]. Abnormal activity of suppressor T cells has been observed in the beginning of active BD, returning to normal when patients became fully active or BD became inactive. This may point to a defect in suppressor cells in the development of BD [122].

Several microbial antigens have been shown to stimulate T cells in patients with BD. This includes streptococcal antigens [57, 84, 85], staphylococcal endotoxin, *E. coli*-derived peptides, but especially importantly heat shock proteins (hsp) of various microbes [86]. Hsps are important for the survival of microbes when they infect an organism. This may lead to an efficient presentation of hsp by host cells, resulting in recognition of infected cells by the immune system. Hsps from specific bacteria show considerable homology with those of both oth-

er bacterial and host hsps. Due to the high conservation among microbial pathogens, hsps can even become major antigens.

The role of hsp in BD remains unclear at the moment. There is an anti-hsp60 T-cell response detectable in BD patients. Four peptides from 65-kDa mycobacterial hsps (111–125, 154–172, 219–233, 311–325) and their homologous peptides from human 60-kDa hsps (136–150, 179–197, 224–258, 336–351) have been identified as specific epitopes for BD. Subcutaneous immunization with hsp peptides (with adjuvant), but also oral and nasal administration (without adjuvant), induced experimental uveitis in rats [55]. Responses against hsps may reflect a common relation between hsps and other autoantigens, but are probably leading to a molecular mimicry between bacterial and self-hsps. The T-cell hypersensitivity seems to be not limited to hsp or other microbial antigens, but also to retinal antigens like retinal-soluble-antigen [24, 151]. It may result from an intrinsic T-cell defect affecting signal transduction through TCR [53, 137]. There are also signs of viral infection in BD patients. Therefore, herpes simplex virus (HSV) genome 1 and serum antibodies against HSV-1 have been found in higher amounts in BD patients compared to controls [31, 85]; also cytotoxic T cells have been found. These findings are probably more related to the T-cell dysfunction than to a direct effect of the virus.

Still badly understood is the role of γδ-T cells in inflammatory disorders, and therefore also in BD, in which these cells are elevated [34, 39, 40, 52, 101, 152]. A majority of these γδ-T cells produce IL-2R (CD25), CD45RA IFN-γ, TNF-α and IL-8 [40, 101, 152]. A subset of γδ-T cells has a proliferative response to four mycobacterial hsp-derived peptides, correlating to the activity of BD and suggesting a regulatory role of the γδ-T cells [52]. These findings have not been confirmed by other groups [55, 64].

Even in the inactive state of BD elevated levels of Th1-type cytokines have been detected following stimulation [94]. This includes TNF-α, TNFR-75, IFN-γ, IL-1, sIL-2R, IL-8, IL-12, but also the Th2 cytokine IL-10 [158]. The cytokine level seems to correlate with activity for IL-8, IL-12, sIL-2 and TNFR-75 [158].

12.4.2 NK Cells

Activity of NK cells is another controversial problem. While CD16+CD56+ NK cells and CD56+ T cells have been found in BD patients by some investigators [63, 138], some have only shown increases of CD4+CD16+ and CD4+CD56+ T cells with a normal CD16+ CD56+ NK level [32], or even a decrease of CD16+ cells [51]. There may also be NK-T cells involved which in a state of activation can express mRNA of macrophage migration inhibitory factor (MIF) [72]. In the serum of BD patients MIF levels are increased [71].

12.4.3 Neutrophils

Neutrophil infiltration is a major finding in BD lesions. They seem to be hyperactive, leading to increased chemotaxis, phagocytosis and superoxide generation [14, 30, 106, 118]. They show a higher expression of CD10, CD11a and CD14. Cytokines like TNF-α, IL-8 and GM-CSF can stimulate neutrophils to a primed state from which they reach complete activity more easily and rapidly than in the quiet state. T cells seem to be important for this stimulation [107].

Incubation of neutrophils from healthy individuals with sera from patients with BD induced an increase in adhesion to endothelial cells and expression of CD11a and CD18 [121].

12.4.4 Endothelial Dysfunction

There is some evidence for an endothelial dysfunction or endothelial injury in BD patients. Blood levels of von Willebrand factor, produced in the endothelium, thrombomodulin and E-selectin, are elevated (review in [80]). A single nucleotide polymorphism in the eNOS (endothelial nitric oxide synthase) gene (Glu298Asp) was found to be associated with BD [125]. Flow-mediated dilation of arteries, which is dependent on the function of eNOS from endothelial tis-

sue, was found to be reduced in BD patients [18]. There is controversy regarding the level of NOS [33, 37, 112], but the higher activity of superoxide dismutase suggests a role for nitric oxide metabolites in the pathogenesis of BD [70].

The role of anti-endothelial antibodies remains unclear. Up to 50 % of patients with BD demonstrate these antibodies, mostly associated with activity of disease [7, 141]. A 44-kDa endothelial cell surface antigen has been found which reacts with IgM anti-endothelial cell antibodies [82]. These findings may reflect an immune mediated response, but probably only demonstrate endothelial defects.

12.4.5 Coagulation and Fibrinolytic Pathway Abnormalities

There are indications for an activation of the coagulation and the fibrinolytic systems in BD patients, partly associated with thrombosis, like an elevation of thrombin-antithrombin III complex and prothrombin 1 and 2, that may lead to intravascular thrombin formation. Defects in protein C, protein S and factor V Leiden have been shown in patients with BD patients having thrombosis [15, 95, 124, 129] for carriers of factor V Leiden and prothrombin gene.

Mutations, giving a severe increase in risk for thrombosis, have been described in patients with BD [43, 44, 89, 145]. Anticardiolipin antibodies have also been reported in up to 25 % of BD patients, but these antibodies seem to play no primary role in BD [139].

Regarding tissue plasminogen activator, no conclusive results have been published [158]. It has to be kept in mind that different clinical situations besides ongoing therapy are influencing such investigations, and that findings in serum only in part reflect the local situation. Despite such limitations it seems that BD patients have an imbalance towards a prothrombotic state which, probably associated with other factors, may lead to vessel inflammation and finally occlusion.

Summary for the Clinician

- **Behçet's disease is at least in parts an immune mediated disorder**
- **T cells play a major role, probably stimulated by microbial antigens, probably heat shock proteins**
- **Neutrophil infiltration and endothelial dysfunction are also major components of BD**
- **Abnormalities of the coagulation and fibrinolytic system have been found**

12.5 General (Extraocular) Manifestations of BD

As a multisystem vasculitis [123, 130], almost any organ system can be affected in BD. However, there is a clear preference for the mucous membranes, the skin and the eye. In this chapter, we briefly describe the extraocular manifestations of BD.

12.5.1 Oral Aphthous Ulcerations

These are present in 90–100 % of all patients. Together with the skin lesions, they represent the most common primary manifestation of the disease. The oral aphthous ulcers of BD occur in localizations which are uncommon in habitual aphthosis, for example sublingual, on the soft and hard palate, in the epipharynx, pharynx and larynx. They last a long time (14 days), tend to relapse (three times a year up to twice monthly) and are very painful. Histologically, leucocytoclastic vasculitis with intraepidermal vesicles, caused by necrosis of keratinocytes or acantholysis, is found. There is a perivascular infiltrate of neutrophils, lymphocytes, mast cells and macrophages. Endothelial cells proliferate and finally become necrotic, resulting in fibrinoid necrosis.

12.5.2 Genital Ulcerations

These are present in 60–80 % of patients and usually manifest on the scrotum and vulva, but may also appear in any other part of the male and female outer genitals, for example the urethra, anus or perineum. Deep ulcerations are painful, whereas superficial lesions, especially in the cervix and vagina, often cause no symptoms. They mostly heal within 10 days up to 4 weeks, sometimes scarring, and relapse less often than oral aphthous ulcers. The bipolar (oral and genital) aphthosis is not specific for BD; it may also occur in patients with inflammatory bowel disease, relapsing polychondritis or chronic myelogenous leukaemia.

12.5.3 Skin Lesions

Skin lesions occur in 41–94 % of cases and are morphologically diverse. Papulopustules, acneiform pseudofolliculitis and erythema nodosa are most common, but pyodermia, ulcerations, necrotizing lesions, Sweet syndrome and superficial thrombophlebitis may also occur. Polymorphic erythema or pyoderma gangrenosum and pernio-like lesions are rare. Different skin lesions can occur in the same patient, either sequentially or at the same time. Histologically, there is a leucocytic vasculitis with perivascular infiltration by neutrophils and fewer lymphocytes. Even histologically, the differentiation between acne vulgaris and papulopustules of BD is difficult. Erythema nodosum, which, when associated with sarcoidosis or other disorders, represents a panniculitis, reveals vasculitic changes and an additional subcutaneous thrombophlebitis in BD. Immunohistologically, the infiltrate consists of T cells, NK cells and macrophages.

12.5.4 Pathergy Phenomenon

Pathergy phenomenon is positive in 19–53 % of all patients and consists of a sterile papulopustule which occurs 24–48 h after an intracutaneous stick with a sterile 21G needle [27, 42]. It is caused by cutaneous hypersensitivity. Pathergy may also occur after other irritations of the skin. “Extended” pathergy occurs after dental surgery (oral pathergy), in the eye (inflammatory flares after surgery), with aneurysms after vascular surgery, and with ulcerations in the area of anastomosis after gastrointestinal surgery. Under immunosuppressive treatment, the test often becomes negative. The pathergy phenomenon is supposed to be specific for BD, although it has also been described in patients with pyoderma gangrenosum, CML and in relatives of patients with BD. Histopathology reveals a predominantly neutrophilic infiltration in combination with memory T cells expressing HLA-DR as activation marker.

12.5.5 Skeletal System

Arthritis occurs in 47–69 % of patients, being mostly oligoarticular (less than five joints affected) and asymmetrical, affecting the lower extremities and non-erosive. Only 1 % of the arthritides in BD lead to erosions which are visible on X-rays. Sacroiliitis may also occur, its prevalence estimated at 7 %. There are marked similarities to spondyloarthropathies, and enthesiopathies also occur. The synovial fluid and histology reveal unspecific findings, but pannus and lymphocyte follicles as in rheumatoid arthritis are unusual.

Another, although rare, manifestation in the skeletal system is myositis, which may be either localized or generalized and which has also been described in juvenile BD. Histology is unspecific with inflammatory infiltrates and degeneration of muscle fibres. Myalgia and fibromyalgia syndrome are much more common, but also unspecific.

12.5.6 Gastrointestinal Manifestations

Gastrointestinal manifestations occur in 3–30 % of patients. Differentiation from inflammatory bowel disease (IBD) is difficult. Main symptoms are abdominal pain (92 %), followed by diarrhoea (29 %) and gastrointestinal bleedings (5 %). Diffuse ulcerations (76 %) are more common than localized aphthous ulcers. The ulcerations are deep, and oesophagus and rectum may be affected. They are mainly localized in the ileocoecal region (96 %). Perforations are common (56 % in a Japanese study). Histopathology reveals lymphocytic and neutrophilic infiltrations in the mucosa, but cryptal abscesses may also occur. Granuloma formation, transmural lymphocytic aggregates and fibrosis, in contrast to IBD, are uncommon. A perivascular infiltration (leucocytoclastic vasculitis) is typical of bowel inflammation associated with BD [81].

12.5.7 Neurological Manifestations

Neurological manifestations are present in 8–31 % of all patients and mostly begin 4–6 years after the first manifestation of BD. In neuro-BD, parenchymal involvement is distinguished from non-parenchymal (vascular) involvement, where the parenchymal lesion, when it occurs, is secondary to another pathological process, such as large arterial or venous occlusion, haemorrhage, etc., and thus corresponds to a major vascular territory. Intracranial hypertension with or without dural sinus thrombosis is the most common manifestation of vascular neuro-BD and comprises 11–35 % of all neuro-Behçet's patients. In parenchymal involvement, which is more common and is seen in 82 % of all cases of neuro-Behçet, the pathological process occurs primarily within the nervous parenchyma, with a tendency to produce focal lesions clustering in the brainstem, basal ganglia, diencephalic structures, internal capsule, etc., and is also disseminated throughout the CNS as a low-grade inflammation [4, 127]. Histologically, there is microglial proliferation, slight glial scarring, and perivascular infiltration of lymphocytes, neutrophils and plasma cells. The site most commonly affected is the brainstem, followed by isolated hemispherical involvement in a subgroup of patients usually presenting with hemiparesis and/or pyramidal signs, and spinal cord involvement in 4–20 % of all cases (only 18 % being isolated). A special, although fortunately rare, form of cerebral involvement in BD is the demyelinating variant, which does not have signs of active vasculitis in MRI or cerebrospinal fluid (except elevated protein levels), but nevertheless leads to progressive mental deterioration and dementia [127]. In the other forms, cerebrospinal fluid analysis mostly reveals neutrophilic pleocytosis, but lymphocytic pleocytosis is also common. Elevated protein concentrations with an increased IgG index are usually found, and oligoclonal bands similar to those in multiple sclerosis (MS) may occur and do not exclude neuro-BD [142].

12.5.8 Vascular Manifestations

Vascular manifestations occur in 28 % and consist of thromboses and arterial aneurysms. Symptoms correlate with the localization and mostly occur 3–4 years after the first manifestation of BD. Venous thrombosis is more common than arterial complications. Most commonly occlusions of the superior or inferior vena cava, femoral veins, cerebral veins, veins of the upper extremities and liver veins (Budd-Chiari syndrome) occur. Rarely, also thrombosis of the renal veins, the portal vein with consecutive portal hypertension and intracardiac thrombus are seen [54, 150]. Pulmonary embolism is diagnosed in 10–15 % of cases. There is an accumulation of thromboembolism in patients with additional coagulation defects such as prothrombin mutation, factor V Leiden mutation, protein C or protein S deficiency or hyperhomocysteinaemia [35, 87, 143]. However, thromboses in BD are due to vascular inflammation and the resulting endothelial dysfunction. The frequency of arterial complications is estimated at 7–

34 %, usually consisting of occlusions, isolated aneurysms or a combination of both. They can be localized anywhere, but most commonly occur in the arteries of the lower extremities with the main symptom of intermittent claudication. The prognosis of arterial aneurysms is worse than that of occlusions. Pulmonary arterial aneurysms bear the greatest risk of death, their major symptoms being dyspnoea and haemoptysis.

12.5.9 Rare Manifestations

Cardiac manifestations are diagnosed in 1–6 % of all patients. A pericarditis is most common, followed by coronary vasculitis with occlusions and/or aneurysms resulting in myocardial infarctions in mostly young patients. In some studies, perfusion scintigraphy of the myocardium revealed silent ischaemia in 25 % of all patients. Furthermore, myocarditis resulting in dilative cardiomyopathy and ventricular aneurysms occurs. Valvular vegetations and insufficiencies are rare, but mitral valve prolapse appears to be significantly more common in BD patients when compared to healthy matched subjects.

Urogenital manifestations are rare. The most common is epididymitis (4–31 %). Renal manifestations (different forms of glomerulonephritis) are seen in less than 1 %; they are almost always asymptomatic and are diagnosed by proteinuria or haematuria. Nephritic syndrome and renal insufficiency are rarities. Single cases of bladder ulcerations and fistulas have been described.

12.5.10 Life Expectancy

Life expectancy is diminished especially in the young and males, because they tend to have severe courses of BD [154]. CNS manifestations, arterial aneurysms and gastrointestinal manifestations bear the worst prognosis [154]. The mortality of pulmonary aneurysms is as high as 50 %. The mortality of myocardial infarctions in BD is much higher than in primary infarctions (29 %). In a study by Park et al., the main causes of death in male patients were gastrointestinal bleeding, bowel perforations, vena cava superior syndromes, aortic insufficiency, CNS manifestations, sepsis and pulmonary abscesses [115]. Not only BD itself, but also the immunosuppressive treatment contributes to overall mortality.

Summary for the Clinician

- **Oral aphthous ulcerations, occurring in uncommon localizations, represent the most common primary manifestation of Behçet's disease**
- **Further frequent extraocular manifestations of BD consist of genital ulcerations, skin lesions and mostly oligoarticular arthritis**
- **The pathergy phenomenon is supposed to be specific for BD**
- **Main causes of diminished life expectancy are CNS manifestations, arterial aneurysma and gastrointestinal manifestations**

12.6 Ocular Involvement

Ocular involvement, frequently termed ocular BD, occurs in 60–80 % of patients on average 4 years after disease onset [11, 21, 135, 156, 157]. It is the initial symptom in 10–20 % of patients [11, 21, 156, 157]. Recurrences are common and the recurrent attacks of ocular inflammation lead to severe, permanent ocular damage unless effective treatment is instituted. Each attack damages the eye; therefore loss of sight occurs in affected BD patients. The reported frequency of ocular involvement in cases of BD is 83–95 % in men and 67–75 % in women [156]. The disease is often more severe in men, and bilateral disease occurs in 80 % of patients.

The primary manifestation in 50–87 % of BD patients may be unilateral and occurs most often as an anterior uveitis, but later, in two-thirds of cases, bilateral panuveitis with a chronic relapsing course is present [11, 21, 96, 156, 157].

Nongranulomatous inflammation with necrotizing obliterative vasculitis may be found either in the anterior or the posterior segment, or, more commonly, in both.

12.6.1 Anterior Segment Changes

Anterior uveitis may be the only ocular manifestation in BD patients. Anterior uveitis is an inflammation, which is limited to the iris and the vitreous. The synonym of anterior uveitis is *iridocyclitis*. In the literature, the classic finding in BD patients is an anterior uveitis described as occurring together with hypopyon (Fig. 12.4) in 30 % of cases [11, 21, 96]. Nowadays, iridocyclitis occurs mostly in isolation, which is probably due to earlier and more aggressive treatment, resulting in dampening inflammatory responses.

The inflammatory response in the anterior chamber in BD is of nongranulomatous nature. The patients often complain of redness, periorbital pain, photophobia, and blurred vision. Slit-lamp biomicroscopy examination reveals conjunctival injection, ciliary flash in the perilimbal region, cells and flare in the anterior chamber, and fine keratic precipitates [135, 156].

In eyes with severe iridocyclitis, in which hypopyon is not seen by direct slit-lamp examination, a small layering of leucocytes can be observed in the angle by gonioscopy. This is termed angle hypopyon [156].

The anterior uveitis may resolve spontaneously over 2–3 weeks even if therapy is not instituted. It is explosive in nature, appearing very rapidly. Some patients with BD may change from feeling perfect one moment to having severe inflammation 2 h later. However, this anterior segment inflammation may not be accompanied by posterior segment involvement [135, 156].

Structural changes of the anterior portion of the eye, including posterior synechiae, iris atrophy, and peripheral anterior synechiae, may develop during the course of repeated ocular inflammatory attacks. The presence of peripheral anterior synechiae or iris bombe from pupillary seclusion may lead to secondary glaucoma.

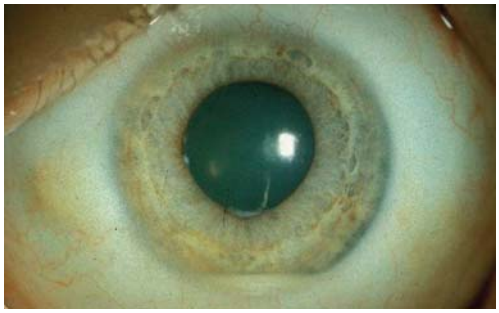

Fig. 12.4. Acute iridocyclitis with hypopyon in a BD patient

Neovascularization of the iris can occur as a result of posterior segment involvement [156].

Uncommon anterior segment findings are conjunctivitis with or without subconjunctival haemorrhage, episcleritis or scleritis, keratitis, and, rarely, extraocular muscle paralysis [21, 156].

12.6.2 Posterior Segment Changes

Changes of the posterior segment include white cell infiltration of the vitreous body, ranging from a moderate number of cells suspended on the vitreous fibrils to a dense plasmoid reaction with sheets of inflammatory cells, especially during the acute phase. An isolated vitreous inflammation is not characteristic of BD.

The essential finding of the posterior pole changes in patients with ocular BD is an *occlusive, necrotizing, retinal vasculitis* [135, 156]. In most patients retinal vasculitis occurs mainly affecting the retinal veins, which is pathognomonic for BD as it is the only systemic vasculitis affecting small and medium sized arteries and also veins. Other typical findings are venous and capillary dilation with engorgement. Involvement of the retinal vessels in the form of acute periphlebitis or thrombangiitis obliterans may lead to massive retinal (Fig. 12.5) and vitreous haemorrhage [135, 156]. Patchy perivascular sheathing (Fig. 12.6) with inflammatory whitish yellow exudates surrounding retinal haemorrhages may be seen. They usually accumulate in

the deeper retinal layers during acute episodes, while the overlying retina shows turbidity and edema. Retinal edema is present in 20–75% of cases, especially in the macula [156]. Retinal atrophy is frequently present after the retinal exudates and haemorrhage resolve, offering testimony to the prior ischaemia. Sheathing of the veins often precedes sheathing of the arteries. Choroidal vascular involvement occurs as well, and choroidal infarcts are probably more common than is generally appreciated [156].

Severe retinal vasculitis may lead to ischaemic changes because of vascular occlusion.

The optic nerve is affected in at least one-fourth of BD patients [156]. Hyperaemia of the optic disc with blurring of the margins (papillitis) is the most frequently observed lesion of the optic nerve. Papilledema is not frequent, but it may occur as a result of microvasculitis of the arterioles supplying the optic disc [156].

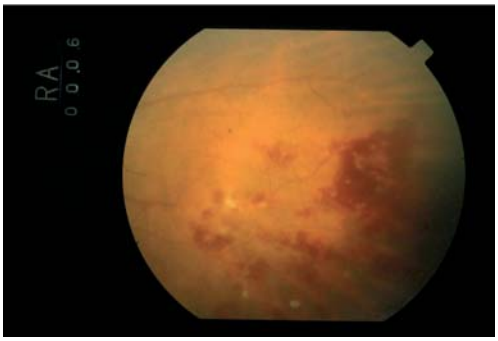

Fig. 12.5. BD patient with retinal haemorrhage due to a venous branch occlusion

12.6.3 Complications

Due to the inflammatory changes in the anterior segment, development of secondary cataract and secondary glaucoma is possible [21, 135, 156, 157].

Repeated inflammatory bouts are the major concern, with the most vision-robbing pathology located in the posterior pole, with fibrotic, attenuated retinal arterioles, narrowed and occluded "silver-wired" vessels, a variable degree of chorioretinal scars, alterations of retinal pigmented epithelium, and optic nerve atrophy being the consequences of repeated inflammatory attacks (Fig. 12.7) [135, 156].

Vascular occlusion causes tissue hypoxia, which stimulates the growth of new vessels at the optic disc or elsewhere. These neovascularizations can induce neovascular glaucoma and they can rupture and bleed. Bleedings into the vitreous can lead to organization with membrane formation, causing retinal holes with subsequent retinal detachment. Phthisis of the eye may finally occur [157].

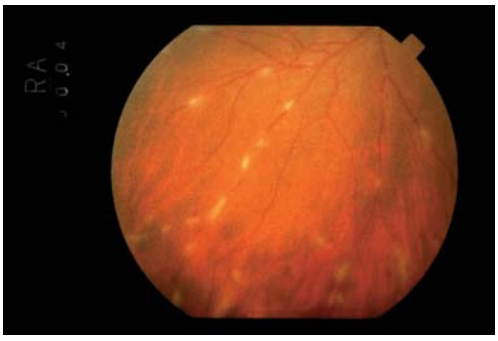

Fig. 12.6. Yellowish-white perivascular sheathing and narrowing of the retinal veins in a BD patient

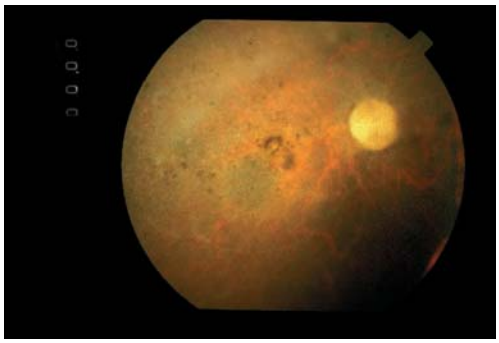

Fig. 12.7. Fundus of a BD patient after multiple inflammatory attacks: narrowed and occluded "silver-wired" vessels, chorioretinal scars, retinal pigment epithelial alterations, and optic nerve atrophy. Visual acuity: hand movement

12.6.4 Diagnosis

Diagnosis of ocular BD is based on clinical observations with slit lamp biomicroscopy and ophthalmoscopy. In addition, helpful examinations might be fluorescein angiography and/or indocyanine green angiography and electrophysiology [74, 156].

12.6.4.1 Fluorescein Angiography/Indocyanine Green Angiography

In 1989, Atmaca [5] demonstrated in her study that in 6.3 % of BD patients who had otherwise no visual loss and no abnormal findings on fundus examination fluorescein angiography (FA) disclosed incipient fundus changes, like discrete fluorescein leakage from the retinal capillaries. Mostly, in patients with recurrent ocular BD, FA demonstrated occlusion and marked dilatation of the retinal capillaries [156].

In patients with active ocular BD, FA discloses diffuse fluorescein leakage from the retinal capillaries, the larger engorged vessels, and the optic disc. Late staining of the vasculature, as a sign of large zones of capillary nonperfusion, collateral vascular formation, secondary retinal teleangiectasia, and retinal neovascularization could also be present [135, 156]. In addition, FA demonstrates central retinal changes which are responsible for poor vision, such as macular ischaemia, cystoid macular edema, macular hole, and epiretinal membranes [12].

Choroidal and retinal pigment epithelial involvement are rarely seen in BD patients. However, since examination of choroidal abnormalities could only be done with indocyanine green angiography (ICG), simultaneous FA and ICG are useful [92, 156].

12.6.4.2 Electrophysiology

Expecting impairment of the central retinal areas in patients with ocular BD, it is useful to perform the electrophysiological examination with the multifocal electroretinogram (mfERG) because any impairment of small, circumscribed areas of the retina usually does not exhibit a pathologic Ganzfeld ERG [80, 133]. Since the mfERG is capable of mapping the central visual field functionally by means of local electroretinographic responses, initial studies in uveitis patients have shown the successful detection of central retinal changes [80, 133]. Therefore, the mfERG may be a good indicator for the monitoring of posterior segment changes as well as for predicting visual prognosis.

Summary for the Clinician

- **In 60–80 % of patients with BD, ocular involvement occurs on average 4 years after disease onset**
- **The presenting symptom often is an anterior uveitis**
- **Later, in most cases, a bilateral panuveitis with a chronic relapsing course is present**
- **Visual loss is mainly caused by occlusive retinal and optic disc vasculitis**
- **Angiographic and electrophysiologic methods are useful in the diagnosis of ocular BD**

12.7 Therapy

Behçet's disease is probably one of the most challenging conditions to treat. Reasons are the still unclear aetiology of this syndrome as well as multiple manifestations possibly affecting all organ systems, moreover complicated by varying appearances of the disease in different ethnic groups. As a consequence diagnosis and therapy of patients with Behçet's disease requires close cooperation between physicians of different medical disciplines, which is usually only found in specialized institutions.

A good therapy for Behçet's disease should be highly effective for preferably manifestations, should work rapidly, should be low in side effects and at the same time be as cheap as possible. At present these contradictory requirements are not compatible. This may be a reason for the fact that standardized treatment regimens for Behçet's disease are still missing. There is a remarkable lack of randomized con-

trolled studies. Today, therapies are mostly based on open uncontrolled trials, pilot studies or case reports. On the other hand, placebo-controlled trials are ethically not justifiable in a disease which untreated may lead to death or blindness.

Due to its poor prognosis, eye involvement is one of the most worrying manifestations for patients with Behçet's disease. Without treatment, vision is usually lost on average 3.4 years after the onset of eye symptoms [90]. Thus it is often necessary to treat the condition earlier and more aggressively than other forms of uveitis. Therapy of ocular Behçet's disease usually consists of systemic corticosteroids and immunosuppressants. However, despite immunosuppressive treatment, loss of useful visual acuity occurs in up to 74% of the affected eyes within 5–10 years [11, 157]. Therefore evaluation of new therapeutic concepts is urgently needed. Fortunately, with novel substances such as interferon-α and TNF-α antagonists, new therapeutic options are being found which are possibly more effective and may lead to a better visual prognosis than conventional anti-inflammatory drugs. On the other hand, these new medications are very expensive and more complicated in the institution and control of treatment. Thus it has to be assumed that in the near future these medications will not be available everywhere for all patients.

In this chapter the authors aim to give both an update on modern therapy and a careful evaluation of the therapeutic options for eye involvement in Behçet's disease.

12.7.1 Local Treatment

Eyedrops or ointments containing corticosteroids, e.g. prednisolone acetate, are indicated during an acute inflammatory attack of the anterior eye segment such as anterior uveitis or scleritis. Application frequency depends on the severity of the inflammation. Subconjunctival steroid injection may be helpful in the case of hypopyon in the anterior chamber. In contrast topical steroids are not effective in treating posterior eye segment involvement such as vitritis, posterior uveitis or cystoid macular edema. For these conditions steroids administered by periocular injection may be helpful in single cases.

Concomitant administration of topical mydriatics both to prevent posterior synechiae and to relieve ocular pain is necessary if a moderate to severe inflammation of the anterior chamber is present.

12.7.2 Systemic Treatment

12.7.2.1 Corticosteroids

Because of their high anti-inflammatory potential, corticosteroids are widely used in the management of ocular Behçet's disease. They are indicated to shorten the duration of an acute uveitis attack, but as a monotherapy they are not effective for long-term treatment, probably because the doses necessary for maintenance of remission would be too high, accompanied by unacceptable side effects [77]. Thus in most cases a steroid sparing immunosuppressive agent has to be added. An exception may represent cases in which immunosuppressive drugs are contraindicated, such as during pregnancy [61].

Initial dosage of orally administered corticosteroids usually amounts to 1–2 mg/kg per day prednisolone equivalent, followed by a gradual decrease of the dose by 5–10 mg/week. For cases of severe sight threatening attacks of posterior uveitis, efficacy of a high dose intravenous steroid therapy prior to initiation of oral corticosteroid treatment has been reported in some patients [140].

12.7.2.2 Nonsteroidal Anti-inflammatory Drugs

Systemic nonsteroidal anti-inflammatory drugs, e.g. indomethacin, may be beneficial in mild joint involvement, but due to their comparatively weak effect they have no relevance in the treatment of eye involvement in Behçet's disease.

12.7.2.3 Immunosuppressive and Cytotoxic Agents

12.7.2.3.1 Cyclosporine A

Cyclosporine A is the most widely used immunosuppressive drug for ocular Behçet's disease. At dosages of 3–5 mg/kg/day or in combination with low dose corticosteroids it has proven to be a rapidly acting and effective medication not only to treat acute attacks but also to reduce recurrence rates of ocular inflammation [108, 113, 136]. However, nephrotoxicity, particularly at dosages higher than 5 mg/kg per day, and relapses after discontinuation of therapy may limit the use of cyclosporine A [77]. Moreover, there is evidence suggesting that cyclosporine A exhibits neurotoxicity in patients with Behçet's disease or accelerates the development of neuro-Behçet's disease [73, 66].

12.7.2.3.2 Azathioprine

Azathioprine is considered as a proven immunosuppressive drug with considerably fewer side effects. Therefore it is acceptable as an alternative to cyclosporine A for the treatment of eye involvement in Behçet's disease. In a placebo controlled double-blind study, azathioprine (dosage of 2.5 mg/kg daily) was effective in controlling intraocular inflammation, maintaining visual acuity as well as preventing an onset or progression of eye disease [153]. Compared to those patients originally allocated to the placebo group in the previous study, long-term visual prognosis improved in the group treated with azathioprine [49]. But this beneficial effect did not extend to those patients who had eye disease for 2 years or more when they first started azathioprine treatment [155]. Using azathioprine it must be noted that the therapeutic effect of this drug usually starts with a delay of 2–3 months. To treat or prevent an acute attack, this period has to be bridged with systemic steroids.

12.7.2.3.3 Colchicine

The therapeutic effect of colchicine in Behçet's disease seems to vary among races. Except for Japan, where it is widely used and has been reported to be very effective, colchicine has been shown to be beneficial only for mucocutaneous and articular manifestations but not for severe ocular involvement [8, 102].

12.7.2.3.4 Tacrolimus (FK506)

Tacrolimus (FK506) develops a similar immunosuppressive activity at lower dosages than cyclosporine A. In a few open studies with small cohorts of Behçet's patients, low dose tacrolimus showed good efficacy in cases of sight-threatening posterior uveitis, in which previous treatment with cyclosporine A had to be discontinued either because of a lack of response or unacceptable side effects [69, 131].

12.7.2.3.5 Mycophenolate Mofetil

Mycophenolate mofetil is a relatively new immunosuppressive drug. It has been successfully used in several autoimmune ocular disorders, e.g. corneal transplant rejection, endogenous uveitis or atopic keratoconjunctivitis. Theoretically one should expect that mycophenolate mofetil is also beneficial for uveitis and retinal vasculitis due to Behçet's disease. However, there are no data published yet as to whether this holds true. Interestingly a prospective clinical proof-of-principle study investigating the effectivity of mycophenolate mofetil in mucocutaneous Behçet's disease had to be discontinued because of treatment failure [2].

12.7.2.3.6 Methotrexate

Methotrexate does not play an important role in the treatment of eye involvement. It is generally believed that this drug has only a weak effect on the manifestations of Behçet's disease [61].

12.7.2.3.7 Others

Cytotoxic drugs such as chlorambucil or cyclophosphamide are used for very severe and life threatening manifestations of Behçet's disease (CNS, arterial aneurysms, gastrointestinal) [110, 148]. Thalidomide is used for therapy resistant aphthous ulcers and skin lesions as well as for exceptional cases of refractory life threatening symptoms of Behçet's disease [50]. In our opinion, for ocular disease these drugs are usually no longer indicated because of their toxic side effects.

12.7.2.4 Immunomodulating Substances

12.7.2.4.1 Interferon-α

Interferon-α is a cytokine which belongs to the so-called type-1 interferons and which can be produced by virtually all somatic cells after viral infection. It exerts antiviral, antiproliferative, antiangiogenetic and immunomodulatory effects. There are two different human recombinant α-interferons (IFN-α-2a and IFN-α-2b) in use for the treatment of viral hepatitis and myeloproliferative syndromes, as well as for certain solid tumours and lymphomas [76].

Since the early 1990s interferon-α has become more important in the treatment of severe ocular Behçet's disease. Several open studies were performed to investigate the efficacy of interferon-α especially in such patients with posterior uveitis, panuveitis, retinal vasculitis or cystoid macular edema who showed to be refractory to previous conventional immunosuppressive treatment. Interferon-α-2a led to a quick response and complete remission of ocular symptoms in more than 90 % of these patients [77, 79, 134, 149]. A significant increase in visual acuity, complete disappearance of cystoid macular edema without additional treatment and an only small recurrence rate even after cessation of interferon therapy were also found. Moreover interferon-α-2a led to reperfusion of occluded retinal vessels as well as to complete regression of retinal neovascularization in some patients. Side effects of interferon-α-2a were frequent but, except for hypothyroidism, dose dependent and reversible. The development of autoimmune phenomena during treatment with interferon may be a major concern. Pre-existing depression and psoriasis may be a contraindication for interferon as well. Recently published data suggest that interferon-α-2a also leads to a better long-term visual prognosis in severe ocular Behçet's disease than conventional immunosuppressants. None out of 15 eyes in 9 patients, who were treated with interferon-α-2a, showed a decrease of visual acuity over a follow-up period of at least 5 years [25].

There exists no consensus yet on the interferon dosage for treatment of Behçet's disease. In our hands, the initial dosage necessary to achieve optimal results is 3–6 million IU daily for 4–8 weeks; a maintenance dosage amounts to 3 million IU 3×/week, with a treatment duration of at least 6 months before cessation of the drug [76]. Previous immunosuppressive treatment should be discontinued and systemic corticosteroids reduced to a maximum of 10 mg prednisolone equivalent per day before the initiation of interferon treatment to avoid possible antagonistic effects.

Regarding published data, there may be no doubt that interferon-α-2a is very effective in the therapy of severe ocular Behçet's disease and moreover superior to conventional immunosuppressants. Nevertheless in Germany a randomized controlled multicentre study to compare the efficacy of interferon-α-2a against cyclosporine A has been initiated.

12.7.2.4.2 TNF-α Antagonists

A promising new approach for the treatment of Behçet's disease is the use of inhibitors of tumour necrosis factor alpha (TNF-α). This cytokine produced by Th1-lymphocytes is assumed to play an important role in Behçet's disease as well as in other chronic inflammatory disorders. In two case series there were a total of ten patients with sight-threatening and refractory posterior uveitis or panuveitis (due to Behçet's disease in eight). Infliximab, a soluble human-mouse chimeric monoclonal antibody to TNF-α

(intravenously in a dosage of 3–5 mg/kg at intervals of 2–8 weeks) led to rapid and effective suppression of the acute inflammatory attacks. In one patient reactivation of tuberculosis, a known adverse effect of infliximab besides the anaphylactic reaction, occurred [58, 128]. In an early phase II clinical study infliximab showed efficacy in Behçet's patients with uveoretinitis who did not respond well to cyclosporine treatment. But approximately 2 months after completion of the study, all subjects had recurrences of uveitis. Consequently infliximab was assumed to remain effective for 2 months only, whereafter uveitis may relapse [105]. In contrast to the majority of the immunosuppressants and to interferon-α, no more data are available so far describing the long-term beneficial effects of infliximab. Whether tachyphylaxia during therapy occurs also remains unclear. On the other hand, in Behçet's disease infliximab seems to be more effective than etanercept, a soluble TNF-α receptor [36]. For adalimumab, the latest representative of the TNF-α antagonists, no data have been published yet regarding its efficacy in ocular Behçet's disease.

12.7.3 Surgical Treatment

In contrast to drug therapy, ocular surgery plays a secondary role in the treatment of eye involvement due to Behçet's disease. It is usually limited to secondary complications of the ocular inflammation like cataract or glaucoma in the anterior eye segment. Complications regarding the posterior eye segment such as macular hole, retinal detachment or persisting vitreous haemorrhage are usually indications for vitreoretinal surgery.

Cataract surgery will be able to be scheduled in most cases. However, it is highly recommended to perform this procedure only when the eye has been at least 3 months free from inflammation in order to keep postoperative inflammation mild [59].

Laser photocoagulation is a widely used procedure which has been reported to be successful in preventing complications of retinal and disc neovascularization due to occlusive vasculitis [6]. On the other hand, interferon-α-2a led to complete regression of retinal neovascularization in some cases [77, 134]. Thus a treatment attempt with this anti-angiogenetic substance may be indicated before deletion of large parts of the retina by laser photocoagulation.

12.7.4 Practical Treatment Recommendations

Treatment of Behçet's disease has to be adapted to patients' most severe symptoms, usually ocular or neurological manifestations. By close cooperation between physicians of different disciplines, a therapy, which should be sufficient for all present manifestations, has to be found.

Regarding ocular involvement exclusive local treatment is restricted to cases with only mild or moderate anterior segment inflammation. With the background of poor visual prognosis, the first onset of vitreous or posterior segment affection normally indicates the initiation of an aggressive systemic treatment. This first-line therapy usually consists of a combination of steroids and an established immunosuppressant like cyclosporine A or azathioprine. If this immunosuppressive therapy fails, not inducing remission of the disease or recurrences under treatment occur, we do not recommend adding a further immunosuppressive drug because of potentiating adverse effects but of switching to interferon-α-2a. In those cases where retinal neovascularizations are present or an acute retinal vessel occlusion occurs, interferon-α-2a may also be first-line therapy without previous steroid or immunosuppressive treatment. In our opinion TNF-α antagonists should be reserved for those patients who either do not respond to interferon-α-2a or in whom interferon has to be discontinued because of side effects, because interferon-α-2a shows a lower recurrence rate at a similar cost. Ocular surgery should be limited to secondary complications of eye involvement. In most patients with ocular Behçet's disease it should be possible today, by early initiation of sufficient medical treatment, both to preserve visual acuity and to prevent secondary complications.

Summary for the Clinician

- **Treatment of Behçet's disease is a challenge and necessitates close cooperation between different medical disciplines**
- **Posterior eye segment involvement is associated with poor visual prognosis and therefore indicates early and aggressive systemic treatment**
- **First-line therapy usually consists of a combination of systemic corticosteroids and an established immunosuppressant such as cyclosporine A or azathioprine**
- **Novel drugs such as interferon-α-2a or TNF-α antagonists may lead to better visual prognosis of ocular Behçet's disease because they are effective even if conventional immunosuppression fails**
- **In contrast to drug therapy, ocular surgery plays an inferior role in the treatment of ocular Behçet's disease and is limited to secondary complications of inflammation**

References

1. Adamantiades B (1931) Sur un cas d'iritis á hypopyon recidivante. Ann Ocul (Paris) 168:271–274
2. Adler YD, Mansmann U, Zouboulis CC (2001) Mycophenolate mofetil is ineffective in the treatment of mucocutaneous Adamantiades-Behçet's disease. Dermatology 203:322–324
3. Ahmad T, Wallace GR, James TE, et al. (2003) Mapping the HLA association in Behcet's disease: a role for tumor necrosis factor polymorphisms? Arthritis Rheum 48:807–813
4. Akman-Demir G, Serdaroglu P, Tasci B (1999) Clinical patterns of neurological involvement in Behcet's disease: evaluation of 200 patients. The Neuro-Behcet Study Group. Brain 122:2171–2182
5. Atmaca LS (1989) Fundus changes associated with Behçet's disease. Arch Clin Exp Ophthalmol 227:340–344
6. Atmaca LS, Batioglu F, Ýdil A (1996) Retinal and disc neovascularisation in Behçet's disease and efficacy of laser photocoagulation. Graefes Arch Clin Exp Ophthalmol 234:94–99
7. Aydintug O, Tokgöz G, D'Cruz DP, Gürler A, Cervera R, Düzgün N, et al. (1993) Antibodies to endothelial cells in patients with Behçet's disease. Clin Immunol Immunopathol 67:157–162
8. Bang D (1997) Treatment of Behçet's disease. Yonsei Med J 38:401–410
9. Behçet H (1937) Über rezidivierende aphtöse, durch ein Virus verursachte Geschwüre am Mund, am Auge und an den Genitalien. Derm Wochenschr 36:1152–1157
10. Ben-Chetrit E, Cohen R, Chajek-Shaul T (2002) Familial Mediterranean fever and Behcet's disease – are they associated? J Rheumatol 29:530–534
11. BenEzra D, Cohen E (1986) Treatment and visual prognosis in Behçet's disease. Br J Ophthalmol 70:589–592
12. Bentley CR, Stanford MR, Shilling JS (1993) Macular ischemia in posterior uveitis. Eye 7:411–414
13. Boiardi L, Salvarani C, Casali B, et al. (2001) Intercellular adhesion molecule-1 gene polymorphisms in Behcet's disease. J Rheumatol 28:1283–1287
14. Carletto A, Pacor ML, Biasi D, Caramaschi P, Zemininan S, Bellavite P, et al. (1997) Changes of neutrophil migration without modification of in vitro metabolism and adhesion in Behçet's disease. J Rheumatol 24:1332–1336
15. Chafa O, Fischer AM, Meriane F, Chellali T, Sternberg C, Otmani F, et al. (1992) Behçet syndrome associated with protein S deficiency. Thromb Haemostasis 67:1–3
16. Chajek T, Fainaru M (1975) Behçet's disease: report of 41 cases and a review of the literature. Medicine 54:179–196
17. Chajek-Shaul T, Pisanty S, Knobler H, et al. (1987) HLA-B*51 may serve as an immunogenetic marker for a subgroup of patients with Behcet's syndrome. Am J Med 83:666–672
18. Chambers JC, Haskard DO, Kooner JS (2001) Vascular endothelial function and oxidative stress mechanisms in patients with Behçet's syndrome. J Am Coll Cardiol 37:517–520
19. Choukri F, Chakib A, Himmich H, Hue S, Caillat-Zucman S (2001) HLA-B*51 and B*15 alleles confer predisposition to Behcet's disease in Moroccan patients. Hum Immunol 62:180–185
20. Chung YM, Yeh TS, Sheu MM (1990) Behcet's disease with ocular involvement in Taiwan: a joint survey of six major ophthalmological departments. J Formos Med Assoc 89:413–417
21. Cochereau-Massin I, Wechsler B, Le Hoang P, et al. (1992) Prognostic oculaire de la maledie de Behçet. J Fr Ophthalmol 15:343–347
22. Criteria for Diagnosis of Behcet's Disease (1990) International Study Group for Behcet's Disease. Lancet 335:1078–1080
23. Davatchi F, Shahram F, Akkbarian M (1992) The prevalence of Behçet's disease in Iran. In: Nasution AR, Darmawan J, Isbagio H (eds) Proceedings of the 7th APLAR Congress of Rheumatology. Churchill Livingstone, Japan, pp 95–98

24. deSmet MD, Dayan M (2000) Prospective determination of T-cell responses to S-antigen in Behçet's disease patients and controls. Invest Ophthamol Vis Sci 41:3480–3484
25. Deuter CME, Kötter I, Günaydin I, Zierhut M, Stübiger N (2004) Ocular involvement in Behçet's disease: first 5-year results for visual development after treatment with interferon alfa-2a. Ophthalmologe 101:129–134
26. Dilsen N, Konice M, Aral O (1985) Our diagnostic criteria of Behcet's disease – an overview: recent advances in Behcet's disease. Royal Society of Medicine Services, London
27. Dilsen N, Konice M, Aral O, Ocal L, Inanc M, Gul A (1993) Comparative study of the skin pathergy test with blunt and sharp needles in Behcet's disease: confirmed specificity but decreased sensitivity with sharp needles. Ann Rheum Dis 52:823–825
28. Dilsen N (1996) History and development of Behçet's disease. VII International Conference on Behçet's Disease. Rev Rheum Engl Ed 63:512–519
29. Dundar SV, Gencalp U, Simsek H (1985) Familial cases of Behçet's disease. Br J Dermatol 113:319–321
30. Efthimiou J, Addison IE, Johnson BV (1989) In vivo leukocyte migration in Behçet's syndrome. Ann Rheum Dis 48:206–210
31. Eglin RP, Lehner T, Subak-Sharpe JH (1982) Detection of RNA complementary to herpes simplex virus in mononuclear cells from patients with Behçet's syndrome and recurrent oral ulcers. Lancet 2:1356–1361
32. Eksioglu-Demiralp E, Direskeneli H, Ergun T, Fresko I, Akoglu T (1999) Increased CD4+CD16+ and CD4+CD56+T cell subsets in Behçet's disease. Rheumatol Int 19:23–26
33. Er H, Evereklioglu C, Cumureu T, Turkoz Y, Ozerol E, Sahin K, et al. (2002) Serum homocysteine level is increased and correlated with endothelin-1 and nitric oxide in Behçet's disease. Br J Ophthalmol 86:653–567
34. Esin S, Gul A, Hodara V, Jeddi-Tehrani M, Dilsen N, Konice M, et al. (1997) Peripheral blood T cell expansions in patients with Behcet's disease. Clin Exp Immunol 107:520–527
35. Espinosa G, Font J, Tassies D, et al. (2002) Vascular involvement in Behcet's disease: relation with thrombophilic factors, coagulation activation, and thrombomodulin. Am J Med 112:37–43
36. Estrach C, Mpofu S, Moots RJ (2002) Behçet's syndrome: response to infliximab after failure of etanercept. Rheumatology 41:1213–1214
37. Evereklioglu C, Turkoz Y, Er H, Inaloz HS, Ozbek E, Cekmen M (2002) Increased nitric oxide production in patients with Behçet's disease: is it a new activity marker? J Am Acad Dermatol 46:50–54
38. Falk K, Rotzschke O, Takiguchi M, et al. (1995) Peptide motifs of HLA-B*51, -B52 and -B78 molecules, and implications for Behcet's disease. Int Immunol 7:223–228
39. Fortune F, Walker J, Lehner T (1990) The expression of γδT cell receptor and the prevalence of primed, activated and Ig-A bound T cell in Behçet's syndrome. Clin Exp Immunol 82:326–332
40. Freydottir J, Lau SH, Fortune F (1999) γδT cells in Behçet's disease (BD) and recurrent aphthous stomatitis (RAS). Clin Exp Immunol 118:451–457
41. Gonzalez-Escribano MF, Rodriguez MR, Walter K, Sanchez-Roman J, Garcia-Lozano JR, Nunez-Roldan A (1998) Association of HLA-B*51 subtypes and Behcet's disease in Spain. Tissue Antigens 52:78–80
42. Gül A, Esin S, Dilsen N, Konice M, Wigzell H, Biberfeld P (1995) Immunohistology of skin pathergy reaction in Behcet's disease. Br J Dermatol 132:901–907
43. Gül A, Özbek U, Öztürk C, Inanç M, Koniçe M, Özçelik T (1996) Coagulation factor V gene mutation increases the risk of venous thrombosis in Behçet's disease. Br J Rheumatol 35:1178–1180
44. Gül A, Aslantas AB, Tekinay T, Koniçe M, Özçelik T (1999) Procoagulant mutations and venous thrombosis in Behçet's disease. Rheumatology (Oxford) 38:1298–1299
45. Gül A, Inanc M, Ocal L, Aral O, Konice M (2000) Familial aggregation of Behcet's disease in Turkey. Ann Rheum Dis 59:622–625
46. Gül A, Hajeer AH, Worthington J, Ollier WE, Silman AJ (2001) Linkage mapping of a novel susceptibility locus for Behcet's disease to chromosome 6p22–23. Arthritis Rheum 44:2693–2696
47. Gül A, Uyar FA, Inanc M, et al. (2001) Lack of association of HLA-B*51 with a severe disease course in Behcet's disease. Rheumatology (Oxford) 40:668–672
48. Gürler A, Boyvat A, Türsen Ü (1997) Clinical manifestations of Behcet's disease: an analysis of 2147 patients. Yonsei Med J 38:423–427
49. Hamuryudan V, Özyazgan Y, Hizli N, Mat C, Yurdakul S, Tüzün Y, [a]enocak M, Yazici H (1997) Azathioprine in Behçet's syndrome: effects on long-term prognosis. Arthritis Rheum 40:769–774
50. Hamuryudan V, Mat C, Saip S, Ozyazgan Y, Siva A, Yurdakul S, Zwingenberger K, Yazici H (1998) Thalidomide in the treatment of the mucocutaneous lesions of the Behçet syndrome. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 128:443–450
51. Hamzaoui K, Ayed K, Hamza M, et al. (1988) Natural killer cells in Behcet's disease. Clin Exp Immunol 71:126–131

52. Hasan A, Fortune F, Wilson A, Warr K, Shinnick T, Mizushima Y, et al. (1996) Role of γδT cells in pathogenesis and diagnosis of Behçet's disease. Lancet 347:789–794
53. Hirohata S, Hashimoto S (1998) Abnormal T cell responses to bacterial superantigens in Behçet's disease. Clin Exp Immunol 112:317–324
54. Houman MH, Ben Ghorbel I, Khiari Ben Salah I, Lamloum M, Ben Ahmed M, Miled M (2001) Deep vein thrombosis in Behcet's disease. Clin Exp Rheumatol 19:S48–50
55. Hu W, Hasa A, Wilson A, Stanford MR, Li-Yang Y, Todryk S, et al. (1998) Experimental mucosal induction of uveitis with 60-kDa heat shock protein-derived peptide 336–351. Eur J Immunol 28:2444–2455
56. Ishihara M, Ohno S, Mizuki N, et al. (1996) Allelic variations in the TAP2 and LMP2 genes in Behcet's disease. Tissue Antigens 47:249–252
57. Isogyi E, Ohno S, Kotake S, Isogai H, Tsurumizu T, Fujii N, et al. (1990) Chemiluminescence of neutrophils from patients with Behçet's disease and its correlation with an increased proportion of uncommon serotypes of *Streptococcus sanguis* in the oral flora. Arch Oral Biol 35:43–48
58. Joseph A, Raj D, Dua HS, Powell PT, Lanyon PC, Powell RJ (2003) Infliximab in the treatment of refractory posterior uveitis. Ophthalmology 110:1449–1453
59. Kadayifcilar S, Gedik S, Eldem B, Irkec M (2002) Cataract surgery in patients with Behçet's disease. J Cataract Refract Surg 28:316–320
60. Kahan A, Hamzaoui K, Ayed K (1992) Abnormalities of T lymphocyte subsets in Behçet's disease demonstrated with anti-CD45RA and anti-CD29 monoclonal antibodies. J Rheumatol 19:742–746
61. Kaklamani VG, Kaklamanis PG (2001) Treatment of Behçet's disease – an update. Semin Arthritis Rheum 30:299–312
62. Kaktos (1993) In: Hippokrates (ed) Third book on epidemiology. Case 7,13:209
63. Kaneko F, Takahashi Y, Muramatsu R, Adachi K, Miura Y, Nakane A (1985) Natural killer cell numbers and function in peripheral lymphoid cells in Behçet's disease. Br J Dermatol 113:313–318
64. Kaneko S, Suzuki N, Yamashita N, Nagafuchi H, Nakajima T, Wakisaka S, et al. (1997) Characterization of T cells specific for an epitope of human 60-kD heat shock protein (hsp) in patients with Behçet's disease (BD) in Japan. Clin Exp Immunol 108:204–212
65. Karasneh J, Hajeer AH, Barrett J, Ollier WER, Thornhill M, Gul A (2003) Association of specific interleukin 1 gene cluster polymorphisms with increased susceptibility for Behcet's disease. Rheumatology 42:860–864
66. Kato Y, Numaga J, Kato S, Kaburaki T, Kawashima H, Fujino Y (2001) Central nervous system symptoms in a population of Behçet's disease patients with refractory uveitis treated with cyclosporine A. Clin Exp Ophthalmol 29:335–336
67. Keino H, Sakai JI, Nishioka K, Sumida T, Usui M (2000) Clonally accumulating T cells in the anterior chamber of Behçet's disease. Am J Ophthalmol 130:243–245
68. Kilmartin DJ, Finch A, Acheson RW (1997) Primary association of HLA-B*51 with Behcet's disease in Ireland. Br J Ophthalmol 81:649–653
69. Kilmartin DJ, Forrester JV, Dick AD (1998) Tacrolimus (FK506) in failed cyclosporine A therapy in endogenous posterior uveitis. Ocul Immunol Inflamm 6:101–109
70. Kiraz S, Ertenli I, Çalgüneri M, Öztürk MA, Haznedaroglu IC, Altun B, et al. (2001) Interactions of nitric oxide and superoxide dismutase in Behçet's disease. Clin Exp Rheumatol 19 (Suppl 24):S25–S29
71. Kitaichi N, Kotake S, Sasamoto Y, Namba K, Matsuda A, Ogasawara K, et al. (1999) Prominent increase of macrophage migration inhibitory factor in the sera of patients with uveitis. Invest Ophthalmol Vis Sci 40:247–250
72. Kitaichi N, Ogasawara K, Iwabuchi K, Nishihira J, Namba K, Onoé K Jr, et al. (1999) Different influence of macrophage migration inhibitory factor (MIF) in signal transduction pathway of various T cell subsets. Immunobiology 201:356–367
73. Kotake S, Higashi K, Yoshikawa K, Sasamoto Y, Okamoto T, Matsuda H (1999) Central nervous system symptoms in patients with Behçet disease receiving cyclosporine therapy. Ophthalmology 106:586–589
74. Kötter I, Stübiger N (1999) Aktuelle Aspekte der Diagnostik und Therapie des Morbus Behçet. Akt Rheumatol 24:51–57
75. Kötter I, Gunaydin I, Stubiger N, et al. (2001) Comparative analysis of the association of HLA-B*51 suballeles with Behcet's disease in patients of German and Turkish origin. Tissue Antigens 58:166–170
76. Kötter I, Stübiger N, Zierhut M (2003) Use of interferon-α in Behçet's disease. In: Zierhut M, Ohno S (eds) Immunology of Behçet's disease. Swets & Zeitlinger, Lisse, Netherlands, pp 155–159
77. Kötter I, Zierhut M, Eckstein AK, Vonthein R, Ness T, Guenaydin I, Grimbacher B, Blaschke S, Meyer-Riemann W, Peter HH, Stübiger N (2003) Human recombinant interferon alfa-2a for the treatment of Behçet's disease with sight threatening posterior or panuveitis. Br J Ophthalmol 87:423–431
78. Krause I, Uziel Y, Guedj D et al. (1998) Mode of presentation and multisystem involvement in Behcet's disease: the influence of sex and age of disease onset. J Rheumatol 25:1566–1569

79. Krause L, Turnbull JR, Torun N, Pleyer U, Zouboulis CC, Förster MH (2003) Interferon alfa-2a in the treatment of ocular Adamantiades-Behçet's disease. Adv Exp Med Biol 528:511–519
80. Kretschmann K, Seeliger MW, Ruether K, et al. (1998) Multifocal electroretinography in patients with macular dystrophy. Br J Ophthalmol 82:267–275
81. Lee CR, Kim WH, Cho YS, et al. (2001) Colonoscopic findings in intestinal Behcet's disease. Inflamm Bowel Dis 7:243–249
82. Lee KH, Bang D, Choi ES, Chun WH, Lee ES, Lee S (1999) Presence of circulating antibodies to a disease-specific antigen on cultured human dermal microvascular endothelial cells in patients with Behçet's disease. Arch Dermatol Res 291:374–381
83. Lee S (1997) Diagnostic criteria of Behcet's disease: problems and suggestions. Yonsei Med J 38:365–369
84. Lehner T, Lavery E, Smith R, von der Zee R, Mizushima Y, Shinnick T (1991) Association between the 65-kilodalton heat shock protein, *Streptococcus sanguis,* and the corresponding antibodies in Behçet's disease. Infect Immun 59:1434–1441
85. Lehner T (1997) The role of heat shock protein, microbial and autoimmune agents in aetiology of Behçet's disease. Intern Rev Immunol 14:21–32
86. Lehner T (2000) Immunopathogenesis of Behçet's disease. In: Bang D, Lee ES, Lee S (eds) Behçet's disease. Design Mecca, Seoul, pp 3–18
87. Leiba M, Sidi Y, Gur H, Leiba A, Ehrenfeld M (2001) Behcet's disease and thrombophilia. Ann Rheum Dis 60:1081–1085
88. Livneh A, Aksentijevich I, Langevitz P, et al. (2001) A single mutated MEFV allele in Israeli patients suffering from familial Mediterranean fever and Behcet's disease (FMF-BD). Eur J Hum Genet 9:191–196
89. Mammo L, Al-Dalaan A, Bahabri SS, Saour JN (1997) Association of factor V Leiden with Behçet's disease. J Rheumatol 24:2196–2198
90. Mamo JG (1970) The rate of visual loss in Behçet's disease. Arch Ophthalmol 84:451–452
91. Mason RM, Barnes CG (1969) Behcet's syndrome with arthritis. Ann Rheum Dis 28:95–103
92. MatsuoT, Sato Y, Shiraga F, et al. (1999) Choroidal abnormalities in Behçet's disease observed by simultaneous indocyanine green and fluorescein angiography with scanning laser ophthalmoscopy. Ophthalmology 106:295–300
93. McLennan N, Masters M (1998) GroE is vital for cell-wall synthesis. Nature 392:1–39
94. Mege JL, Dilsen N, Sanguedolce V, Gul A, Bongrand P, Roux H, et al. (1993) Overproduction of monocyte derived tumor necrosis factor α, interleukin (IL) 6, IL-8 and increased neutrophil superoxide generation in Behçet's disease. A comparative study with familial Mediterranean fever and healthy subjects. J Rheumatol 20:1544–1549
95. Misgav M, Goldberg Y, Zeltser D, Eldor A, Berliner AS (2000) Fatal pulmonary artery thrombosis in a patient with Behçet's disease, activated protein C resistance and hyper-homocysteinemia. Blood Coagul Fibrinolysis 11:421–423
96. Mishima S, Masuda K, Izawa Y, et al. (1979) Behçet's disease in Japan: Ophthalmological aspects. Trans Am Ophthalmol Soc 76:225–279
97. Mizuki N, Ohno S, Ando H, et al. (1996) HLA-C genotyping of patients with Behcet's disease in the Japanese population. Hum Immunol 50:47–53
98. Mizuki N, Inoko H, Ohno S (1997) Molecular genetics (HLA) of Behcet's disease. Yonsei Med J 38:333–349
99. Mizuki N, Ota M, Yabuki K, et al. (2000) Localization of the pathogenic gene of Behcet's disease by microsatellite analysis of three different populations. Invest Ophthalmol Vis Sci 41:3702–3708
100. Mizushima Y (1988) Revised diagnostic criteria for Behcet's disease in 1987. Ryumachi 28:66–70
101. Mochizuki M, Suzuki N, Takeno M, Nagafuchi H, Harada T, Kaneoka H, et al. (1994) Fine antigen specificity of human γδT cell lines Vγ9+ established by repetitive stimulation with a serotype (KTH-1) of a gram-positive bacterium, *Streptococcus sanguis*. Eur J Immunol 24:1536–1543
102. Mochizuki M (1997) Immunotherapy for Behçet's disease. Intern Rev Immunol 14:49–66
103. Müftüoglu AÜ, Yazici H, Yurdakul S, et al. (1981) Behçet's disease: lack of correlation of clinical manifestations with HLA antigens. Tissue Antigens 17:226–230
104. Nakae K, Masaki F, Hashimoto T, et al. (1993) Behçet's disease. In: Wechsler B, Godeau P (eds) International Congress Series 1037. Excerpta Medica, Amsterdam, pp 145–151
105. Nakamura S, Ohno S (2003) The efficacy of anti-TNF-alpha antibody in the treatment of uveitis patients with Behçet's disease. In: Zierhut M, Ohno S (eds) Immunology of Behçet's disease. Swets & Zeitlinger, Lisse, Netherlands, pp 151–154
106. Niwa Y, Miyake S, Sakane T, Shingu M, Yokoyama M (1982) Auto-oxidative damage in Behçet's disease – endothelial cell damage following the elevated oxygen radicals generated by stimulated neutrophils. Clin Exp Immunol 49:247–255
107. Niwa Y, Mizushima Y (1990) Neutrophil-potentiating factors released from stimulated lymphocytes; special reference to the increase in neutrophil-potentiating factors from streptococcus-stimulated lymphocytes of patients with Behçet's disease. Clin Exp Immunol 79:353–360
108. Nussenblatt RB, Palestine AG, Chan CC, Mochizuki M, Yancey K (1985) Effectiveness of cyclosporine therapy for Behçet's disease. Arthritis Rheum 28:671–679
109. O'Duffy JD (1974) Suggested criteria for diagnosis of Behçet's disease. J Rheumatol 1:18

110. O'Duffy JD, Robertson DM, Goldstein NP (1984) Chlorambucil in the treatment of uveitis and meningoencephalitis of Behçet's disease. Am J Med 76:75–84
111. Ohno S, Ohguchi M, Hirose S, et al. (1982) Close association of HLA-Bw51 with Behcet's disease. Arch Ophthalmol 100:1455–1458
112. Orem A, Vanizor B, Cimsit, Kiran E, Deger O, Malkoc M (1999) Decreased nitric oxide production in patients with Behçet's disease. Dermatology 198:33–36
113. Özdal PÇ, Ortaç S, Taskintuna I, Firat E (2002) Long-term therapy with low dose cyclosporine A in ocular Behçet's disease. Doc Ophthalmol 105:301–312
114. Palimeris G, Papakonstantinou P, Mantas M (1984) The Adamantiades-Behçet's syndrome in Greece. In: Saari KM (ed) Uveitis update. Excerpta Medica, Amsterdam, p 321
115. Park KD, Bang D, Lee ES, Lee SH, Lee S (1993) Clinical study on death in Behcet's disease. J Korean Med Sci 8:241–245
116. Park SH, Park KS, Seo YI, et al. (2002) Association of MICA polymorphism with HLA-B*51 and disease severity in Korean patients with Behcet's disease. J Korean Med Sci 17:366–370
117. Paul M, Klein T, Krause I, Molad Y, Narinsky R, Weinberger A (2001) Allelic distribution of HLA-B*5 in HLA-B5-positive Israeli patients with Behcet's disease. Tissue Antigens 58:185–186
118. Pronai L, Ichikawa Y, Nakzawa H, Arimori S (1991) Enhanced superoxide generation and the decreased scavenging activity of peripheral blood leukocytes in Behçet's disease – effect of colchicines. Clin Exp Rheumatol 9:227–233
119. Rammensee HG, Friede T, Stevanovic S (1995) MHC ligands and peptide motifs: first listing. Immunogenetics 41:178–228
120. Robinson J, Waller MJ, Parham P, Bodmer JG, Marsh SG (2001) IMGT/HLA database – a sequence database for the human major histocompatibility complex. Nucleic Acids Res 29:210–213
121. Sahin S, Akoglu T, Direskeneli H, Sen LS, Lawrence R (1996) Neutrophil adhesion to endothelial cells and factors affecting adhesion in patients with Behçet's disease. Ann Rheum Dis 55:128–133
122. Sakane T, Kotani H, Takada S, Tsunematsu T (1982) Functional aberration of T cell subsets in patients with Behçet's disease. Arthritis Rheum 25:1343–1351
123. Sakane T, Takeno M, Suzuki N, Inaba G (1999) Behcet's disease. N Engl J Med 341:1284–1291
124. Salavarani C, Calamia K, Silingardi M, Ghirarduzzi A, Olivieri I (2000) Thrombosis associated with the prothrombin GA20210 mutation in Behçet's disease. J Rheumatol 27:515–516
125. Salvarani C, Boiardi L, Casali B, Olivieri I, Ciancio G, Cantini F, et al. (2002) Endothelial nitric oxide synthase gene polymorphisms in Behçet's disease. J Rheumatol 29:535–540
126. Saylan T, Özarmargan G, Azizlerli G, et al. (1986) Behçet disease in Turkey. N Zbl Hautkr 1120–1122
127. Serdaroglu P (1998) Behcet's disease and the nervous system. J Neurol 245:197–205
128. Sfikakis PP, Theodossiadis PG, Katsiari CG, Kaklamanis P, Markomichelakis NN (2001) Effect of infliximab on sight-threatening panuveitis in Behçet's disease. Lancet 358:295–296
129. Shehto NM, Ghosh K, Abdul Kader B, al Assad HS (1992) Extensive venous thrombosis in a case of Behçet's disease associated with heterozygous protein C deficiency. Thromb Haemostasis 67:283
130. Shimizu T, Matsumura N (1972) [Behcet's disease]. Nippon Rinsho 30:416–420
131. Sloper CML, Powell RJ, Dua HS (1999) Tacrolimus (FK506) in the treatment of posterior uveitis refractory to cyclosporine. Ophthalmology 106:723–728
132. Stratigos AJ, Laskaris G, Stratigos JD (1992) Behçet's disease. Semin Neurol 12:346–357
133. Stübiger N, Besch D, Deuter CME, et al. (2003) Multifocal ERG changes in patients with ocular Behçet's disease during therapy with interferon alpha 2a. In: Zouboulis CC (ed) Adamantiades-Behçet's disease (Proceedings of the 10th International Conference on Behçet's disease, Berlin), vol 528. Kluwer Academic/Plenum Publishers, New York, pp 529–532
134. Stübiger N, Kötter I, Zierhut M (2000) Complete regression of retinal neovascularisation after therapy with interferon alfa in Behçet's disease. Br J Ophthalmol 84:1437–1438
135. Stübiger N, Zierhut M, Kötter I (2003) Ocular manifestations in Behçet's disease. In: Zierhut M, Ohno S (eds) Immunology of Behçet's disease. Swets & Zeitlinger, Lisse, Netherlands, pp 36–45
136. Süllü Y, Öge I, Erkan D, Aritürk N, Mohajeri F (1998) Cyclosporin-A therapy in severe uveitis of Behçet's disease. Acta Ophthalmol Scand 76:96–99
137. Sumita K, Yamashita N, Takeno M, Sakane T (1997) Dysregulated tyrosine phosphorylation cascade in TCR/CD3 signaling pathway leads to T cell irritability in patients with Behçet's disease. Arthritis Rheum 40 (Suppl):S232
138. Suzuki Y, Hoshi K, Matsuda T, Mizushima Y (1992) Increased peripheral blood gδ T cells and natural killer cells in Behçet's disease. J Rheumatol 19:588–592
139. Tokay S, Direskeneli H, Yurdakul S, Akoglu T (2001) Anticardiolipin antibodies in Behçet's disease: a reassessment. Rheumatology (Oxford) 40:192–195

140. Toker E, Kazokoglu H, Acar N (2002) High dose intravenous steroid therapy for severe posterior segment uveitis in Behçet's disease. Br J Ophthalmol 86:521–523
141. Triolo G, Accardo-Palumbo A, Triolo G, Carbone MC, Ferrante A, Giardina E (1999) Enhancement of endothelial cell E-selection expression by sere from patients with active Behçet's disease: moderate correlation with anti-endothelial cell antibodies and serum myeloperoxidase levels. Clin Immunol 91:330–337
142. Trojano M, Paolicelli D (2001) The differential diagnosis of multiple sclerosis: classification and clinical features of relapsing and progressive neurological syndromes. Neurol Sci 22 Suppl 2:S98–102
143. Tursen U, Kaya TI, Eskandari G, et al. (2001) Association of factor V Leiden and prothrombin gene mutation with Behcet's disease. Arch Dermatol Res 293:537–539
144. Vaiopoulos G, Sfikakis PP, Hatzinikolaou P (1996) Adamantiades-Behçet's disease in sisters. Clin Rheumatol 15:382–384
145. Verity DH, Vaughan RW, Madanat W, Kondeatis E, Zureikat H, Fayyad F, et al. (1997) Factor V Leiden mutation is associated with ocular involvement in Behçet's disease. Am J Ophthalmol 128:352–356
146. Verity DH, Marr JE, Ohno S, Wallace GR, Stanford MR (1999) Behcet's disease, the Silk Road and HLA-B*51: historical and geographical perspectives. Tissue Antigens 54:213–220
147. Verity DH, Wallace GR, Vaughan RW, et al. (1999) HLA and tumour necrosis factor (TNF) polymorphisms in ocular Behcet's disease. Tissue Antigens 54:264–272
148. Watanabe T, Shimoji S, Sugiyama Y, Tsukamoto T, Yamamoto T (1995) Pulse-dose cyclophosphamide therapy for steroid-refractory autoimmune neurological diseases. Rinsho Shinkeigaku 35:611–616
149. Wechsler B, Bodaghi B, Huong DL, Fardeau C, Amoura Z, Cassoux N, Piette JC, LeHoang P (2000) Efficacy of interferon alfa-2a in severe and refractory uveitis associated with Behçet's disease. Ocul Immunol Inflamm 8:293–301
150. Wechsler B, Du LT, Kieffer E (1999) Cardiovascular manifestations of Behcet's disease. Ann Med Interne (Paris) 150:542–554
151. Yamamoto JH, Minami M, Inaba G, Masuda K, Mochizuki M (1990) Cellular autoimmunity to retinal specific antigens in patients with Behçet's disease. Br J Ophthalmol 77:584–589
152. Yamashita N, Kaneoka H, Kaneke S, Takeno M, Oneda K, Koizumi H, et al. (1997) Role of γδT lymphocytes in the development of Behçet's disease. Clin Exp Immunol 107:241–247
153. Yazici H, Pazarli H, Barnes CG, Tüzün Y, Özyazgan Y, Silman A, Serdaroglu S, Oguz V, Yurdakul S, Lovatt GE, Yazici B, Somani S, Müftüoglu A (1990) A controlled trial of azathioprine in Behçet's syndrome. N Engl J Med 322:281–285
154. Yazici H, Basaran G, Hamuryudan V, et al. (1996) The ten-year mortality in Behcet's syndrome. Br J Rheumatol 35:139–141
155. Yazici H, Ozyagan Y (1999) Medical management of Behçet's syndrome. Dev Ophthalmol 31:118–131
156. Zafirakis P, Foster CS (2002) Adamantiades-Behçet disease. In: Foster CS, Vitale AT (eds) Diagnosis and treatment of uveitis. Saunders, Philadelphia, pp 632–652
157. Zierhut M, Saal J, Pleyer U, Kötter I, Dürk H, Fierlbeck G (1995) Behçet's disease: epidemiology and eye manifestations in German and Mediterranean patients. Ger J Ophthalmol 4: 246–251
158. Zierhut M, Mizuki N, Ohno S, et al. (2003) Human genome and diseases: review. Immunology and functional genomics of Behcet's diseases. CMSL Cell Mol Life Sci 60:1903–1922
159. Zouboulis CC, Buttner P, Djawari D, et al. (1993) The HLA pattern in Adamantiades-Behcet's disease in Germany. Association of occurrence, clinical symptoms and follow-up in 39 patients. Hautarzt 44:81–85
160. Zouboulis CC, Djawari D, Kich W (1993) Adamantiades-Behçet's disease in Germany. In: Godeau P, Wechsler B (eds) Behçet's disease. Elsevier Science, New York, pp 193–196
161. Zouboulis CC, Kötter I, Djawari D, et al. (1997) Epidemiological features of Adamantiades-Behçet's disease in Germany and in Europe. Yonsei Med J 38:411–422
162. Zouboulis CC (1999) Epidemiology of Adamantiades-Behcet's disease. Ann Med Interne (Paris) 150:488–498
163. Zouboulis CC (2003) Epidemiology of Adamantiades-Behçet's disease. In: Zierhut M, Ohno S (eds) Immunology of Behçet's disease. Swets & Zeitlinger, Nisse, Netherlands, pp 1–16

Choroiditis: General Considerations and Classification

Carl P. Herbort

Core Messages

- The choroid is the site of origin of posterior intraocular inflammation at least as often as the retina
- Before indocyanine green angiography (ICGA) became available, subtle inflammatory changes in the choroid were not detectable and appraisal of choroiditis was based on clinical appearance alone, leading to an amalgamation of unrelated conditions under the term "white dot syndromes"
- By giving imaging access to the choroid and by enabling lesions to be shown at an incipient stage, ICGA has contributed to an understanding of disease mechanisms and has helped to classify choroiditides
- The two principal properties of the indocyanine green molecule are fluorescence in the infrared wavelengths and a macromolecular behaviour because it is nearly 100% linked to proteins
- Infrared fluorescence can be detected through the retinal pigment epithelium and gives imaging access to the choroid
- The macromolecular ICG-protein complex egresses from the vascular bed only when capillaries are fenestrated (or when larger vessels are severely inflamed) and so impregnates the choroidal stroma. Absence of ICG fluorescence can mean non-perfusion of the choriocapillaris or impaired diffusion of the molecule because of the presence of inflammatory foci
- If choroidal involvement is suspected, dual fluorescein and ICG angiography is indicated (allows complete and thorough work-up of inflammatory involvement of all structures and is essential for follow-up of choroidal inflammation)
- Use a conventional fundus camera coupled to an image digitalizing system (easier for peripheral imaging) or a scanning laser ophthalmoscopy system
- Use one vial of 25 mg indocyanine green diluted in 7.5 ml of physiologic saline solution (Cardiogreen, Akorn, Inc., Buffalo Grove, IL, USA). In case of iodine allergy use iodine-free Infracyanine (SERB Laboratories, Paris, France)
- Angiographic procedure: (1) exclude autofluorescence by taking frames with the highest flash intensity previous to the dye injection; (2) perform early frames of the posterior pole up to 2–3 min (early phase); (3) perform posterior pole and 360 degree periphery panoramic frames at 8–12 min (intermediate phase); (4) perform fluorescein angiography between intermediate and late ICGA phases; (5) perform posterior pole and 360 degree periphery panoramic frames at 28–35 min (late phase)
- Choroidal inflammation may involve either the choriocapillaris or the choroidal stroma, two disease mechanisms that should be distinguished. In the first group, including diseases such as APMPPE, MEWDS and multifocal choroiditis, the inflammatory process causes choriocapillaris non-perfusion, whereas in the second group, including Vogt-Koyanagi-Harada (VKH) disease and birdshot chorioretinopathy (BC), inflammatory foci develop in the choroidal stroma and are associated with inflammation of larger stromal vessels

Core Messages

- Stromal choroiditis should further be subdivided into two groups. In the first group including diseases such as VKH and BC, the choroidal stroma is the primary target of the inflammatory process whereas in most other diseases, such as sarcoidosis and tuberculous choroiditis, the choroid is only one of the sites of a multisystem disease

13.1 Introduction

The choroid is the site of origin of posterior intraocular inflammations at least as often if not more often than the retina. Unlike the retina, where lesions are accessible to funduscopy at an early stage of disease and can be analysed in fine detail by fluorescein angiography, exploration of the choroid has been very gross and limited so far. Only choroidal foci of sufficient importance causing yellow-white discoloration of the fundus red reflex have been detected through the screen of the retinal pigmentary epithelium by fundoscopy. Fine alterations caused by choroiditis or the early stages of disease have, however, not been accessible to imaging exploration unless they produced alterations on the adjacent structures such as the overlying retinal pigmentary epithelium and/or retina.

Therefore appraisal of choroiditis has been mainly descriptive based on fundoscopy, with little information on early inflammatory lesions, their site of origin and the potential sequence of inflammatory events. This approach, purely based on clinical examination, has led to amalgamate conditions that are similar on fundoscopy but are the result of completely diverse pathologic processes [1]. This is well illustrated by the inadequate introduction of the term "white dot syndromes" purely based on the fundus appearance of posterior segment inflammatory diseases [1]. Although the diseases covered by the term "white dot syndromes" vary from one author to the other, this terminology brings together under the same denomination disease entities that obviously have different origins and pathologic processes such as Vogt-Koyanagi-Harada disease where the primary insult is at the level of the choroidal stromal melanocytes and acute posterior multifocal placoid epitheliopathy (APMPPE) most probably due to inflammation of the choriocapillaris [1].

Since the availability of indocyanine green angiography, more detailed investigation of the choroid has been possible, giving information on early and/or subclinical disease, and on the structures involved in the inflammatory process, leading to a more appropriate classification based on the mechanisms of choroidal inflammation. Some of these mechanisms have been verified histopathologically while others are still only presumed to exist and need proof.

13.2 Indocyanine Green Angiography (ICGA)

Since the introduction of digitalized imaging systems and the use of performing infrared cameras, indocyanine green angiography (ICGA) has been the object of renewed interest because of the good quality and reproducibility of images allowing their systematic analysis [2, 3]. A basic introduction to the principles and interpretation of ICGA in inflammatory diseases is indispensable for a comprehensive appraisal of choroiditis going beyond the usual purely descriptive approach.

13.2.1 Physicochemical Properties of Indocyanine Green (ICG)

Indocyanine green fluoresces at 830 nm and therefore gives access to the choroidal vascular structures through the retinal pigment epithelium. The molecular weight difference between ICG (775 daltons) and fluorescein (354 daltons) molecules does not account for the specific angiogram pattern obtained with ICG as compared with fluorescein. Besides the different wavelength at which ICG fluoresces, the crucial difference between these two fluorescing mole-

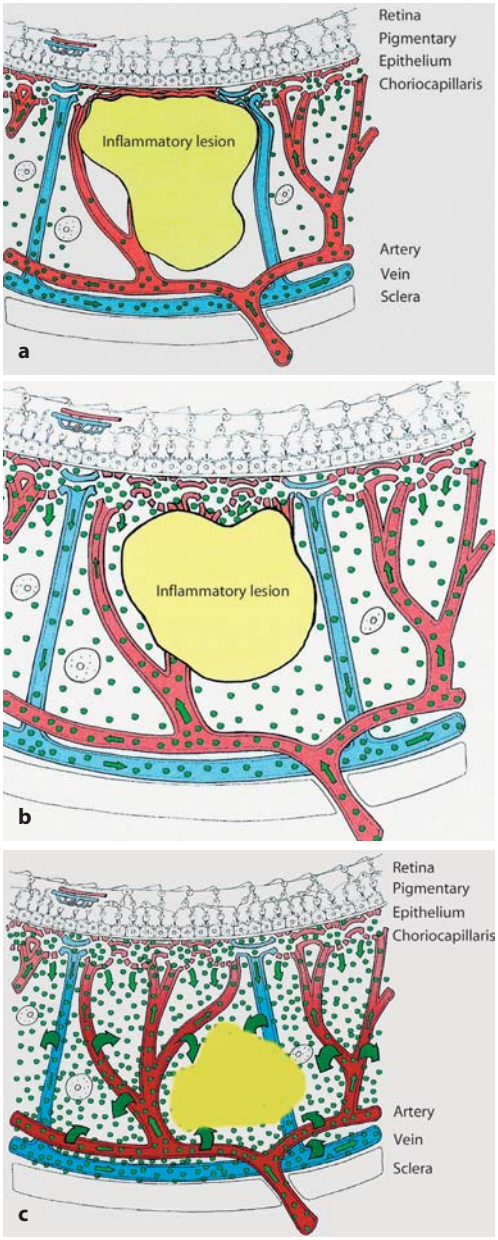

Fig. 13.1. **a** Full-thickness choroidal stromal inflammatory lesion. Diagram illustrating the situation of a full-thickness stromal granuloma. On both parts of the lesion the large complex of the ICG molecule linked to proteins is coming out from the fenestrated choriocapillaris and produces the physiological choroidal fluorescence except in the area of the inflammatory lesion (mass effect), explaining the hypofluorescence seen up to the late angiographic phase. **b** Partial-thickness choroidal stromal inflammatory lesion. Diagram illustrating the ICGA situation of hypofluorescent dots seen at the intermediate angiographic phase, but becoming isofluorescent in the late phase. Extrusion of the ICG complex from the fenestrated choriocapillaris occurs unimpaired, producing the physiological choroidal fluorescence except in the area of the inflammatory lesion (mass effect). With time the ICG complex tends to accumulate around the lesion while it is washed out in non-inflamed areas, resulting in isofluorescence in the late phase. **c** Diffuse choroidal hyperfluorescence. Diagram showing additional leakage from precapillary and large choroidal vessels in stromal choroiditis. Inflammation of larger vessels is usually associated with inflammatory lesions and explains why partial thickness lesions are erased and become isofluorescent in the late angiographic phase

cules comes from their binding affinity to proteins [4, 5]. The ICG molecule is nearly completely protein bound and predominantly to large-sized proteins (lipoproteins) [6], whereas only about 80 % of fluorescein is protein bound and predominantly to smaller proteins such as albumins. Fluorescein leaks readily from slightly altered retinal vessels with minor damage to the blood-retinal barrier and readily impregnates tissues, whereas only major damage to retinal vessels allows ICG to leak [7]. In the choroid, however, ICG leaks unimpaired but slowly from the fenestrated choriocapillaris [8] (Fig. 13.1). During recirculation more and more ICG is entrapped in the choroidal tissue as the ICG-protein complex is only slowly reabsorbed into the circulation. Gradual impregnation of the choroid occurs with time, causing intermediate and late choroidal background fluorescence. This choroidal impregnation by ICG fluorescence is disturbed by choroidal inflammatory lesions, causing mostly areas of decreased or absent fluorescence and/or increased fluorescence. It is this alteration of the slow choroidal impregnation process that is the main parameter studied in ICGA performed for posterior uveitis.

13.2.2 Standard ICG Angiographic Protocol for Inflammatory Diseases

A standard ICGA protocol to analyse choroiditis has been designed [9]. The angiographic procedure comprises three main phases, the early phase up to 2–3 min showing superimposed retinal and choroidal large vessels and incipient exudation of the dye through the choriocapillaris into the choroidal stroma. The intermediate phase at about 10 min shows maximum choroidal stromal background fluorescence and the late phase at about 32 min shows wash-out of the dye from the general circulation with the large choroidal vessels appearing dark against the background stromal fluorescence [9].

13.2.3 Principles for the Interpretation of ICGA [9, 10]

When analysing ICG in posterior inflammatory disorders crucial differences with fluorescein angiography interpretation have to be borne in mind to analyse correctly the images obtained [10]. During initial circulation, ICG is comparable to fluorescein showing the passage through arteriovenous compartments except that it shows superimposed retinal and choroidal circulation. The difference occurs during recirculation time when ICG is progressively leaking out from the fenestrated choriocapillaris, gradually and physiologically impregnating the whole choroidal thickness.

This process can be altered in two ways that can be associated in the same disease; either there is a decreased fluorescence or an increased fluorescence.

13.2.3.1 ICG Hypofluorescence (Fig. 13.2)

The impregnation of the choroidal space can be absent (*hypofluorescence*) either: (1) by a decrease of the physiological extrusion of the ICG from the choriocapillaris (non-perfusion or hypoperfusion) or (2) by the impairment of the filling of the choroidal tissue by the ICG molecule because of the presence of space-occupying lesions (inflammatory foci). These lesions are hypofluorescent in the intermediate angio-

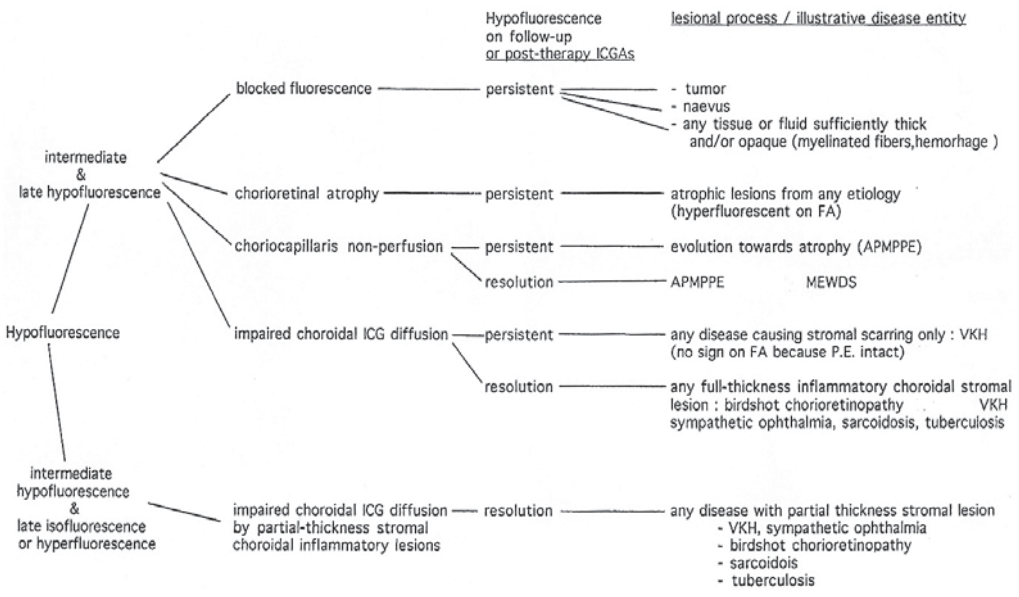

Fig. 13.2. Schematic interpretation of ICGA hypofluorescence in ocular inflammatory disease

graphic phase. If they remain hypofluorescent in the late phase, this signifies that the inflammatory lesion occupies the whole thickness of the choroidal stroma (Fig. 13.1a). When lesions become isofluorescent in the late angiographic phase inflammation causes only partial thickness infiltrates (Fig. 13.1b). Therefore, in ICGA performed for inflammatory disorders, the main information is obtained less from the analysis of the early circulatory phase than from the analysis of the altered pattern of the filling of the choroidal space.

13.2.3.2 ICG Hyperfluorescence

Impregnation of the choroidal space can be enhanced (*hyperfluorescence*) by increased leakage from the larger choroidal vessels. The vessels appear fuzzy in the intermediate time frames and extrusion of the dye from large vessels causes late diffuse hyperfluorescence (Fig. 13.1c).

In the case of the presence of inflammatory foci in the choroidal stroma, hyperfluorescence is associated with hypofluorescent dark dots due to inflammatory infiltrates.

Hyperfluorescence occurs in three relevant situations: (1) diffuse late phase hyperfluorescence due to leakage from precapillary or larger non-fenestrated choroidal vessels, (2) disc hyperfluorescence indicating severe inflammation and (3) hyperfluorescent pinpoints occurring in granulomatous choroidal disease.

In most cases, unlike in fluorescein angiography, where most pathologies produce hyperfluorescence, the lesions in ICGA are mostly seen in a negative dark pattern due to impaired physiological choroidal fluorescence.

13.2.4 Differences Between Fluorescein and Indocyanine Green Angiography

When analysing ICGA it is of the utmost importance to abstract in most inflammatory situations two factors that are important in the interpretation of fluorescein angiograms, namely blockage and window defect. Because infrared fluorescence can be seen through structures that are a screen for visible light, blockage has to be considered only if the interfering structures in front of the choroid are sufficiently thick and/or heavily pigmented. Similarly the notion of window defect does not usually apply for ICGA as the retinal pigment epithelium does mostly not act as a screen as in fluorescein angiography.

13.2.5 Clinico-pathologic-angiographic Correlations

The pathologic processes at the origin of the ICGA images we see have been verified histopathologically for some diseases such as the primary stromal choroiditides, Vogt-Koyanagi-Harada disease, sympathetic ophthalmia and birdshot chorioretinopathy, as well as the choroidal lesions of sarcoidosis, while others can still only be hypothesized, needing ICGA-clinico-pathologic correlations.

13.2.6 Relevance of ICGA in Ocular Inflammatory Diseases

Indocyanine green angiography showed occult choroidal lesions not shown by fundoscopy and/or fluorescein angiography in 100% of patients with a well-established diagnosis known to involve the choroid and these findings had an essential impact either on diagnosis or management in 12.3% of these cases, stressing the importance of ICGA for the proper management of most inflammatory processes of the back of the eye [11].

13.2.6.1 Indocyanine Angiography: Indication, Technique and Characteristics for Inflammatory Diseases of the Fundus

- If choroidal involvement is suspected, dual fluorescein and ICG angiography is indicated (allowing complete and thorough work-up of inflammatory involvement of all structures and essential for follow-up of choroidal inflammation)

- Use of a conventional fundus camera coupled to an image digitalizing system (easier for peripheral imaging) or a scanning laser ophthalmoscopy system
- Use of one vial of 25 mg indocyanine green diluted in 7.5 ml of physiologic saline solution (Cardiogreen, Akorn, Inc., Buffalo Grove, IL, USA). In the case of iodine allergy use iodine-free Infracyanine (SERB Laboratories, Paris, France)
- Angiographic procedure: (1) exclude autofluorescence by taking frames with the highest flash intensity previous to the dye injection; (2) perform early frames of the posterior pole up to 2–3 min (early phase); (3) perform posterior pole and 360 degree periphery panoramic frames at 8–12 min (intermediate phase); (4) perform fluorescein angiography between the intermediate and late ICGA phases; (5) perform posterior pole and 360 degree periphery panoramic frames at 28–35 min (late phase).

Summary for the Clinician

- **To investigate all the superficial fundus structures, the retinal vessels, the retinal pigmentary epithelium (RPE, investigate using the FA principles of blockage and window effect) and the choriocapillaris in the early angiographic phase, fluorescein angiography is the examination of choice**
- **ICGA is indicated, unavoidable and the examination of choice for the proper investigation of choroidal inflammatory involvement**
- **ICGA hypofluorescence results from two mechanisms:**
  1. **Choriocapillaris non-perfusion (patchy/geographic disposition; persistent or even increased hypofluorescence on late frames)**
  2. **Stromal inflammatory infiltration (more regular dots and more even distribution)**
     - **Hypofluorescence up to late frames (full-thickness lesion)**
     - **Isofluorescence on late frames (partial-thickness lesions)**
     - **Usually surrounded by leakage of large choroidal vessels (fuzzy aspect in intermediate phase followed by difuse choroidal fluorescence in late phase)**
- **ICGA hyperfluorescence:**
  1. **In its diffuse form results from increased leakage from larger inflamed choroidal vessels**
  2. **When present at the level of disc indicates severe inflammation**
  3. **When present in the form of numerous hyperfluorescent pinpoints indicates granulomatous disease**
- **FA principle of window effect not applicable for ICGA as infrared fluorescence is perceived through the RPE that is not a screen in ICGA**
- **FA principle of blockage only rarely plays a role in ICGA (unless thick or strongly pigmented screen) in which hypofluorescence is mostly caused by choriocapillaris non-perfusion or choroidal stromal infiltration**

13.3 The Concepts of Inflammatory Choriocapillaropathy and Stromal Choroiditis [12]

Indocyanine green angiography has allowed the reclassification of choroidal inflammation according to the structure that is preponderantly or initially involved. At the present stage of our knowledge there seem to be at least two main mechanisms of inflammation touching the choroid.

13.3.1 Primary Inflammatory Choriocapillaropathy (PICCP)

This first group of diseases, formerly mostly included in the inadequate term of "white dot syndromes", results from inflammation at the level of the choriocapillaris causing areas of choriocapillaris non-perfusion and its ischaemic consequences both at the level of the choroid but also at the level of the outer retina that depends on the choriocapillaris for oxygen and nutrients. Acute posterior multifocal placoid pigmentary epitheliopathy (APMPPE) is a disease typically illustrating this type of choroidal inflammation.

13.3.2 Stromal Choroiditis

In the second group of diseases, the primary mechanism is the development of inflammatory foci, mostly granulomatous at the level of the stroma appearing hypofluorescent on ICGA, usually associated with inflammation of larger non-fenestrated stromal vessels appearing on ICGA as fuzzy vessels in the intermediate phase followed by diffuse late choroidal hyperfluorescence. Vogt-Koyanagi-Harada disease and birdshot chorioretinopathy are typical illustrations of this type of choroidal inflammation. Although the mechanism is completely different from the first type of disease, these conditions have also been included by some authors in the "white dot syndromes" [1].

13.3.3 Secondary Inflammatory Choriocapillaropathy

As usual in inflammatory disorders, the inflammation is not strictly limited to the primary site of involvement but can also touch adjacent structures. For example severe retinitis caused by *Toxoplasma gondii* usually extends to the choriocapillaris, causing secondary inflammatory choriocapillaropathy [13]. Likewise severe stromal disease can cause inflammation at the level of the choriocapillaris and beyond to the retinal pigmentary epithelium and to the retina as in Vogt-Koyanagi-Harada disease.

Choroiditis can be summarized following these new principles as follows:

Summary for the Clinician

Classification of Choroiditis

- **Two main lesional mechanisms determine the classification of choroiditis:**
  1. **Choriocapillaris inflammation (primary inflammatory choriocapillaropathies)**
     - **MEWDS/AIBSE**
     - **APMPPE**
     - **Multifocal choroiditis/PIC**
     - **Serpiginous choroiditis**
     - **Rare entities: AMN, AZOOR**
  2. **Stromal inflammation (stromal choroiditis) further subdivided into two categories**
     - 2.1. **Primary obligatory stromal choroiditiis**
       - **Vogt-Koyanagi-Harada disease**
       - **Sympathetic ophthalmia**
       - **Birdshot chorioretinopathy**
     - 2.2. **Stromal choroiditis as a random location of systemic disease**
       - **Sarcoidosis**
       - **Tuberculosis**
       - **Syphilis**
       - **Other infectious choroiditides**

References

1. Ben Ezra D, Forrester JV (1995) Fundal white dots: the spectrum of a similar pathological process. Br J Ophthalmol 856–860
2. Yannuzzi LA, Sorenson JA, Guyer DR, Slakter JS, Chang B, Orlock D (1994) Indocyanine green videoangiography: current status. Eur J Ophthalmol 4:69–81
3. Guyer DR, Yannuzzi LA, Slakter JS, Sorenson JA, Ho AC, Orlock D (1994) Digital indocyanine green videoangiography of central serous chorioretinopathy. Arch Ophthalmol 112:1057–1062
4. Herbort CP, Borruat FX, de Courten C, Jaccard L (1996) Angiographie au vert d'indocyanine dans les uvéites postérieures. Klin Monatsbl Augenheilk 208:321–326
5. Slakter JS, Giovannini A, Yannuzzi LA, Sforzolini B, Guyer DR, Orlock DR (1994) Indocyanine green videoangiography of multifocal choroiditis and the presumed ocular histoplasmosis syndrome. Invest Ophthalmol Vis Sci 35:1982
6. Baker KJ (1966) Binding of sulfobromophthalein (BSP) sodium and indocyanine green (ICG) by plasma alpha-1 lipoproteins. Proc Soc Exp Biol Med 122:957–963
7. Guex-Crosier Y, Herbort CP (1997) Prolonged retinal arteriovenous circulation time by fluorescein but not by indocyanine green angiography in birdshot chorioretinopathy. Ocular Immunol Inflamm 5:203–206
8. Lim JI, Flower RW (1995) Indocyanine green angiography. Int Ophthalmol Clin 35:59–70
9. Herbort CP, Guex-Crosier Y, LeHoang P (1994) Schematic interpretation of indocyanine green angiography. Ophthalmology 2:169–176

10. Herbort CP, Bodaghi B, LeHoang P (2001) Angiographie au vert d'indocyanine au cours des maladies inflammatoires: principes, interpretation schématique, sémiologie et intérêt clinique. J Fr Ophtalmol 24:423–447
11. Herbort CP, Auer C, Wolfensberger TJ, Ambresin A, Bouchenaki N (1999) Contribution of indocyanine green angiography (ICGA) to the appraisal of choroidal involvement in posterior uveitis. Invest Ophthalmol Vis Sci 40:S383
12. Bouchenaki N, Cimino L, Auer C, Tran VT, Herbort CP (2002) Assessment and classification of choroidal vasculitis in posterior uveitis using indocyanine green angiography. Klin Monatsbl Augenheilkd 219:243–249
13. Auer C, Bernasconi O, Herbort CP (1999) Indocyanine green angiography features in toxoplasmic retinochoroiditis. Retina 19:22–29

Primary Inflammatory Choriocapillaropathies

14

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Core Messages

- The common denominator in primary inflammatory choriocapillaropathies is choriocapillaris non-perfusion and secondary ischaemia of the outer retina. Indocyanine green angiograhy and early fluorescein angiographic frames show choriocapillaris non-perfusion. Electroretinography shows outer retinal dysfunction and late fluorescein angiographic hyperfluorescent frames are explained by the ischaemia of the outer retina causing retinal capillaries to leak
- The clinical differences between the primary inflammatory capillaropathies (PICCP) could possibly be explained by the level and the severity of the inflammatory insult to the choriocapillaris circulation
- Angiographic signs in inflammatory choriocapillaropathies: ICGA 1: in the acute phase of disease, patchy or geographical ICGA hypofluorescent areas of variable sizes present in the early, intermediate and late angiographic frames but are usually more clearly visible on the late frames after partial wash-out of ICG from choroid; ICGA 2: in the post-acute phase, ICGA hypofluorescent areas represent choroidal atrophy and scarring; ICGA 3: there are hyperfluorescent ICGA rims around progressing (serpiginous) lesions; FA 1: in the acute phase of disease, early FA hypofluorescent areas indicate choriocapillaris non-perfusion; FA 2: in the acute phase of the disease, late FA hyperfluorescent areas go from faint to profuse depending on the severity of ischaemic process at the level of the outer retina; FA 3: in the postacute phase, there are zones of alternating areas of window effects and masking effects indicating chorioretinal atrophy and scars; in the convalescent phase of disease, regression of FA signs lags behind normalization of ICGA signs
- Multiple evanescent white dot syndrome (MEWDS) and acute idiopathic blind spot enlargement (AIBSE): (1) viral flu-like syndrome often found in preceding fortnight in up to 60% of cases; (2) usually unilateral and unique episode; (3) symptoms: photopsias, scotomata, visual loss that can be severe in some cases; (4) visual loss found in >90% of cases: drop of visual acuity from slight to severe, visual field changes and blind spot enlargement; (5) fundus findings: discrete discolorations in midperiphery and granular aspect of macula; (6) ERG abnormal in 80% of cases; (7) ICGA: hypofluorescent dots and peripapillary hypofluorescence in acute phase resolving in 4–8 weeks; (8) FA: hypofluorescence (early), discretely hyperfluorescent foci (late) or absent FA findings ± cystoid macular edema; disc hyperfluorescence; (9) spontaneous resolution of signs and symptoms within 6–10 weeks in ± all cases; (10) AIBSE: entity described before the ICGA era behaving as MEWDS without fundus signs that have probably resolved or are subclinical
- APMPPE/AMIC: (1) viral flu-like syndrome often found in patient history; (2) symptoms: visual loss, scotomata, photopsias; (3) yellowish deep bilateral discoloration at posterior pole (with serous exudative retinal detachments in hyperacute cases);

Core Messages

(4) vitreitis and slight anterior uveitis that can also be more severe; (5) ICGA: geographic hypofluorescent areas (early, intermediate and late) = choriocapillaris nonperfusion; (6) FA: hypofluorescence (early) and hyperfluorescence (late); geographic aspect; (7) ERG: usually normal; (8) lesions and functional disturbances usually reversible without therapy (steroids sometimes given); chorioretinal scars possible

- Multifocal choroiditis: (1) recurrent chorioretinal inflammatory disease: photopsias, scotoma, visual loss; (2) bilateral involvement; usually unilateral in the case of PIC (in more myopic eye); (3) small faintly apparent active foci and older chorioretinal scars; (4) vitreous cells in posterior vitreous during active stage; (5) ICGA: hypofluorescent areas (early, intermediate and late): scars and active foci (the latter only detectable by ICG); (6) FA: early hypo- and late hyperfluorescence (scars); active foci rarely seen by FA; (7) subretinal inflammatory neovascularization frequent (up to 30%); (8) therapy: corticosteroids (and immunosuppressive therapy); not always successful
- Serpiginous choroiditis: (1) acute atrophying peripapillary choriocapillaritis progressing centrifugally, usually bilateral but with asymmetric involvement; (2) fundus findings: acute stage: geographic white-grey to yellow peripapillary lesions ± serous detachments; chronic stage: atrophic pigmented scars; (3) ICGA: acute: geographic hypofluorescence (= choriocapillaris nonperfusion) surrounded by choroidal hyperfluorescence. Chronic: hypofluorescence (= atrophy); (3) FA: acute: early hypofluorecence and late impregnation; chronic: early hypofluorescence and late hyperfluorescence (window effect) (= atrophy); (4) evolution: slowly progressing, prognosis can be reserved; (5) treatment: empirical corticosteroids/immunosuppressants
- Acute zonal occult outer retinopathy (AZOOR): (1) rare disorder affecting mainly young myopic women. Share clinical similarities with MEWDS, PIC, multifocal choroiditis and may be a variant in the spectrum of these entities; (2) photopsia, scotoma, visual loss are the main complaints; (3) fundus findings: acute stage: minimal in contrast to electroretinogram alterations; chronic stage: retinal pigment epithelium hypopigmentation and mottling; (3) electroretinography and visual field: generally impaired before visible fundus modifications; (4) FA: normal at the onset when no fundus alterations present. Then RPE alterations appear as mottling fluorescence; (5) evolution: can be self-limited without treatment; (6) empirical corticosteroids and acyclovir treatment have been suggested

14.1 Introduction

The amount of evidence indicating that primary inflammation at the level of the choriocapillaris is a common denominator for several diseases of the fundus has increased recently particularly since indocyanine green angiography (ICGA) has become available [1–3]. The ICGA pattern correlated with functional (visual field testing) and electrophysiologic parameters strongly points towards choriocapillaropathy and consequent ischaemic dysfunction of the outer retina in the group of diseases formerly classified within the purely descriptive potpourri group of "white dot syndromes" [4, 5]. Included in this group are multiple evanescent white dot syndrome (MEWDS), acute posterior multifocal placoid pigment epitheliopathy or acute multifocal ischaemic choroiditis (APMPPE/AMIC), multifocal choroiditis/punctate inner choroidopathy (MFC/PIC), serpiginous choroiditis as well as rarer entities such as acute macular neuroretinopathy (AMN) and possibly acute zonal occult outer retinopathy (AZOOR). Between these rather well determined clinical entities, intermediary forms occur that are diffi-

cult to fit into these categories. Thus, an intermediary form of disease between APMPPE and serpiginous choroiditis has been reported to which the name of AMPPiginous choroiditis was given [6]. Recently another new name has been given to a similar disease pattern having the lesional aspect of APMPPE combined with the evolutionary pattern of serpiginous choroiditis with recrudescences and progression [7]. The authors called these cases relentless placoid chorioretinitis and asked themselves whether it was a new entity or a variant of serpiginous choroiditis [7]. This question is of limited relevance as far as its mechanism, choriocapillaris inflammation, is understood. It is however relevant to determine further characteristic disease patterns to gather information on the evolutionary pattern of such or such "new entity" in order to determine treatment strategies. A further argument for a common disease mechanism is patients presenting two or more of these diseases at different times as stated by Jampol and Becker [8]. It is therefore useful to consider both the well-determined specific pathologies as well as the non-classifiable intermediary cases as a disease spectrum, regrouping these conditions under the physiopathologic terminology of primary inflammatory choriocapillaropathies. This disease spectrum goes from a benign disease such as MEWDS at one end to a disease such as serpiginous choroiditis destroying the chorioretina at the other end. The reason the disease behaviours are so different from one condition to the other, regarding severity, recurrence and progression, although the same structure seems to be involved, is unknown and still has to be elucidated.

Primary inflammation of the choriocapillaris was suspected long before ICGA became routinely available. Deutman and colleagues were among the first authors to incriminate the choriocapillaris rather than the pigment epithelium as the primary site of injury in APMPPE and he suggested renaming the disease acute multifocal ischaemic choroidopathy (AMIC) [9, 10]. He based his hypothesis partly on the observation of choriocapillaris non-perfusion seen at the very early phase of fluorescein angiography. Fluorescein angiography is, however, incapable of giving information on the choriocapillaris beyond the early phases of angiography and does not give imaging access to the rest of the choroid. With the availability of ICG angiography, imaging of the choroid has become possible and particularly the choriocapillaris can be analysed angiographically not only in the very early angiographic phases but also during later angiographic phases [11–13]. As was suspected earlier, ICG angiography has clearly shown that the lesional process in APMPPE/AMIC occurs at the level of the choriocapillaris, showing areas of choriocapillaris non-perfusion from early to late angiographic times [1]. ICG angiography has also shown that the same physiopathologic mechanism seems to prevail in other entities such as multifocal choroiditis, MEWDS and serpiginous choroiditis [14–16]. The specific entities will be analysed in detail below and well-determined conditions will be presented first, going from the benign end (MEWDS) to the severe types of evolution (serpiginous choroiditis), followed by the less well analysed rare forms such as acute zonal occult outer retinopathy (AZOOR) and acute macular neuroretinopathy (AMN) and finally the overlapping and intermediary forms which cannot be classified into known entities.

14.2 Angiographic Signs in Inflammatory Choriocapillaropathies

Indocyanine green angiographic signs in inflammatory choriocapillaropathies have been well determined and have contributed to the recognition of the common mechanism involved and to the regrouping of these entities formerly classified under the term of "white dot syndromes".

The following *ICGA signs* need to be looked for:

1. The hallmark sign of inflammatory choriocapillaropathy is patchy or geographic ICGA hypofluorescent areas of variable sizes present in the early, intermediate and late angiographic phases but usually more clearly visible on the late frames, indicating choriocapillaris non-perfusion or hypoperfusion (Fig. 14.1 a–c).

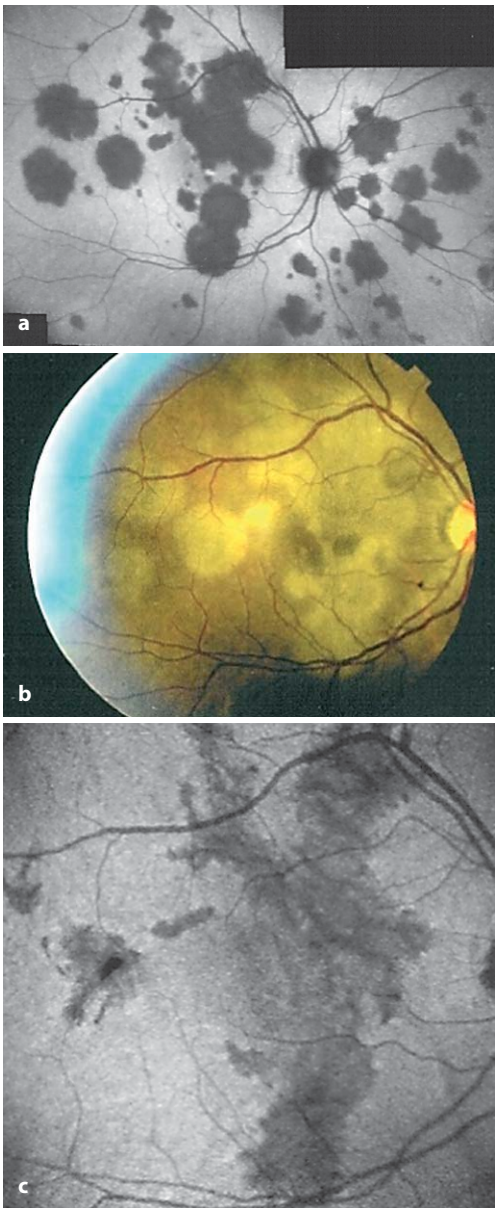

Fig. 14.1 a–c. ICGA signs of choriocapillaris non-perfusion. Areas of patchy or geographic ICGA hypofluorescent areas of variable size present in the early, intermediate and late angiographic phases (**a**) in a case of APMPPE/AMIC, corresponding to confluent plaques of deep fundal discoloration (**b**), leaving atrophic areas typical of serpiginous choroiditis in the convalescent phase (**c**)

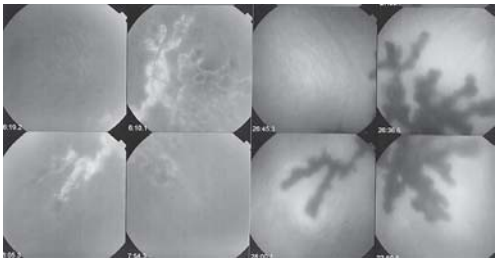

Fig. 14.2. Diffuse perilesional choroidal hyperfluorescence in subclinically progressing serpiginous choroiditis. ICGA (*right quartet of pictures*) shows many more involved areas than shown by fluorescein angiography (*left quartet of pictures*) and shows hyperfluorescence around progressing lesions

2. Complete or partial regression of the ICGA hypofluorescence or absence of regression in the convalescent phase. The areas remaining hypofluorescent in the convalescent phase represent chorioretinal atrophy and correspond to areas of window effect and masking effect on fluorescein angiography.
3. In diseases with a progressing course such as serpiginous choroiditis, ICGA can show diffuse choroidal hyperfluorescence at the edges of the progressing lesions in areas having no translation on funduscopy or fluorescein angiography (Fig. 14.2).

Whereas the ICGA signs are quite uniform, the *FA angiographic signs* depend on the severity and extension of the choriocapillaris non-perfusion and on the outer retinal damage.

1. On FA there is likewise early hypofluorescence showing the choriocapillaris non-perfusion identified on ICGA.
2. Depending on the severity of the choriocapillaris non-perfusion seen on ICGA, late FA frames either show no hyperfluorescence (for instance in mild MEWDS), discrete patchy late hyperfluorescence seen in MEWDS or extensive late hyperfluorescence seen in APMPPE (Fig. 14.3 a, b). To understand the genesis of the FA signs, it is important to be aware that late FA fluorescence is coming from retinal vessels overlying areas of ischaemic outer retina that present increased permeability in response to the ischaemia due to choriocapillaris non-perfusion.

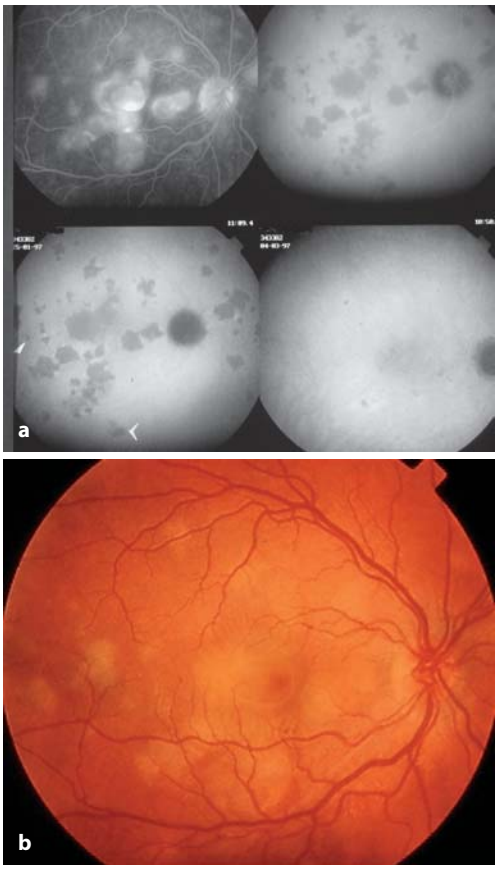

Fig. 14.3 a, b. Choriocapillaris non-perfusion in a case of APMPPE/AMIC. Choriocapillaris non-perfusion is shown by patchy geographic areas of hypofluorescence in the intermediate phase of angiography (*top right picture*) and in the late angiographic phase (*bottom left picture*) that resolve almost completely in the convalescent stage of disease 2 months later (*bottom right picture*). The late fluorescein frame on the *top left* shows hyperfluorescence, corresponding to the ICG areas of choriocapillaris, non-perfusion, that did not develop from the periphery of the lesions and that can only be explained by leakage from the capillaries from the inner retina in response to ischaemic signals from the outer retina (**a**). The deep discoloured plaques seen on funduscopy are explained by the retinal edema (**b**)

3. In the convalescent phase there is a delayed regression of FA signs (hyperfluorescence and staining) as compared to the regression of ICGA signs. In the case of chorioretinal atrophy, window effect and masking effect due to chorioretinal scarring are seen.

Any severe inflammation in an adjacent structure to the choriocapillaris (retina or choroidal stroma) can cause inflammation at the level of the choriocapillaris and produce similar angiographic signs. In that situation we speak of secondary inflammatory choriocapillaropathy.

14.2.1 Angiographic Signs in Inflammatory Choriocapillaropathies

- ICGA 1: in the acute phase of disease, patchy or geographical ICGA hypofluorescent areas of variable sizes present in the early, intermediate and late angiographic frames but are usually more clearly visible in the late frames after partial wash-out of ICG from choroid.
- ICGA 2: in post-acute phase, ICGA hypofluorescent areas representing choroidal atrophy and scarring
- ICGA 3: hyperfluorescent ICGA rims around progressing (serpiginous) lesions
- FA 1: in acute phase of disease, early FA hypofluorescent areas indicating choriocapillaris non-perfusion
- FA 2: in acute phase of disease, late FA hyperfluorescent areas going from faint to profuse depending on the severity of the ischaemic process at the level of the outer retina
- FA 3: in post-acute phase, zones of alternating areas of window effects and masking effects indicating chorioretinal atrophy and scars
- In convalescent phase of disease, regression of FA signs lags behind normalization of ICGA signs

14.3 Patient History, Systemic and Ocular Symptoms and Signs in Primary Inflammatory Choriocapillaropathies

Most cases of primary inflammatory choriocapillaropathies occur in young adults. Systemic symptoms such as a flue-like and/or febrile episode preceding the ocular disease can be found in 50–60 % of cases, indicating a possible

viral or other infectious or systemic trigger at the origin of inflammatory choriocapillaropathies. We have previously described a case of APMPPE that developed just after an episode of mumps in a young adult [17]. APMPPE has also been described following acute group A streptococcal infection [18]. Furthermore both MEWDS and APMPPE have been described after hepatitis vaccinations [19–21]. Rather than choriocapillaris disease caused by one given infectious trigger, the pathologic process is probably that of a common pathway in response to several different triggers. A genetic predisposition has been put forward and it might well be that some individuals and even some races are more or less susceptible to presenting choriocapillaris inflammation and occlusion or the reverse [8]. We found the human leucocyte antigen HLA-B*51 haplotype to be significantly more frequently present in MEWDS patients when compared to a normal control population [22]. The symptoms to be searched for are photopsias that are usually sufficiently pronounced for the patient to report them spontaneously. Frequently patients also report scotomata and visual disturbance that can be objectively documented by testing visual acuity and visual fields. In the acute phase patchy or geographic yellow-white-grey discoloration of the fundus occurs in the deep retina, recovering in the convalescent stage or producing definitive chorioretinal scars. Usually discrete signs (cells in the posterior vitreous) orient towards an inflammatory process. In rare cases there can be a mild to moderate and rarely a severe anterior uveitis associated with the fundus findings.

14.4 Practical Attitude in Primary Inflammatory Choriocapillaropathies (PICCP)

As a first step the characteristic ICGA patterns for primary inflammatory choriocapillaropathies (PICCP) should be identified and the FA signs should be recorded to stage the severity of the involvement (strong versus mild choriocapillaris ischaemia). Before the diagnosis of PICCP is retained, an infectious cause, a neoplastic process or a systemic vasculitis causing choroidal ischaemia has to be excluded. The two most frequent infectious causes that can mimic a PICCP are syphilitic and tuberculous chorioretinitis. The neoplastic cause that has to be excluded is intraocular lymphoma and any systemic vasculitis, in particular systemic lupus erythematosus, can cause choroidal ischaemia. On the other hand, it is easy to exclude central serous chorioretinopathy that produces choroidal hyperfluorescence and not hypofluorescence on ICG angiography.

Finally it is useful to try to classify the case into any of the known entities (MEWDS, APMPPE, multifocal choroiditis, etc.) in order to anticipate evolutionary patterns and determine a therapeutic approach (monitoring only versus therapeutic intervention using corticosteroid and/or immunosuppressive therapy). In the case of severe visual impairment, even if the condition is known to recover spontaneously, e.g. APMPPE, corticosteroids with or without immunosuppressants should probably be given as it is not known whether a well-known entity is going to develop into an atypical intermediary form possibly more deleterious to the chorioretina than initially anticipated.

Summary for the Clinician

Practical Approach of PICCP

- **Identify ICGA and FA angiographic signs and their compatibility with the diagnosis of primary inflammatory choriocapillaropathy; establish severity of involvement**
- **Exclude any infectious cause (syphilis and tuberculosis, etc.), neoplastic process (ocular lymphoma) or systemic vasculitis before proposing the diagnosis of PICCP**
- **Try to classify each case into one of the well-defined entities (MEWDS, APMPPE, serpiginous choroiditis) to anticipate evolution and define therapeutic attitude**
- **In the case of the intermediary non-classifiable form of disease, monitor lesional process closely (dual FA and ICG angiography, ERG, visual field) and introduce corticosteroid and/or immunosuppressive therapy in the case of persistence or extension of the ischaemic process**

14.5 Specific Entities

Some of the primary inflammatory choriocapillaropathies (PICCP) were sufficiently characteristic to have been described as individual diseases in the past such as MEWDS and APMPPE/AMIC. It is important to try to classify each individual case into one of the well-determined entities because it will help predict the evolution of the case. Very often, however, it is difficult to fit the case into a given category and close monitoring including ICGA is of crucial importance to determine the evolutionary pattern. Of the 28 cases of PICCP seen in our centre from 1995 to 2003, 11 (40 %) could not be classified into known entities and were followed as intermediary forms.

14.5.1 Multiple Evanescent White Dot Syndrome (MEWDS)

MEWDS was first described by Lee Jampol et al. in 1984 [23]. It is predominant in young women, who are 2–3 times more frequently affected than men. A preceding flue-like episode or upper respiratory tract infection occurs in up to 50 % of patients if a directed history is performed [24]. Hepatitis B as well as hepatitis A vaccinations have been associated with MEWDS [19, 20]. An association with the human leucocyte antigen, HLA-B*51, has been found [22].

14.5.1.1 Clinical Symptoms and Findings

Patients with MEWDS usually present with unilateral visual impairment consisting of visual loss of diverse importance (severe visual loss in some cases) and scotomatas objectively identified by visual field testing. Characteristic symptoms include photopsias that are usually spontaneously reported by the patient. The disease usually hits only once and the evolution is spontaneously favourable with restoration of visual function within 6–12 weeks.

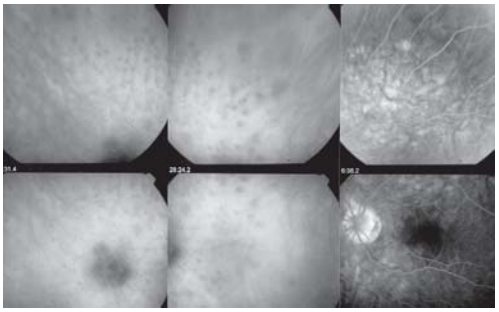

Fig. 14.4. Multiple evanescent white dot syndrome (MEWDS). Patchy hypofluorescent areas geographically confluent in the macula and around the optic disc (*left quartet of frames*), corresponding to discrete hyperfluorescent areas on fluorescein angiography due to retinal leakage in response to ischaemia caused to the outer retina by choriocapillaris non-perfusion

Funduscopy shows numerous very faint white dots in the mid-periphery seen at disease onset. These dots can disappear very rapidly and are missed by the clinician if the patient does not consult at an early stage. ICGA is especially precious in reaching a diagnosis in the situation when the patient is seen in the postacute phase. At the convalescent stage the macula takes a granular aspect. Inflammation is usually moderate and limited to the vitreous but the optic disc can be involved.

14.5.1.2 ICG Angiography

Indocyanine green angiography shows numerous hypofluorescent dark dots predominant in the mid-periphery and around the optic disc where hypofluorescence is confluent (Fig. 14.4). Interestingly these dark dots are much more clearly delineated in the late phase and less so in the early angiographic times, speaking more for choriocapillaris hypoperfusion than for strict non-perfusion, which possibly could also explain the usual favourable outcome of MEWDS. The disposition of the lesions is more of the patchy type except around the optic disc, where the lesions have a more geographic form. These ICGA lesions almost integrally resolve without treatment in the convalescent phase of disease after 6–10 weeks of evolution. The discrepancy

between the very prominent ICGA signs compared to the very discrete FA signs in many cases is a characteristic of MEWDS. To monitor disease evolution, ICGA is the most precise parameter to follow.

14.5.1.3 Fluorescein Angiography

The intensity of FA signs depends on the severity of choriocapillaris non-perfusion. Signs can be quasi-absent or very minimal, consisting of faint patchy areas of late hyperfluorescence. They can also be very pronounced in some cases. There can be an associated disc hyperfluorescence. Because of the discrete and sometimes absent FA signs, fluorescein angiography is of limited use in MEWDS.

14.5.1.4 Visual Field Testing

Visual field anomalies are present in up to 90 % of cases [25]. They consist of a decrease in the central mean sensitivity, with enlargement of the blind spot being present in 90 % of patients associated with visual field disturbance by more or less deep scotomata within the 30 degree zone. Visual field disturbance is well correlated with the ICG hypofluorescent dark dots and peripapillar hypofluorescence and recovery occurs in parallel with resolution of ICGA dark dots or the peripapillary hypofluorescent area usually within 6–8 weeks after the acute period.

14.5.1.5 Electroretinography and Pathophysiologic Implications

The lesional impact has been clearly localized at the level of the outer retina by electrophysiologic analysis of the first series of MEWDS patients. During the acute stage of MEWDS the electroretinogram a-wave and the early receptor potential (ERP) were profoundly decreased, indicating outer retina/photoreceptor dysfunction [26]. These findings were confirmed by other groups also clearly pointing towards outer retinal dysfunction [5, 25, 27]. The authors of the first report localized the primary lesional event at the level of the retinal pigment epithelium (RPE) [26]. This statement, however, relies on no sound evidence and is pure speculation. A more logical sequence of events begins with choriocapillaris hypo- or non-perfusion leading to ischaemic damage at the level of the RPE and outer retina and depending on choriocapillaris perfusion for oxygen and nutrients, followed by functional effects measured by ERG and visual field testing. It has been shown that ICGA and multifocal ERG are more sensitive than visual field testing to detect lesions, being able to detect subclinical disease [27, 28].

14.5.2 Acute Idiopathic Blind Spot Enlargement (AIBSE)

Acute idiopathic blind spot enlargement (AIBSE) was described in 1988 in a report including seven patients that presented a peripapillary scotoma producing symptomatic enlargement of the blind spot objectively identified by visual field testing. They were all young patients aged from 25 to 39 years with a 5/2 female predominance and 2/7 patients had previous episodes [29]. Visual acuity, colour vision, pupillary responses, funduscopy and fluorescein angiography were all normal. The only additional abnormal finding was an abnormal focal ERG indicating retinal dysfunction around the optic disc at the origin of the visual field defect. Most probably AIBSE and MEWDS are the same disease with the exception of the usual fundus findings that were not found in this and other reports on AIBSE. As indicated by Hamed et al., the retinal lesions may already have subsided at the time of examination or were subclinical [30]. If ICGA had been available and performed by the authors of this and subsequent reports, it is probable that AIBSE would never have been described as a separate entity, as ICGA is presently the method of choice for diagnosing atypical MEWDS in patients consulting at a later stage of the disease or presenting subclinical disease [31]. The numerous reports on associated blind spot enlargement in many of the diseases presently regrouped in the category of primary inflammatory choriocapillaropathy are an

indication for a common physiopathogenic mechanism [32–35]. Furthermore, the reports including ICGA investigations in their work show that visual field alterations are related to peripapillary hypofluorescence, indicating choriocapillaris non-perfusion as the physiopathogenic process at the origin of blind spot enlargement [31, 36, 37]. This pathologic characteristic, found in many of the PICCPs, seems to indicate a common weakness of the peripapillary choriocapillaris, possibly more sensitive to closure secondary to inflammation, with subsequent outer retinal ischaemia and dysfunction producing a peripapillary scotoma [35].

Summary for the Clinician

Multiple Evanescent White Dot Syndrome (MEWDS) and Acute Idiopathic Blind Spot Enlargement (AIBSE)

- Viral flu-like syndrome often found in preceding fortnight in up to 60 % of cases
- Usually unilateral and unique episode
- Symptoms: photopsias, scotomata, visual loss that can be severe in some cases
- Visual loss found in >90 % of cases: drop in visual acuity from slight to severe, visual field changes and blind spot enlargement
- Fundus findings: discrete discolorations in midperiphery and granular aspect of macula
- ERG abnormal in 80 % of cases
- ICGA: hypofluorescent dots and peripapillary hypofluorescence in acute phase resolving in 4–8 weeks
- FA: hypofluorescence (early), discretely hyperfluorescent foci (late) or absent FA findings ± cystoid macular edema; disc hyperfluorescence
- Spontaneous resolution of signs and symptoms within 6–10 weeks in ± all cases
- AIBSE: entity described before the ICGA era behaving as MEWDS without fundus signs that have probably resolved or are subclinical

14.5.3 Acute Posterior Multifocal Placoid Pigment Epitheliopathy (APMPPE) or Acute Multifocal Ischaemic Choriocapillaropathy (AMIC)

Acute posterior multifocal placoid pigment epitheliopathy (APMPPE) was first described by Donald Gass in 1988 in a report of three female patients aged 19, 22 and 22 years, showing rapid loss of central vision associated with multifocal, yellow-white placoid lesions occurring in one eye, with sequential involvement of the second eye, followed by resolution of the fundus lesions and visual improvement over weeks or months thereafter [38]. The hypothesis that was put forward was an acute cellular response on the part of the pigment epithelium to a local noxious agent. Deutman and colleagues, based on the choriocapillaris non-perfusion on early FA frames, clearly indicated that it was the choriocapillaris rather than the pigment epithelium that was primarily involved and he suggested renaming the disease acute multifocal ischaemic choroidopathy (AMIC) [9, 10]. As for other PICCPs, APMPPE/AMIC is preceded by a febrile, infectious or flue-like episode in at least half of the patients if a careful history is taken [28, 39]. It was associated with diverse infectious episodes preceding the ocular involvement such as mumps, streptococcal group A infection and a hepatitis vaccination [17–19]. A clinical picture identical to APMPPE was seen in a patient with Lyme disease and a patient with secondary syphilis with resolution of the acute lesions following treatment of the underlying cause [40, 41]. Like other PICCPs, APMPPE is predominant in young individuals that are in the 2nd–4th decade of life. Concerning disease mechanism, there is an appreciable body of information and evidence that the lesional process in APMPPE is a vasculitis. On the one hand, there is a report of systemic vasculitis that produces a clinical picture exactly similar to APMPPE [42]. On the other hand, there are many reports of APMPPE associated with a vasculitis elsewhere, in the cerebellum, with associated nephritis and meningoencephalitis [43–47].

14.5.3.1 Clinical Symptoms and Findings

As in other PICCPs, patients present with complaints of photopsias and visual disturbance. Usually both eyes are affected. Involvement may, however, be asymmetric and sequential in time from one eye to the other. Visual loss is diverse from minimal to severe and depends on the location of the lesions. Visual field testing objectively identifies the scotoma the patients report and that is localized to the areas of fundus involvement. Classically, absent or only slight anterior segment inflammation is described, but in some cases frank anterior uveitis can be associated with the fundus lesions. Cells in the vitreous can be found most of the time.

On *fundus examination,* lesions in the acute phase are characterized by deep plaques of yellow-white discoloration of the fundus of diverse size, sometimes confluent (Fig. 14.3b). In the convalescent phase the lesions progressively lose their dense aspect, leaving a mottled RPE behind in the areas of maximal involvement. Serous retinal detachment can rarely be seen in severe cases resembling Vogt-Koyanagi-Harada disease that should be excluded.

14.5.3.2 ICG Angiography

ICGA signs in APMPPE/AMIC are characterized by unevenly sized geographic hypofluorescent areas randomly scattered in the posterior pole present in the early and intermediate phase of angiography but very distinctly visible in the late ICG angiographic phase (Fig. 14.3a). On follow-up angiograms these signs resolve almost completely with the exception of a few areas of persisting hypofluorescence due to chorioretinal atrophy [48]. Because the pattern of hypofluorescence is so characteristic, most authors that published ICGA studies on APMPPE concluded that lesions were due to primary choroidal vascular disease [2], choroidal vascular occlusion [48] or hypoperfusion [1], confirming what some authors had already suspected on the base of fluorescein angiography showing choriocapillaris perfusion delay [9, 10]. ICGA gave us the additional information not available from fluorescein angiography, namely that the choriocapillaris perfusion delay seen on fluorescein angiography corresponds indeed to choriocapillaris non-perfusion.

14.5.3.3 Fluorescein Angiography

Fluorescein angiography shows equally consistent and characteristic findings as ICGA. In the acute phase there is early choriocapillaris hypofluorescence, indicating perfusion delay. In the late angiographic phase there is usually pronounced hyperfluorescence corresponding to the areas of perfusion delay seen on FA and to the hypofluorescent areas on ICGA, reflecting choriocapillaris non-perfusion (Fig. 14.3a). This leakage leading to late hyperfluorescence cannot come from the choroid as we now know that the ICGA hypofluorescent areas correspond to choriocapillaris non-perfusion. If it were coming from the choroid we would see hyperfluorescent pinpoints as in exudative VKH retinal detachments signalling the sites of leakage of liquid through the RPE-Bruch's membrane complex. Moreover, if leakage and staining were coming from the choroid it would be expected that the placoid lesions would stain from their periphery inward. Therefore it makes sense to attribute the leakage leading to the late FA hyperfluorescence to the vessels of the inner retina that respond to ischaemic signals from the outer retina which depends on the choriocapillaris for oxygen and nutrients. In the convalescent phase most FA signs regress except for the mixture of window and masking effects corresponding to cicatrized lesions of the RPE.

14.5.3.4 Visual Field Testing

Visual field testing objectively identifies the scotomata the patients report. They correspond to the areas of fundus discoloration that correspond also to the hypofluorescent zones on ICGA. If the size of the lesions is small, no scotomata and minimal fluorescein signs are present and the only way to make the diagnosis is by ICGA showing the typical dark dots up to the late phase of angiography without functional translation.

Resolution of dark dots occurs in parallel with visual field recovery in the convalescent phase, 4–8 weeks after onset, whereas fluorescein angiography is less well correlated with the evolution of the functional parameters such as visual fields. Often fluorescein angiographic signs, unlike in MEWDS, have not yet resolved at the time of ICGA normalization.

14.5.3.5 Electroretinography

Astonishingly the full-field electroretinogram shows moderate and transient abnormalities in APMPPE [49]. This is in contrast to MEWDS, where electroretinogaphy shows abnormalities indicating outer retinal dysfunction in up to 80 % of cases. This is possibly explained by the fact that lesions in MEWDS are diffuse whereas in APMPPE they are usually more limited and focal.

14.5.3.6 Treatment

Corticosteroid and/or immunosuppressive treatment were not proven to be useful in APMPPE. Visual acuity and visual fields are known to recover spontaneously in most patients. In cases with profound bilateral visual loss, being aware of the vasculitic component in APMPPE, we empirically prescribed systemic corticosteroids in some severe cases with a consecutive good functional recovery. Recovery could probably also have occurred without treatment, but the high level of anxiety of the patient has to be taken into account, which is moreover enhanced by his knowing of the inflammatory nature of the disease.

Summary for the Clinician

APMPPE/AMIC

- **Viral flu-like syndrome often found in patient history**
- **Symptoms: visual loss, scotomata, photopsias**
- **Yellowish deep bilateral discoloration at posterior pole (with serous exudative retinal detachments in hyperacute cases)**
- **Vitreitis and slight anterior uveitis that can also be more severe**
- **ICGA: geographic hypofluorescent areas (early, intermediate and late) = choriocapillaris nonperfusion**
- **FA: *hypofluorescence* (early) and *hyperfluorescence* (late); geographic aspect**
- **ERG: usually normal**
- **Lesions and functional disturbances usually reversible without therapy (steroids sometimes given); chorioretinal scars possible**

14.5.4 Multifocal Choroiditis (MFC), Including Punctate Inner Choroiditis (PIC), Diffuse Subretinal Fibrosis and Presumed Ocular Histoplasmosis Syndrome (POHS)

14.5.4.1 Introduction

The boundaries and nosological characteristics are less well determined for multifocal choroiditis than for MEWDS and APMPPE. This entity is more heterogeneous than the previously described diseases. When seen by the clinician most of the cases already show chorioretinal scars, as if preceding silent episodes occur before the disease becomes symptomatic at the time of a recurrence. The cases described as POHS in patients with a negative hypersensibility skin test to histoplasma capsulatum and coming from non-endemic areas for histoplasmosis should probably be assimilated with multifocal choroiditis. The characteristics of all the subtypes of multifocal choroiditis are: (1) the numerous small randomly distributed chorioretinal scars and the recurrent behaviour of the disease as well as the propensity to develop secondary neovascular membranes, which is much more frequent than in all other PICCPs.

14.5.4.2 Multifocal Choroiditis

Multifocal choroiditis occurs in the same age groups as other PICCPs, namely in young to middle-aged adults with women being predom-

inantly affected [50]. Lesions tend to leave scars and are not spontaneously reversible but seem to respond to corticosteroid therapy. Subretinal neovascular membranes occur in one-third of patients [50].

14.5.4.2.1 Clinical Symptoms and Findings

The symptoms that connect multifocal choroiditis to all other PICCPs are the photopsias. They are usually much more disturbing for the patient than in other PICCPs and their duration is protracted, being present also when there is no clinical evidence of reactivation of the disease. The patients also report scotomata. Multifocal choroiditis can be bilateral, with involvement usually being asymmetric, or it can be unilateral and is then usually included in the subtype of punctate inner choroidopathy (PIC). Visual loss depends on the localization of the lesions and can be severe when lesions are close to the fovea. Visual field testing objectively identifies the scotomata the patients report and that are localized to the areas of fundus involvement.

Classically, only slight non-granulomatous anterior segment inflammation is present. Therefore if anterior granulomatous uveitis is present, a specific diagnosis, such as sarcoidosis, syphilis or tuberculosis, has to be excluded. Cells in the vitreous can be found most of the time when the disease is active but can be absent in quiet disease.

On *fundus examination,* the typical lesions are small randomly distributed choroidal mostly atrophic yellow-white foci with pigment spots that can sometimes become adjacent to each other and form a ribbon of pearls (Fig. 14.5a). These lesions involve the posterior pole as well as the periphery or both. In the active phases of disease new lesions are not always visible and can be very discreet on FA, whereas ICGA is the most sensitive method for detecting new lesions [28, 51]. One particular feature of multifocal choroiditis is the high proportion of neovascular membranes complicating the disease.

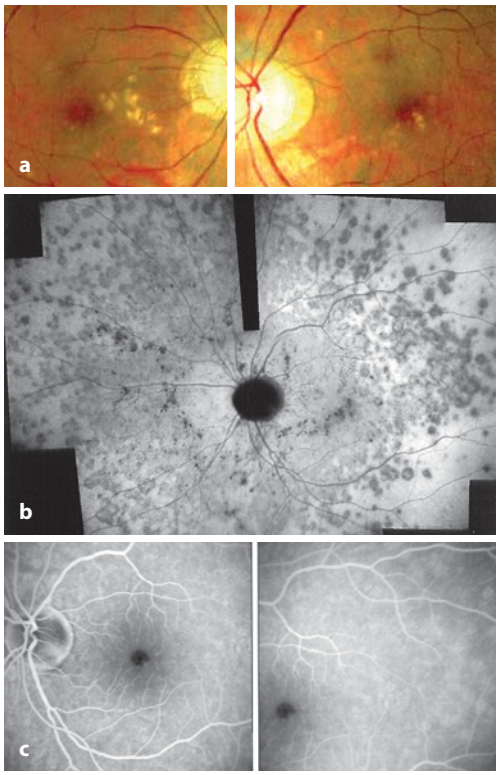

Fig. 14.5 a–c. Multifocal choroiditis (MFC). Numerous depigmented foci in both the right and left fundus (**a**) in a patient presenting a second episode of visual field loss. ICGA shows an extensive area of patchy and geographically confluent choriocapillaris nonperfusion (**b**), whereas fluorescein angiography is quasi normal except for patchy areas of late hyperfluorescence (**c**)

14.5.4.2.2 ICG Angiography

The first set of signs identifies old scarred chorioretinal lesions and consists of hypofluorescent areas persisting up to the late angiographic phase, distributed at random in the fundus, corresponding to hyperfluorescence on the late fluorescein angiographic times, typical for chorioretinal atrophy from scars of previous inflammatory episodes seen on fundus examination. The second set of signs can be seen in addition to the previously described signs when choroiditis recurs or they can be seen in their absence when it is the first episode of multifocal

choroiditis. The signs consist of hypofluorescent areas, almost silent on fluorescein angiography and not visible on fundus examination, representing areas of new inflammatory involvement (Fig. 14.5b). As in MEWDS, some cases may present peripapillary hypofluorescence translating functionally into an enlarged blind spot [52] (Fig. 14.5b). The latter signs respond to systemic corticosteroids and can regress completely if therapy is started early.

14.5.4.2.3 Fluorescein Angiography

Fluoresein angiography shows mainly signs of chorioretinal scarring, with window effects being associated with masking effects where there is pigment clumping. In the active phase FA may show faint late hyperfluorescence in areas corresponding to ICGA hypofluorescent dark dots, corresponding to new lesions (Fig. 14.5c). The use of fluorescein angiography is, however, of little use for assessing and following active disease, as FA angiographic signs are often absent or faint in new areas of inflammatory involvement, which are, however, clearly shown by ICGA.

14.5.4.2.4 Visual Field Testing

Visual field testing can show small scotomata corresponding to chorioretinal scars. In the active phase, however, scotomata are larger and correspond to the choriocapillaris non-perfusion shown on ICGA. Visual field recovery is well correlated with the regression of ICGA hypofluorescent areas that occurs following sub-Tenon's or systemic cortocosteroid therapy [28].

14.5.4.2.5 Electroretinography

Electrophysiology in active MFC has shown that the multifocal ERG (MERG) is diffusely depressed in addition to focal areas of greater loss corresponding to scotomata on the visual field. In contrast to MEWDS, where recovery of the MERG occurred in 100% of patients, MERG recovered partially only in 78% of patients [53]. This might be the explanation for patients continuing to be symptomatic and having photopsias despite apparent quiescence of the disease.

14.5.4.2.6 Treatment

There is probably sufficient anecdotal evidence to favour corticosteroid therapy in the case of newly diagnosed active disease or reactivation of multifocal choroiditis. Monitoring of therapy is best done by ICGA, which is more sensitive than visual field testing for detecting actively involved areas and response to therapy showing regression of hypofluorescent areas [28]. Corticosteroids can be given by sub-Tenon's injections if the reactivation is unilateral or systemically if there is bilateral involvement. Corticosteroids improve visual function in up to 60% of patients with active disease [50]. Corticosteroids are especially useful if there is an associated CME. They are also given routinely in neovascular membranes complicating MFC, before considering additional treatment modalities as a proportion of vessels regress spontaneously or following corticosteroid therapy. If neovessels do not regress under corticosteroids, photodynamic therapy (PDT) should be considered [54]. Overall the prognosis is reasonably good as a majority of patients retain a visual acuity above 0.5 (20/40) [50].

14.5.4.3 Punctate Inner Choroidopathy (PIC)

Punctate inner choroidopathy (PIC) is a subset of multifocal choroiditis characterized by a similar clinical picture as far as symptoms, fundus signs and neovascular complications are concerned except that the lesions are smaller. In the original description by Watzke and colleagues the disease is reported to be bilateral and predominant in myopic women [55]. In our experience the disease tended to be unilateral involving predominantly the more myopic of the two eyes. Like multifocal choroiditis, in our hands, new lesions responded to systemic or sub-Tenon's corticosteroids and additional immunosuppressive therapy was not necessary. Corticosteroids were also thought to have an effect on the neovascular membrane.

14.5.4.4 Subretinal Fibrosis

In some patients the evolution of multifocal choroiditis is associated with subretinal fibrosis sometimes linking the atrophic scars. This type of evolution might be due to subclinical progression of the disease. It might also reflect the propensity of the disease to develop subretinal neovascularization that might result in spontaneous fibrosis in some cases. Histopathology has shown that active choroidal inflammation is found in such cases [56].

14.5.4.5 Presumed Ocular Histoplasmosis Syndrome (POHS)

Histoplasma capsulatum is a fungal organism endemic in the Mississippi and Ohio river valleys. Other known endemic regions are Italy, Central America, Turkey, Israel and Australia. In these geographic areas there is a type of multifocal choroiditis that has distinctive features, called presumed ocular histoplasmosis syndrome (POHS) probably caused by the dissemination of the organism after pulmonary inhalation [57]. Diagnosis of exposure to histoplasma capsulatum is performed by performing histoplasma capsulatum hypersensitivity skin testing. In the case of a positive skin test and the presence of multifocal choroiditis the diagnosis of POHS has to be considered. In non-endemic areas this terminology should not be applied and the diagnosis of POHS should be questioned unless there is a positive skin test. The criteria that differentiate POHS from multifocal choroiditis are the absence of anterior chamber reaction, punched-out multifocal lesions also in the periphery (histospots) and peripapillary scarring and an asymptomatic course unless neovascular membranes develop. ICG angiography has shown that POHS tends not to show the hypofluorescent areas seen in MFC in the case of active disease but shows pinpoint hyperfluorescent ICGA spots not detected by funduscopy or fluorescein angiography [58]. We feel that the term POHS should not be used in patients with a negative skin hypersensitivity test and/or patients not living in endemic areas and/or patients not showing the typical hyperfluorescent pinpoints. In such cases, patients should be classified as multifocal choroiditis for the sake of clarity.

Summary for the Clinician

Multifocal Choroiditis

- **Recurrent chorioretinal inflammatory disease: photopsias, scotoma, visual loss**
- **Bilateral involvement; usually unilateral in the case of PIC (in more myopic eyes)**
- **Small faintly apparent active foci and older chorioretinal scars**
- **Vitreous cells in posterior vitreous during active stage**
- **ICGA: hypofluorescent areas (early, intermediate and late): scars and active foci (the latter only detectable by ICG)**
- **FA: early hypo- and late hyperfluorescence (scars); active foci rarely seen by FA**
- **Subretinal inflammatory neovascularization frequent (up to 30 %)**
- **Therapy: corticosteroids (and immunosuppressive therapy); not always successful**

14.5.5 Serpiginous Choroiditis

Serpiginous choroiditis is a progressive recurrent primary inflammatory choriocapillaropathy that leads to non-reversible destruction of the chorioretina and is situated at the "malignant" end of the spectrum of PICCPs [59]. It is also called geographical or helicoid choroidopathy [60, 61] and affects more elderly patients in addition to the young healthy adult age group traditionally affected by the other PICCPs. In India, however, patients are of the same young adult age group as all other PICCPs [62]. Before retaining the diagnosis of serpiginous choroiditis, infectious chorioretinitis, in particular tuberculosis and syphilis, has to be excluded [41, 63]. Presumed choroidal tuberculosis may present as a multifocal progressive choroiditis resembling serpiginous choroiditis [63]. Serpiginous choroiditis seems to be kept from progressing by combined corticosteroid and immunosuppressive therapy.

14.5.5.1 Clinical Symptoms and Findings

Patients usually consult because of loss of vision, metamorphopsias and scotomata. Photopsias seem to be less in the foreground in serpiginous choroiditis than in other PICCPs. The anterior segment is usually uninflamed whereas minimal to mild vitreitis is usually associated with the choroiditis. The active lesions appear as grey-yellow-white deep lesions beginning in the peripapillary region and progressing in a centrifugal fashion. The type of progression of lesions and scar formation can have a serpiginous (pseudopodial) or a geographic (map-like) pattern [60, 61]. The lesions are usually bilateral but involvement is asymmetric. In India involvement tends to be more often unilateral [62]. Visual function impairment depends on the location and progression of the lesions (Fig. 14.6). Curiously, the fovea seems to be spared for some time by the process, leading, however, to severe visual loss when it is ultimately involved. Subretinal neovascular membranes occur in up to 30 % of cases and can have the same aspect as recurrent active lesions. The best diagnostic procedure to distinguish neovascular membranes from reactivating disease is ICGA, showing early hyperfluorescence in neovascular membranes instead of the hypofluorescence characteristic of new serpiginous lesions [48].

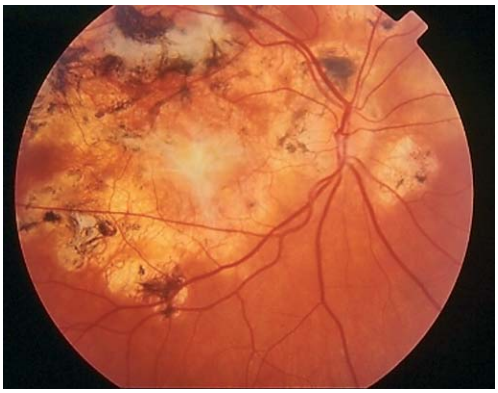

Fig. 14.6. Serpiginous choroiditis. Typical aspect of chorioretinal atrophy and scarring that originated from the optic disc and progressed despite therapy, progressively involving the whole posterior pole

14.5.5.2 ICG Angiography (Fig. 14.2)

Indocyanine green angiography of old lesions shows mainly hypofluorescent areas up to the late angiographic phase, indicating chorioretinal scarring and atrophy [64]. In areas of active disease progression, ICGA shows hypofluorescent areas that go beyond the lesions apparent on funduscopy and/or fluorescein angiography [28]. Another ICGA sign that can give information on the activity of the process is diffuse perilesional hyperfluorescence [28].

14.5.5.3 Fluorescein Angiography (Fig. 14.2)

Fluorescein angiography of the active progressing edges appears as hypofluorescent in the early phase with progressive late staining, a pattern compatible with outer retina ischaemia. Older lesions appear as window defects associated with blockage caused by pigment clumping, a typical pattern for chorioretinal atrophy and scars [61].

14.5.5.4 Visual Field Testing and Electrophysiology

Visual field testing shows scotomata corresponding to the atrophic lesions but is not sensitive enough to detect functional impairment caused by new active lesions [64]. If the central macula is at risk of being involved, follow-up with the Amsler grid is probably of utility. Electrophysiology is of little help in the appraisal of serpiginous choroiditis, often being normal unless extensive areas are involved [60, 65].

14.5.5.5 Treatment

There is no therapeutic regimen proven to be efficient on the basis of controlled studies in serpiginous choroiditis. Corticosteroids and immunosuppressants are often used although the results of such treatments are conflicting [66]. Maintenance of remission has been reported using early immunosuppressive therapy [67]. Reports of recurrence of disease in cases where

these treatments were discontinued, however, represent a strong argument for proposing such treatments, explaining to the patient the lack of scientifically proven evidence of efficacy and the potential side effects of therapy. Because of the deleterious prognosis of serpiginous choroiditis, we favour the attitude to introducing systemic corticosteroid therapy and monitoring disease response using dual FA/ICGA angiography. In the case of insufficient response or efficacy, we tend to introduce empirically immunosuppressive agents beginning with azathioprine (2.5–3 mg/kg/day), alternatives being mycophenolate mofetyl (1–2 g/day) and cyclosporine (3–4 mg/kg per day) with "à la carte" tapering. In the case of visual threat to the fovea, triple immunosuppressive therapy should be considered followed by cautious tapering of therapy [64].

In the case of the development of neovascular membranes complicating serpiginous choroiditis, an increase in corticosteroid and/or immunosuppressive therapy should be considered before additional therapy such as photodynamic therapy (PDT) is considered. Photodynamic therapy seems to be of some use in subretinal neovascular membranes of inflammatory origin, but studies and large series are lacking [54].

Summary for the Clinician

Serpiginous Choroiditis

- **Acute atrophying peripapillary choriocapillaritis progressing centrifugally, usually bilateral but with asymmetric involvement**
- **Fundus findings: *acute stage:* geographic white-grey to yellow peripapillary lesions ± serous detachments; *chronic stage:* atrophic pigmented scars**
- **ICGA: *acute:* geographic hypofluorescence (= choriocapillaris non-perfusion) surrounded by choroidal hyperfluorescence. *Chronic:* hypofluorescence (= atrophy)**
- **FA: *acute:* early hypofluorescence and late impregnation; *chronic:* early hypofluorescence and late hyperfluorescence (window effect) (= atrophy)**
- **Evolution: slowly progressing, prognosis can be reserved. Treatment: empirical corticosteroids/immunosuppressants**

14.6 Rare Entities

14.6.1 Acute Zonal Occult Outer Retinopathy (AZOOR) and Acute Annular Outer Retinopathy (AAOR)

Gass described in 1993 a syndrome characterized by photopsias, acute bilateral loss of outer retinal function, minimal initial fundus changes and an abnormal ERG, which he called acute zonal occult outer retinopathy (AZOOR). This entity shares clinical similarities with MEWDS, acute idiopathic blind spot enlargement, punctate inner choroidopathy, multifocal choroiditis with panuveitis and acute macular neuroretinopathy [68]. All these conditions predominantly affect young myopic women complaining of photopsias with scotomata and ERG abnormalities, which led Gass to speculate that they could be variants of the same pathology [8, 69].

14.6.1.1 Clinical Symptoms and Findings

Patients complain of photopsia and scotomata involving one or several zones of the visual field. A preceding flu-like illness is found in about 20 % of patients [69]. Acute visual loss is present at the onset, which can stabilize within months or progress over years. On *fundoscopy,* there is characteristically a discrepancy between the acute visual complaints and the normal appearance of the fundus in the majority of cases (91 %) [69]. But with the progression of the disease, retinal pigment epithelium (RPE) changes such as hypopigmentation and mottling of the RPE simulate retinitis pigmentosa like evolution, with vascular sheathing and retinal vascular narrowing in more severe cases.

14.6.1.2 Angiography

To date there is limited or no information on ICGA signs in AZOOR, but choriocapillaris disease can be anticipated. Fluorescein angiography is normal at the onset when fundoscopy

shows no alterations and then with chronicity, alterations of the RPE become manifest and are objectively shown by fluorescein angiography.

14.6.1.3 Visual Field Testing

There is variability in visual field loss, the most frequent being an enlarged blind spot but peripheral visual field contraction can also occur. Visual field scotoma can stabilize within months in some cases but progress in evolutive cases.

14.6.1.4 Electroretinography

The full-field electroretinogram (ERG) shows extensive abnormalities that contrast with the limited fundus involvement but are correlated to the extent of visual field alterations. Both scotopic and photopic responses are generally affected and ERG shows the extent of involvement of the outer retina dysfunction [69].

14.6.1.5 Treatment

Systemic corticosteroids have been used to treat AZOOR sometimes in association with acyclovir based on a very hypothetical theory on a possible viral involvement, but interpretation of the therapeutic effect is difficult because spontaneous stabilization of the disease can occur [69].

Acute annular outer retinopathy (AAOR) was first described by Luckie and colleagues and is characterized by visual field loss corresponding to a ring of white-grey discoloration of the retina evolving towards chorioretinal thinning [70], and should probably be classified with AZOOR in the acute zonal outer retinopathies [71].

Summary for the Clinician

Acute Zonal Occult Outer Retinopathy (AZOOR)

- **Rare disorder affecting mainly young myopic women. Shares clinical similarities with MEWDS, PIC, multifocal choroiditis and may be a variant in the spectrum of these entities**
- **Photopsia, scotoma, and visual loss are the main complaints**
- **Fundus findings: *acute stage:* minimal in contrast to electroretinogram alterations; *chronic stage:* retinal pigment epithelium hypopigmentation and mottling**
- **Electroretinography and visual field: generally impaired before visible fundus modifications**
- **FA: normal at the onset when no fundus alterations are present. Then RPE alterations appear as mottling fluorescence**
- **Evolution: can be self-limited without treatment. Empirical corticosteroids and acyclovir treatment have been suggested**

14.6.2 Acute Macular Neuroretinopathy (AMN)

Acute macular neuroretinopathy (AMN) is a rare chorioretinopathy affecting young adults mono- or bilaterally, producing disturbing symptoms of photopsia and often occurring after a flu-like illness like many of the other PICCPs [72]. Visual loss is variable and fundus changes are characterized by large macular orange-brown plaques (Fig. 14.7). Fluorescein angiography shows early choroidal hypofluorescence or can be unremarkable. The visual field can show a central scotoma. The photopsias, clinical signs and functional tests usually recover without sequels within weeks or months. AMN has been described in association with MEWDS in the same patient linking the disease to the other PICCPs [73]. The whole picture of this entity corresponds perfectly to and is characteristic of PICCPs, but the rarity of its occurrence has prevented ICGA analysis so far. We recently saw a patient examined in the early phase showing the typical fundus colour changes associated with ICGA hypofluorescence of the whole macular area, indicating choriocapillaris pathology and linking the disease to the PICCPs (Fig. 14.8).

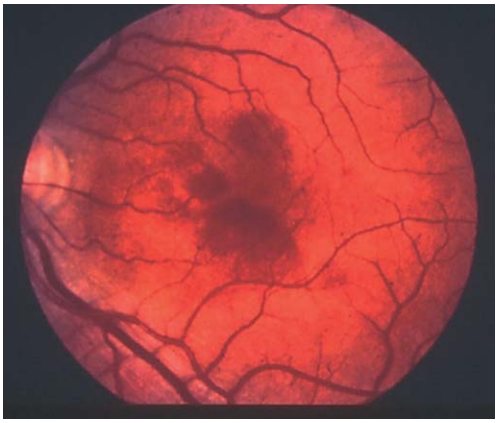

Fig. 14.7. Acute macular neuroretinopathy (AMN). Typical dark discoloration of the posterior pole

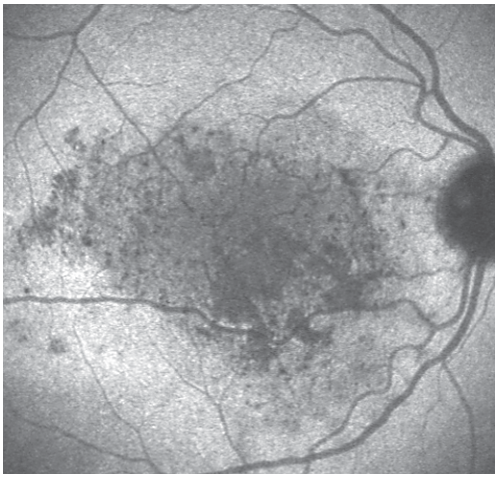

Fig. 14.8. Choriocapillaris non-perfusion in AMN. Patient presenting with visual field changes and darkened posterior pole, showing geographic hypofluorescence compatible with choriocapillaris non-perfusion

14.7 Overlapping Clinical Pictures in PICCPs: The Choriocapillaris as the Common Denominator of PICCPs

14.7.1 Association of Different PICCPs in the Same Patient

Several articles have reported clinical cases combining two or more of the PICCP entities described here above. Holz and colleagues described acute zonal occult outer retinopathy (AZOOR) associated with multifocal choroidopathy [74]. Association of MEWDS and multifocal choroiditis in the same patient has been described in several reports and evolution from MEWDS to MFC as well as the reverse has been reported [35, 75, 76]. Association in the same patient of acute macular neuroretinopathy and MEWDS has been described by Gass, who suggested that AMN was part of the MEWDS/AIBSE spectrum [73]. These and other reports represent a considerable body of evidence suggesting a common denominator for all the PICCPs which has to be sought at the level of the choriocapillaris.

14.7.2 Intermediary Forms of PICCP

In addition to the cases in which more than one clinical entity are associated in one patient, it is not uncommon to have intermediary forms that are difficult to classify within one or the other subset of PICCP. Either they have a hybrid presentation or they have the morphology of one disease and the evolution of another, such as APMPPE, with a disease course characterized by recurrences behaving like serpiginous choroiditis, or there are atypical cases that cannot be classified within a determined subset but for which ICGA shows that the pathology is definitively situated at the level of the choriocapillaris (Fig. 14.1 a–c). When a sufficient number of cases of an intermediary or non-described form behaving similarly are collected, this allows the description of a new entity as has happened

with a series of cases presenting as APMPPE having a subsequent evolution more compatible with serpiginous choroiditis first termed AMPPiginous choroiditis and later described as relentless placoid chorioretinitis [7]. In this report of six patients the acute retinal lesions were similar to APMPPE or serpiginous choroiditis but had a prolonged progressive course and widespread distribution of lesions [7]. Along the same line of evidence, Gupta and colleagues, in one of the largest series of serpiginous choroiditis reported so far, indicate that in their part of the world all cases having the initial features of APMPPE showed progression resembling serpiginous choroiditis during follow-up [61].

14.7.3 Unclassifiable Primary Inflammatory Choriocapillaropathies

It is important to realise that this is a spectrum of diseases involving the choriocapillaris in diverse fashions and diverse degrees of severity and that even when the entity seems to be defined it is important to perform a close follow-up.

This situation is well illustrated by a case recently seen in our centre presenting with macular chorioretinal atrophy (Fig. 14.9 a). The 42-year-old male patient had presented an acute decrease of visual acuity and a central scotoma in his left eye 3 weeks after a flu-like febrile illness that occurred while the patient was staying in Southeast Asia. He consulted an Emergency Ophthalmology Department and a drop of visual acuity to 0.1 was noted OS with full vision OD. The ICGA showed severe central choriocapillaris non-perfusion (Fig. 14.9 b). A complete uveitis work-up was performed but was negative; in particular there was no evidence of syphilis and tuberculosis and no action was undertaken. Two weeks later a decrease of visual acuity and scotoma occurred OD and the patient was seen in a neighbouring country and again no action was undertaken as the diagnosis made was APMPPE. During the whole episode the patient complained of moderate to severe headaches. The patient was seen in our centre 3 months later with a visual acuity reduced to 0.1 OU and central chorioretinal atrophy well seen on FA and ICGA (Fig. 14.9 c). High-dose steroid therapy was undertaken that

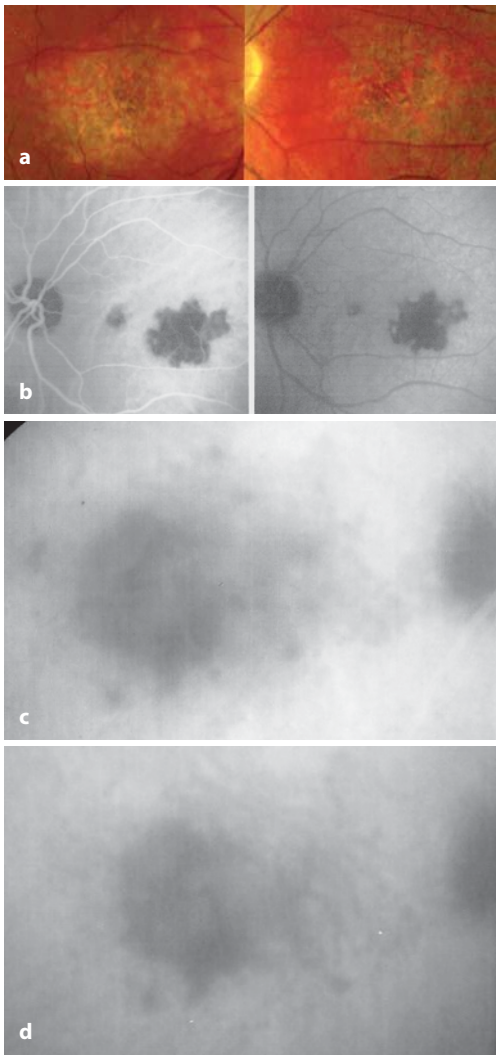

Fig. 14.9 a–d. Unclassifiable primary inflammatory choriocapillaropathy (PICCP). Patient presenting with bilateral macular chorioretinal cicatricial atrophy (**a**), low visual acuity and central scotomata, 3 months after the acute episode during which ICGA had been performed showing macular choriocapillaris non-perfusion (**b**). The recent ICGA shows fuzzy edges indicating residual activity (**c**) that responds to corticosteroid therapy with delineation of the atrophy and resolution of some of the dark dots (**d**)

contributed to stabilization of ICGA lesions and improvement of visual field findings, but visual acuity remained low (Fig. 14.9 d). The first ICGA clearly showed choriocapillaris disease in this case; however, it cannot be classified into one of the known entities. Close follow-up of all inflammatory choriocapillaropathies is mandatory and empiric corticosteroid and/or immunosuppressive therapy should probably be tried in those cases with a deleterious functional evolution.

14.8 Conclusion

Primary inflammatory choriocapillaropathies are caused by an inflammatory lesional process producing disturbance in the perfusion of the choriocapillaris and functional disturbance in the outer retina [1–5, 10, 14, 19]. The lesional pattern of non-perfusion is different from one PICCP disease to another, producing benign conditions at one end of the spectrum such as MEWDS and deleterious conditions at the other end of the spectrum such as serpiginous choroiditis, with numerous intermediary forms including well-described entities and unclassifiable cases as well as an association of different entities. The factor determining the behaviour of each type of PICCP is not known but probably has to do with the level and severity of vascular damage to the choriocapillaris. Although the precise inflammatory scenario is not known, we now know what structure is primarily involved and we have now the means with the advent of ICGA to monitor the choriocapillaris closely and with multifocal ERG to measure better the functional impact on the outer retina and possibly prevent visual loss by introducing potentially effective immunosuppressive therapy until more appropriate therapies are found, in order to try to preserve vision in those cases that take a deleterious course.

References

1. Howe LJ, Woon H, Graham EM, Fitzke F, Bhandari A, Marshall J (1995) Choroidal hypoperfusion in acute posterior multifocal placoid pigment epitheliopathy. Ophthalmology 102:790–798
2. Dhalival RS, Maguirre AM, Flower RW, Arribas NP (1993) Acute posterior multifocal placoid pigment epitheliopathy, an indocyanine green angiographic study. Retina 13:317–325
3. Borruat FX, Auer C, Piguet B (1995) Choroidopathy in multiple evanescent white dot syndrome. Arch Ophthalmol 113:1569–1571
4. Hansen RM, Fulton AB (1981) Cone pigments in acute posterior multifocal placoid pigment epitheliopathy. Am J Ophthalmol 91:465–468
5. Horiguchi M, Miyake Y, Nakamura M, Fujii Y (1993) Focal electroretinogram and visual field defect in multiple evanescent white dot syndrome. Br J Ophthalmol 77:452–455
6. Moshirfar M, Zimmermann PL (1998) A case of AMPPiginous choroidopathy. In: Meisler DM, Chern KC (eds) American Uveitis Society Annual Meeting Abstracts. Ocular Immunol Inflamm 6:135–138
7. Jones BE, Jampol LM, Yannuzzi LA, Tittl M, Johnson MW, Han DP, Davis JL, Williams DF (2000) Relentless placoid chorioretinitis. Arch Ophthalmol 118:931–938
8. Jampol LM, Becker KG (2003) White spot syndromes of the retina: a hypothesis based on the common genetic hypothesis of autoimmune/inflammatory disease. Am J Ophthalmol 135:376–379
9. Deutman AF (1983) Acute multifocal ischaemic choroidopathy and the choriocapillaris. Int Ophthalmol 6:155–160
10. Deutman AF, Lion F (1977) Choriocapillaris nonperfusion in acute multifocal placoid pigment epitheliopathy. Am J Ophthalmol 84:652–657
11. Bischoff PM, Flower RW (1985) Ten years experience with choroidal angiography using indocyanine green dye: a new routine examination or an epilogue. Doc Ophthamol 60:235–291
12. Herbort CP (1998) Posterior uveitis: new insights given by indocyanine green angiography. Eye 12: 757–759
13. Orlock DA, Scheider A, Lachenmayer B (1997) Acquisition of ICG angiograms in indocyanine green angiography, Chap 5. In: Yannuzzi LA, Flower RW, Slakter JS (eds) Mosby Yearbook, St. Louis, MO, pp 50–62

14. Slakter JS, Giovannini A, Yannuzzi LA, Scassellati-Sforzolini B, Guyer D, Sorenson JA, Spaide RF, Orlock D (1997) Indocyanine green angiography of multifocal choroiditis. Ophthalmology 104:1813–1819
15. Borruat FX, Auer C, Piguet B (1995) Choroidopathy in multiple evanescent white dot syndrome. Arch Ophthalmol 113:1569–1571
16. Giovannini A, Ripa E, Scassellati-Sforzolini B, Ciardella A, Tom D, Yannuzzi L (1996) Indocyanine green angiography in serpiginous choroidopathy. Eur J Ophthalmol 6:299–306
17. Borruat FX, Piguet B, Herbort CP (1998) Acute posterior multifocal placoid pigment epitheliopathy following mumps. Ocular Immunol Inflamm 6:39–41
18. Lowder CY, Foster RE, Gordon SM, Gutman FA (1996) Acute posterior multifocal placoid pigment epitheliopathy after acute group A streptococcal infection. Am J Ophthalmol 122:115–117
19. Baglivo E, Safran AB, Borruat FX (1996) Multiple evanescent white dot syndrome after hepatitis B vaccine. Am J Ophthalmol 122:431
20. Fine L, Fine A, Cunningham ET (2001) Multiple evanescent white dot syndrome following hepatitis A vaccination. Arch Ophthalmol 119:1856–1857
21. Brézin AP, Massin-Korobelnik P, Boudin M, Gaudric A, LeHoang P (1995) Acute posterior multifocal placoid pigment epitheliopathy after hepatitis B vaccine. Arch Ophthalmol 113:297–300
22. Desarnaulds AB, Borruat FX, Herbort CP, Spertini F (1996) Multiple evanescent white dot syndrome: a genetic disorder? Kl Monatsbl Augenheilkd 208:301–302
23. Jampol LM, Sieving PA, Pugh D, Fishman GA, Gilbert H (1984) Multiple evanescent white dot syndrome. 1. Clinical findings. Arch Ophthalmol 102:671–674
24. Borruat FX, Othenin-Girard P, Safran AB (1991) Multiple evanescent white dot syndrome. Kl Monatsbl Augenheilkd 198:453–456
25. Desarnaulds AB, Herbort CP, Borruat FX (1996) Multiple evanescent white dot syndrome: visual dysfunction and evolution. Ophthalmologie 10: 95–101
26. Sieving PA, Fishman GA, Jampol LM, Pugh D (1984) Multiple evanescent white dot syndrome. 2. Electrophysiology of the photoreceptors during retinal pigment epithelial disease. Arch Ophthalmol 102:675–679
27. Oh KT, Folk JC, Maturi RK, Moore P, Kardon RH (2001) Multifocal electroretinography in multifocal choroiditis and the multiple evanescent white dot syndrome. Retina 21:581–589
28. Cimino L, Auer C, Herbort CP (2000) Sensitivity of indocyanine green angiography for the follow-up of active inflammatory choriocapillaropathies. Ocular Immunol Inflamm 8:275–283
29. Fletcher WA, Imes RK, Goodman D, Hoyt WF (1988) Acute idiopathic blind spot enlargement. A big blind spot syndrome without optic disc edema. Arch Ophthalmol 106:44–49
30. Hamed LA, Schatz NJ, Glaser JS, Gass DJM (1988) Acute idiopathic blind spot enlargement without optic disc edema. Arch Ophthalmol 106:1030–1031
31. Pece A, Sadun F, Trabucchi G, Brancato R (1998) Indocyanine green angiography in enlarged blind spot syndrome. Am J Ophthalmol 126:604–607
32. Singh K, de Frank MP, Shults WT, Watzke RC (1991) Acute idiopathic blind spot enlargement. A spectrum of disease. Ophthalmology 98:497–502
33. Reddy CV, Brown J, Folk JC, Kimura AE, Gupta S, Walker J (1996) Enlarged blind spots in chorioretinal inflammatory disorders. Ophthalmology 103:606–617
34. Khorram KD, Jampol LM, Rosenberg MA (1991) Blind spot enlargement as a manifestation of multifocal choroiditis. Arch Ophthalmol 109: 1403–1407
35. Callanan D, Gass DJM (1992) Multifocal choroiditis and choroidal neovascularization associated with multiple evanescent white dot and acute idiopathic blind spot enlargement syndrome. Ophthalmology 99:1678–1685
36. Slakter JS, Giovannini A, Yannuzzi LA, Scassellati-Sforzolino B, Guyer DR, Sorenson JA, Spaide RF, Orlock D (1997) Indocyanine green angiography of multifocal choroiditis. Ophthalmology 104: 1813–1819
37. Desarnaulds AB, Borruat FX, Herbort CP, de-Courten C (1998) L'angiographie au vert d'indocyanine dans le "Multiple Evanescent white dot syndrome" (MEWDS). Klin Monatsbl Augenheilkd 212:318–322
38. Gass JDM (1968) Acute posterior multifocal placoid pigment epitheliopathy. Arch Ophthalmol 80:177–185
39. Azar P, Gohd RS, Waltman D, Gitter KA (1975) Acute posterior multifocal placoid pigment epitheliopathy associated with an adenovirus type 5 infection. Am J Ophthalmol 80:1003–1005
40. Bodine SR, Marino J, Camisa T, Salvate AJ (1992) Multifocal choroiditis with evidence of Lyme disease. Ann Ophthalmol 24:169–173
41. Gass JDM, Braunstein RA, Chenoweth RG (1990) Acute syphilitic posterior placoid chorioretinitis. Ophthalmology 97:1288–1297

42. Hsu CT, Harlan JB, Goldberg MF, Dunn JP (2003) Acute posterior multifocal placoid pigment epitheliopathy associated with a systemic necrotizing vasculitis. Retina 23:64–68
43. Seigelmann J, Behrens M, Hilal S (1979) Acute posterior multifocal pigment epitheliopathy associated with cerebral vasculitis and homonymous hemianopsia. Am J Ophthalmol 88:919–924
44. Smith CH, Savina PJ, Beck RW (1983) Acute posterior multifocal pigment epitheliopathy and cerebral vasculitis. Arch Neurol 40:48–50
45. Wilson CA, Choromokos EA, Sheppard R (1988) Acute posterior multifocal pigment epitheliopathy and cerebral vasculitis. Arch Ophthalmol 106:796–800
46. Laatikainen LT, Immonen IJR (1988) Acute posterior multifocal placoid pigment epitheliopathy in connection with acute nephritis. Retina 8:122–124
47. Kersten DH, Lessel L, Carlow TJ (1987) Acute posterior multifocal placoid pigment epitheliopathy and late onset meningoencephalitis. Ophthalmology 94:393–396
48. Herbort CP, LeHoang P, Guex-Crosier Y (1998) Schematic interpretation of indocyanine green angiography in posterior uveitis using a standard angiographic protocol. Ophthalmology 105:432–440
49. Park D, Shatz H, McDonald R, Johnson RN (1995) Indocyanine green angiography of acute multifocal posterior placoid pigment epitheliopathy. Ophthalmology 102:1877–1883
50. Brown J, Folk JC, Reddy CV, Kimura AE (1996) Visual prognosis of multifocal choroiditis, punctate inner choroidopathy, and diffuse subretinal fibrosis syndrome. Ophthalmology 103:1100–1105
51. Weinberger AWA, Kube T, Wolf S (1999) Dark spots in late-phase indocyanine green angiographic studies in a patient with presumed ocular histoplasmosis syndrome. Graefes Arch Clin Exp Ophthalmol 237:524–526
52. Slakter JA, Giovannini A, Yannuzzi LA, Scassellati-Sforzolini B, Guyer DR, Sorenson JA, Spaide RF, Orlock D (1997) Indocyanine green angiography in multifocal choroiditis. Ophthalmology 104:1813–1819
53. Oh KT, Folk JC, Maturi RK, Moore P, Kardon RH (2001) Multifocal electroretinography in multifocal choroiditis and the multiple evanescent white dot syndrome. Retina 21:581–589
54. Perentes Y, Tran VT, Sickenberg M, Herbort CP (2004) Subretinal neovascular membranes complicating uveitis: frequency, treatments and visual outcome. Ocular Immunol Inflamm 12 (in press)
55. Watzke RC, Parker AJ, Folk JC, Benson WE, Burgess D, Ober RR (1984) Punctate inner choroidopathy. Am J Ophthalmol 98:572–584
56. Palestine AG, Nussenblatt RB, Chan CC, Hooks JJ, Friedman L, Kuwabara T (1985) Histopathology of the subretinal fibrosis and uveitis syndrome. Ophthalmology 92:838–844
57. Smith RE, Ganley JP (1972) Presumed ocular histoplasmosis: I. Histoplasmin skin test sensitivity in cases identified during a community survey. II. Patterns of peripheral and peripapillary scarring in persons with non macular disease. Arch Ophthalmol 87:245–257
58. Slakter JS, Giovannini A (1997) Multifocal choroiditis and the presumed ocular histoplasmosis syndrome. In: Yannuzzi LA, Flower RW, Slakter JS (eds) Indocyanine green angiography, chapter 20. Mosby, St. Louis, pp 271–278
59. Laatikainen L, Erkkila H (1974) Serpiginous choroiditis. Br J Ophthalmol 58:777–783
60. Hamilton AM, Bird AC (1974) Geographical choroidopathy. Br J Ophthalmol 58:784–797
61. Schatz H, Maumenee AE, Patz A (1974) Geographic helicoid peripapillary choroidopathy. Clinical presentation and fluorescein angiographic findings. Trans Am Acad Ophthalmol Otolaryngol 78:747–761
62. Gupta V, Agarwal A, Gupta A, Bambery P, Narang S (2002) Clinical characteristics of serpiginous choroidopathy in North India. Am J Ophthalmol 134:47–56
63. Gupta V, Gupta A, Arora S, Bamberry P, Dogra MR, Agarwal A (2003) Presumed tubercular serpiginouslike choroiditis. Clinical presentations and management. Ophthalmology 110:1744–1749
64. Giovannini A, Ripa E, Scassellati-Sforzolini, Ciardella A, Tom D, Yannuzzi LA (1996) Indocyanine green angiography in serpiginous choroidopathy. Eur J Ophthalmol 6:299–306
65. Chisholm IH, Gass JDM, Hutton WL (1982) The late stage of serpiginous (geographic) choroiditis. Am J Ophthalmol 82:343–351
66. Hooper PL, Kaplan HJ (1991) Triple agent immunosuppression in sepiginous choroiditis. Ophthalmology 98:944–952
67. Araujo AAQ, Well AP, Dick AD, Forrester JV (2000) Early treatment with cyclosporin in serpiginous choroidopathy maintains remission and good visual outcome. Br J Ophthalmol 84:979–982
68. Gass JD (1993) Acute zonal occult outer retinopathy. J Clin Neurol Ophthalmol 13:79–97
69. Gass JD, Agarwal A, Scott IU (2002) Acute zonal outer retinopathy: a long-term follow-up study. Am J Ophthalmol 134:329–339
70. Luckie A, Ai E, Del Piero E (1994) Progressive zonal outer retinitis. Am J Ophthalmol 118:583–588
71. Gass JDM (2000) The acute zonal outer retinopathies. Am J Ophthalmol 130:655

72. Bos PJM, Deutmann AF (1975) Acute macular neuroretinopathy. Am J Ophthalmol 80:573–584
73. Gass JDM, Hamed LM (1989) Acute macular neuroretinopathy and multiple evanescent white dot syndrome in the same patient. Arch Ophthalmol 107:189–193
74. Holz FG, Kim RY, Schwartz SD, Harper CA, Wroblewski J, Arden GB, Bird AC (1994) Acute zonal occult outer retinopathy (AZOOR) associated with multifocal choroidopathy. Eye 8:77–83
75. Bryan RG, Freund KB, Yannuzzi LA, Spaide RF, Huang SJ, Costa DL (2002) Multiple evanescent white dot syndrome in patients with multifocal choroiditis. Retina 22:317–322
76. Kozielec GF, Wyhinny GJ, Jampol LM (2001) Evolution of distinct chorioretinal scars in recurrent MEWDS. Retina 21:180–182

Stromal Choroiditis

Nadia Bouchenaki, Carl P. Herbort

Core Messages

- Histopathology has shown that stromal choroiditis is characterized by inflammatory, mostly granulomatous, foci of different sizes
- These foci appear as hypofluorescent areas on indocyanine green angiography (ICGA)
- ICGA has shown, in addition to the hypofluorescent dark areas, hyperfluorescent large choroidal vessels and late diffuse hyperfluoresence indicating choroidal vasculitis
- Vogt-Koyanagi-Harada disease is a good example of primary stromal choroiditis as the immune mediated process is specifically targeting the stromal melanocytes
- ICGA can detect the early inflammatory events when disease is still subclinical and has not yet caused secondary inflammation to neighbouring structures such as the retina (exudative retinal detachment)
- ICGA is essential in optimal follow-up of VKH
- Birdshot chorioretinopathy (BC) is a particular disease, as the choroid and the retina are involved in parallel, inflammation of one structure not being secondary to inflammation occurring in the neighbouring structure
- Choroidal involvement in BC should be considered a primary stromal choroiditis, although the target has not yet been identified
- Choroiditis due to sarcoidosis illustrates the second group of stromal choroiditides where the choroid is only the innocent bystander of an inflammatory reaction that involves the choroid at random but is not specifically directed towards the choroid

15.1 Introduction

Since indocyanine green angiography (ICGA) has become available, it has become possible to investigate stromal choroidal inflammatory disease in a more subtle way and detect lesions even at an early, often subclinical stage of evolution which has not been accessible so far to investigational tests [1].

The primary lesion of stromal choroiditis is the inflammatory, mostly granulomatous focus. Depending on the extension of the granuloma, full-thickness or partial thickness, the ICGA image will be influenced. In contrast to primary inflammatory choriocapillaropathies, the mechanism of stromal choroiditis has been proven with the help of anatomo-clinical-angiographic correlations. Histopathology has been available for some time for Vogt-Koyanagi-Harada disease, sympathetic ophthalmia and sarcoidosis and recently granulomas have been found in an autopsy case of birdshot chorioretinopathy [2–4].

Different pathologic behaviours have to be distinguished to better understand the lesions seen by fundus examination and FA and ICG angiography. They will help us progressively gain a physiopathogenic rather than a descriptive approach to choroiditis. At least three lesional events can take place in the choroidal stroma. The first group of diseases includes the entities where the choroidal stroma is the elective target of the inflammatory process. They can be called the primary obligatory choroitides and include Vogt-Koyanagi-Harada disease, sympathetic ophthalmia and birdshot chorioretinopathy. Their lesions are characterized by

multiple even sized regularly distributed lesions on ICGA. They initially have a minimal effect on the adjacent structures, the retinal pigment epithelium and the retina, which become only secondarily involved when disease severity progresses, resulting in the exudative retinal detachments seen in severe Vogt-Koyanagi-Harada disease. The second group of diseases includes systemic inflammatory or infectious diseases that can involve the choroid by chance, including sarcoidosis, tuberculosis and syphilis. Unlike the first group, the size of the lesions is uneven and distributed more at random. The third group includes infectious choroitides developing after haematogenous spread of the infectious agents that become trapped because of the sponge-like structure of the choroid and produce foci, endogenous candida choroiditis being one example.

The specific ICGA signs that can be seen in varying proportions according to the type of disease on which this classification relies are as follows:

1. Even, regularly distributed ICGA hypofluorescent dots in the case of birdshot chorioretinopathy, Vogt-Koyanagi-Harada disease and sympathetic ophthalmia, or variously sized hypofluorescent areas in the case of sarcoidosis or tuberculosis present in the early and intermediate phase remaining hypofluorescent in the late phase (full thickness inflammatory foci) or becoming isofluorescent in the late phase in the case of partial thickness inflammatory foci, indicating a mass effect [5].
2. Around the inflammatory lesions seen as hypofluorescent dark dots the larger choroidal vessels lose their normal aspect and appear fuzzy, indicating choroidal vasculitis that allows pathologic extrusion of the ICG complex at the origin of late diffuse choroidal hyperfluorescence [5].

15.2 Specific Entities

15.2.1 Primary Stromal Choroiditis

Primary obligatory stromal choroiditis comprises several conditions where the inflammatory process is selectively targeting choroidal stromal structures. In Vogt-Koyanagi-Harada disease the target is probably a melanin-associated protein and a similar process is probably occurring in sympathetic ophthalmia. The regular pattern and the even distribution of choroidal lesions of birdshot chorioretinopathy shown in a recent histopathological report also strongly suggest targeted stromal inflammatory involvement in birdshot chorioretinopathy.

15.2.1.1 Vogt-Koyanagi-Harada Disease

Vogt-Koyanagi-Harada (VKH) disease is a bilateral, granulomatous panuveitis with exudative retinal detachments associated with systemic manifestations such as meningeal signs and cutaneous signs (poliosis, alopecia, vitiligo) and dysacusis [6]. There is now enough evidence to indicate that the disease is caused by an autoimmune process against melanocytes or an antigen present in these cells, namely tyrosinase or tyrosinase related protein [7]. As for most of these autoimmune diseases an infectious event due to one or several different commonly occurring pathogens probably triggers the reaction in susceptible individuals [7]. The disease is more prevalent in Asians, in particular Japanese [8, 9], in Hispanics and native Americans, but can occur in any individual of any race. A genetic predisposition to the disease that can be suspected as an association with class II human leucocyte antigens (HLA) has been established, mainly HLA-DR4 locus for Japanese [10], and HLA-A31 and HLA-B55 for Koreans [11].

Histopathologic evaluation reveals thickening of the choroid with stromal cellular inflammation constituted by macrophages, lymphocytes and epithelioid cells containing melanin. Chronic inflammation and recurrences lead to

the loss of choroidal melanocytes and to the appearance of sunset glow fundus [12].

In the posterior segment of the eye the primary insult is known to occur at the level of the stromal melanocytes of the choroid. The lesional process in this disease defines VKH as a strictly primary choroidal stromal inflammatory disease and involvement of the adjacent structures is only secondary to choroidal disease. VKH is therefore the typical example of a primary stromal choroiditis as is the case for sympathetic ophthalmia and birdshot chorioretinopathy (see below).

15.2.1.1.1 Clinical Symptoms and Findings

VKH disease classically evolves in four phases: *prodromal* with a flu-like illness, headache, tinnitus, and vertigo, followed within a few days by an *ocular phase* consisting of acute uveitis with optic disc hyperaemia and swelling, and characteristic multifocal exudative serous retinal detachment (Fig. 15.1a). Lesions are mostly bilateral and can resolve if adequate and prolonged corticosteroid therapy is introduced at an early stage [19]. If not, the inflammation develops into a *chronic recurrent* disease leading after months or years to a *convalescent* stage characterized by depigmentation at the level of the choroid (sunset glow fundus), and peripheral yellow-white small round chorioretinal atrophic lesions corresponding to scars of Dalen-Fuchs nodules (small yellowish lesion corresponding histopathologically to foci of epithelioid cells containing pigment located between Bruch's membrane and the retinal pigment epithelium) (Fig. 15.1b). With chronic evolution, diffuse retinal pigment epithelium alterations classically appear as a "salt-and-pepper" fundus appearance [13].

In 1999 an international VKH disease committee was created and reintroduced diagnostic criteria for VKH disease [14, 15] (Table 15.1).

15.2.1.1.2 Indocyanine Green Angiography [16]

The main characteristics seen on ICGA in the acute phase of the disease are the presence of disseminated, even, regularly distributed hypofluorescent dark dots in the intermediate phase (Fig. 15.1c). For the most part these hypofluorescent dots remain hypofluorescent in the late angiographic phase, but some become isofluorescent, indicating partial thickness choroidal infiltration. In association there are fuzzy choroidal leaking vessels indicating choroidal vasculitis that can be very severe in some cases (Fig. 15.1d). The corollary of fuzzy vessels in the intermediate phase is late phase diffuse choroidal hyperfluorescence. In very acute disease the disc that is usually hypofluorescent can become hyperfluorescent, indicating severe papillitis. In the case of exudative detachments, ICGA shows the same hyperfluorescent pinpoints seen on fluorescein angiography. These signs all regress with treatment. ICGA was found to be essential not only for the diagnosis in the atypical cases but also for the follow-up, where it can detect subclinical recurrences. It also contributes to reducing the number of apparently unilateral cases by showing fellow eye subclinical involvement.

15.2.1.1.3 Fluorescein Angiography

In the acute stage of the disease or during recurrences, serous exudative detachments appear as multifocal hyperfluorescent pinpoints showing the leaking points at the level of pigment epithelium also seen on ICGA. FA also shows late pooling of the dye in the subretinal space (Fig. 15.1e). Optic disc staining and leakage also appears in the acute phase. In the chronic stage, FA clearly shows the diffuse retinal pigment epithelium alterations classically appearing as a mixture of window and masking effects as a consequence of the exudative retinal detachment, the limits of which are well shown and called highwater marks. In the chronic phase a hyperfluorescent "hot" disc may be the only fluorescein angiographic sign indicating inflammatory activity, whereas ICGA can show subclinical choroidal granuloma.

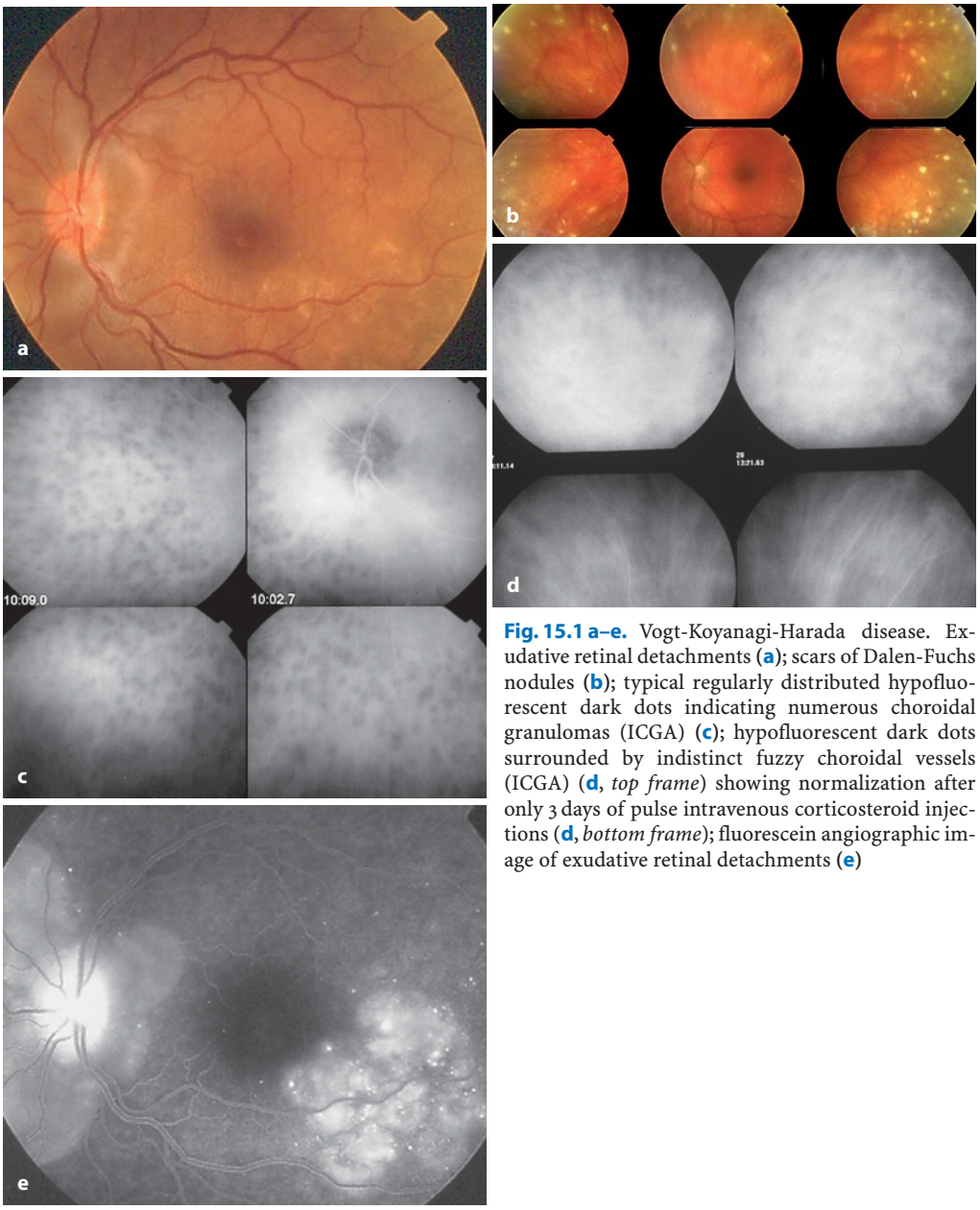

Fig. 15.1 a–e. Vogt-Koyanagi-Harada disease. Exudative retinal detachments (**a**); scars of Dalen-Fuchs nodules (**b**); typical regularly distributed hypofluorescent dark dots indicating numerous choroidal granulomas (ICGA) (**c**); hypofluorescent dark dots surrounded by indistinct fuzzy choroidal vessels (ICGA) (**d**, *top frame*) showing normalization after only 3 days of pulse intravenous corticosteroid injections (**d**, *bottom frame*); fluorescein angiographic image of exudative retinal detachments (**e**)

15.2.1.1.4 Additional Diagnostic Modalities and Differential Diagnosis (Table 15.1)

Echography may be of interest in the work-up of VKH when the media are opacified or pupillary dilatation is difficult. It can show thickening of the posterior choroid and serous retinal detachments. Echography is, however, not sufficient to detect subtle intrachoroidal inflammatory lesions. In order to standardize the appraisal of VKH cases new diagnostic criteria have been worked out to better determine the stage and severity of the disease internationally [14, 15]. These criteria are, however, still inadequate because cerebrospinal pleiocytosis is not explicitly required in the absence of other neurologic symptoms or signs. In non-endemic areas, lumbar puncture remains essential for diagnosis. Without it the diagnosis of VKH could not have been done in over 80 % of our cases. On the other hand, ICGA is not mentioned at all as a means of identifying subclinical choroidal inflammation. ICGA would contribute to reducing the frequency of apparently unilateral VKH by detecting subclinical involvement in the fellow eye and so avoiding the cases that do not fulfil the diagnostic criteria only because the investigational tools with proper sensitivity are not used.

15.2.1.1.5 Treatment

Systemic high-dose corticosteroids are the mainstay of VKH treatment [17, 18]. The introduction of therapy should be prompt and aggressive to shorten the duration of the disease and possibly avoid the progression into a chronic stage [19]. Intravenous pulse methylprednisolone injections should be given for 3 days in acute initial or recurrent disease to be followed by high-dose (1.5–2 mg/kg prednisone) oral therapy. Slow tapering over 9–12 months is necessary. Additional immunosuppressants can be discussed if clinical or ICGA recurrence predicts a high response threshold to corticosteroid therapy. In chronically evolving disease, it was shown that despite apparent clinical control the disease was slowly progressing towards sunset glow fundus [20]. This is due to smouldering subclinical disease that is best avoided by performing ICGA follow-up, the only means of monitoring subclinical evolution [21].

Summary for the Clinician

Vogt-Koyanagi-Harada Disease

- **Bilateral granulomatous uveitis with exudative retinal detachment associated with cutaneous and neurological signs**
- **Autoimmune process against a melanocyte associated antigen = primary target of inflammation**
- **Fundus findings: serous exudative detachment, papillitis in acute phase; recurrences can lead to convalescent phase characterized by diffuse depigmentation (sunset glow fundus), peripheral yellow-white chorioretinal atrophic lesions and diffuse retinal pigment epithelium alterations**
- **ICGA: *at onset:* fuzzy and indistinct choroidal vessels, disseminated, even, regularly distributed hypofluorescent dark dots marking presence of choroidal granulomas, disc hyperfluorescence in severe cases, exudative detachments appearing as hyperfluorescent pinpoints. *Recurrent disease* shows similar features to acute disease**
- **FA: *at onset:* multiple hyperfluorescent pinpoints and dye pooling in the late phase due to exudative detachment; disc hyperfluorescence. *Chronic stage:* window and blocking effects caused by diffuse retinal pigment epithelium alterations**
- **Systemic high-dose corticosteroids started promptly with slow tapering and close ICGA monitoring control the disease and can sometimes prevent the progression to chronicity**

15.2.1.2 Sympathetic Ophthalmia

Sympathetic ophthalmia is a bilateral granulomatous uveitis of unknown origin occurring following a penetrating eye injury. The interval between ocular injury and the clinical manifestations may vary widely, from a few days to several decades, but 90 % of cases occur within 1 year after the injury [22]. The aetiology is not

Table 15.1. Diagnostic criteria for Vogt-Koyanagi-Harada disease [14]

Complete Vogt-Koyanagi-Harada disease (criteria 1–5 must be present)

1. No history of trauma or surgery
2. No clinical or laboratory evidence of other ocular disease entities
3. Bilateral ocular involvement
   (a or b must be met, depending on the stage of disease when the patient is examined)
   a. Early manifestations of disease
      (1) There must be evidence of a diffuse choroiditis
      (with or without anterior uveitis, vitreous inflammatory reaction,
      or optic disc hyperhemia), which may manifest as one of the following:
         (a) Focal areas of subretinal fluid, or
         (b) Bullous serous retinal detachments
      (2) With equivocal fundus findings; both of the following must be present as well:
         (a) Focal areas of delay in choroidal perfusion, multifocal areas of pinpoint leakage,
         large placoid areas of hypofluorescence, pooling within subretinal fluid,
         and optic nerve staining (listed in order of sequential appearance)
         by fluorescein angiography, and
         (b) Diffuse choroidal thickening without evidence of posterior scleritis by ultrasonography
   b. Late manifestations of disease:
      (1) History suggestive of prior presence of findings from 3a and either both (2) and below,
      or multiple signs from (3):
         (a) Nummular chorioretinal depigmented scars, or
         (b) Retinal pigment epithelial clumping/or migration, or
         (c) Recurrent or chronic anterior uveitis
4. Neurological/auditory findings (may have resolved by time of examination)
   a. Meningismus (malaise, fever, headache, nausea, abdominal pain, stiffness of the neck and back,
   or a combination of these factors: headache alone is not sufficient to meet definition
   of meningismus, however), or
   b. Tinnitus, or
   c. Cerebrospinal fluid pleocytosis
5. Integumentary finding (not preceding onset of central nervous system or ocular disease)
   a. Alopecia, or
   b. Poliosis, or
   c. Vitiligo

Incomplete Vogt-Koyanagi-Harada disease (criteria 1–3 and either 4 or 5 must be present)

1. No history of penetrating ocular trauma or surgery
2. No clinical or laboratory evidence suggestive of other ocular disease entities, and
3. Bilateral ocular involvement
4. Neurological/auditory findings: as defined for complete Vogt-Koyanagi-Harada disease above, or
5. Integumentary finding: as defined for complete Vogt-Koyanagi-Harada disease above

Probable Vogt-Koyanagi-Harada disease (isolated ocular disease: criteria 1–3 must be present)

1. No history of penetrating ocular trauma or surgery
2. No clinical or laboratory evidence suggestive of other ocular disease entities
3. Bilateral ocular involvement as defined for complete Vogt-Koyanagi-Harada disease above

clear but an autosensitivity to an antigenic protein from the uvea or uveal pigment has been suggested. Interruption of the self-tolerance process is probably caused by a penetrating trauma permitting a release of antigen and its presentation to lymphocytes, leading to a T-cell-mediated immune response, eventually resulting in bilateral uveitis [23]. Clinical manifestations and histopathologic changes are similar to those of the Vogt-Koyanagi-Harada disease [24, 25].

15.2.1.2.1 Clinical Symptoms and Findings

The onset is usually insidious with blurring of vision, loss of accommodation, photophobia, and slight pain in both eyes. The exciting eye is usually chronically inflamed, often phthisical. There is a bilateral granulomatous panuveitis with mutton-fat keratic precipitates, posterior synechiae, a thickened iris, vitreous cells, choroidal infiltration and thickening, perivasculitis, papillitis and serous detachments if severe inflammation is present. Dalen-Fuchs nodules are classically described in sympathetic ophthalmia and consist of small yellowish spots, corresponding histopathologically to foci of epithelioid cells containing pigment located between Bruch's membrane and the retinal pigment epithelium [25]. The natural course of the disease used to lead to a poor visual prognosis, with as many as 70 % of the eyes becoming blind in some reports [25]. Prevention of sympathetic ophthalmia consists of enucleation of the injured eye before involvement of the sympathizing eye, but this is only performed exceptionally. The benefit of enucleation more than 2 weeks after initiation of uveitis is doubtful if not counterproductive and should probably be avoided [22, 26].

15.2.1.2.2 Indocyanine Green Angiography

Hypofluorescent dark dots are seen in the intermediate angiographic phase with the usual two patterns of evolution; some become isofluorescent at the late phase of ICGA and resolving after long-term corticosteroid therapy and probably correspond to active choroidal partial thickness inflammatory space occupying lesions; others remain hypofluorescent until the late phase and probably correspond to full thickness granulomas [27] (Fig. 15.2).

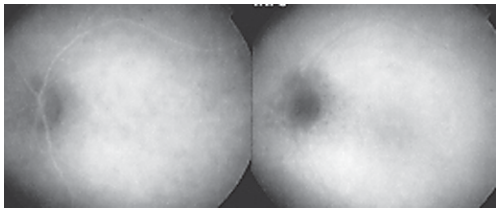

Fig. 15.2. ICGA signs in sympathetic ophthalmia. Numerous hypofluorescent dots in posterior pole and hypofluorescent rim around optic disc

15.2.1.2.3 Fluorescein Angiography

Fluorescein angiography shows multiple areas of leakage at the level of the retinal pigment epithelium and retinal serous detachments in the acute phase as well as disc hyperfluorescence.

15.2.1.2.4 Treatment

Early and systemic corticosteroid therapy with slow tapering over at least 6–9 months can lead to a good visual outcome [25, 26]. Some patients may require other immunosuppressive agents such as azathioprine, mycophenolate mofetyl, cyclosporine or others and with such a two-step approach a good prognosis can be anticipated [25].

Summary for the Clinician

Sympathetic Ophthalmia

- **Bilateral granulomatous uveitis occurring after a penetrating ocular injury. Unknown aetiology but similar process to VKH suspected**
- **Clinical and histopathological manifestations identical to Vogt-Koyanagi-Harada disease**
- **Fundus findings: multifocal choroidal infiltrates, serous exudative detachment, papillitis, Dalen-Fuchs nodules; recurrences can**

lead to convalescent phase characterized by sunset glow fundus, peripheral yellow-white chorioretinal scars of Dalen-Fuchs nodules and diffuse retinal pigment epithelium alterations

- **ICGA: hypofluorescent dark dots indicating stromal granulomas which can resolve after corticosteroid therapy**
- **FA: *acute phase:* multifocal hyperfluorescent pinpoints and retinal serous detachments, disc hyperfluorescence**
- **Treatment: systemic high-dose corticosteroids with slow tapering. Immunosuppressants may be needed. Enucleation has controversial benefits and tends to be avoided nowadays**

15.2.1.3 Birdshot Chorioretinopathy

Birdshot chorioretinopathy (BC) is an intraocular inflammatory condition linked to the presence of the HLA-A29 tissue histocompatibility antigen but without any known systemic association that was first described in 1980 by Ryan and Maumenee [28]. A year later Gass published a series of 11 patients and called it vitiliginous chorioretinitis [29]. The characteristic of BC is that both the choroid and the retina are independent primary targets and sites of an inflammatory reaction, in contrast to most uveitides, where inflammation originates in one structure and causes secondary inflammation in the surrounding structures [30, 31]. A recent histopathological report has shown that in the choroid, as for Vogt-Koyanaga-Harada disease and sympathetic ophthalmia, it is the choroidal stroma that is the primary target of BC while the choriocapillaris and the pigment epithelium are intact and it should therefore not be classified or assimilated with the PICCPs [4]. In contrast to a disease like sarcoidosis that affects the choroid at random, the choroidal stroma is the obligatory primary site of inflammation in birdshot chorioretinopathy. However, the functional deleterious impact comes from the retinal inflammatory involvement [32].

15.2.1.3.1 Clinical Symptoms and Findings

The patients' presenting complaints are usually floaters, decreased visual acuity and sometimes dimness of vision. In the absence of macular edema, however, central vision remains excellent in most cases and is not a good functional parameter for monitoring disease activity, whereas visual field changes more appropriately identify activity of the disease [32, 33].

Involvement is always bilateral but can be asymmetric and BC occurs predominantly in women in their 4th–6th decades. Human leucocyte tissue antigen HLA-A29 is present in nearly all cases and its absence should cast serious doubts on the correctness of diagnosis. Subtyping showed that it is the HLA-A29.2 subtype that is found in BC patients [34].

Anterior chamber inflammation is absent to slight at most. Vitreous infiltration is usually present and can be prominent in some cases. At the onset of disease, *fundus examination* shows papillitis, vasculitis of veins but very few cream-coloured lesions so typical for BC. However, subclinical choroidal involvement is clearly shown by ICGA that reveals numerous hypofluorescent dark dots indicating choroidal stromal infiltration (Fig. 15.3a). The extent of retinal involvement is shown by FA, which, in addition to vasculitis of large vessels, reveals massive capillary exudation to the point that there is not enough fluorescein to mark the large veins [35] (Fig. 15.3b). In our experience, cystoid macular edema is less frequent than reported in the past and occurs in up to 40 % of cases at any time of the evolution of the disease. For ongoing disease the fundus examination reveals progressively more cream-coloured oval fundus lesions despite corticosteroid/immunosuppressive therapy, which goes in parallel with regression of the ICGA dark dots under therapy (Fig. 15.3c). It is supposed that this corresponds to the resolution of the granuloma without stromal scarring, but bearing with it the depletion of melanocytes corresponding to the cream-coloured lesions. Therapy usually allows the stabilization of the disease, but in some cases the process can evolve towards a pseudoretinitis pigmentosa when extensive damage is caused to the retina (Fig. 15.3d).

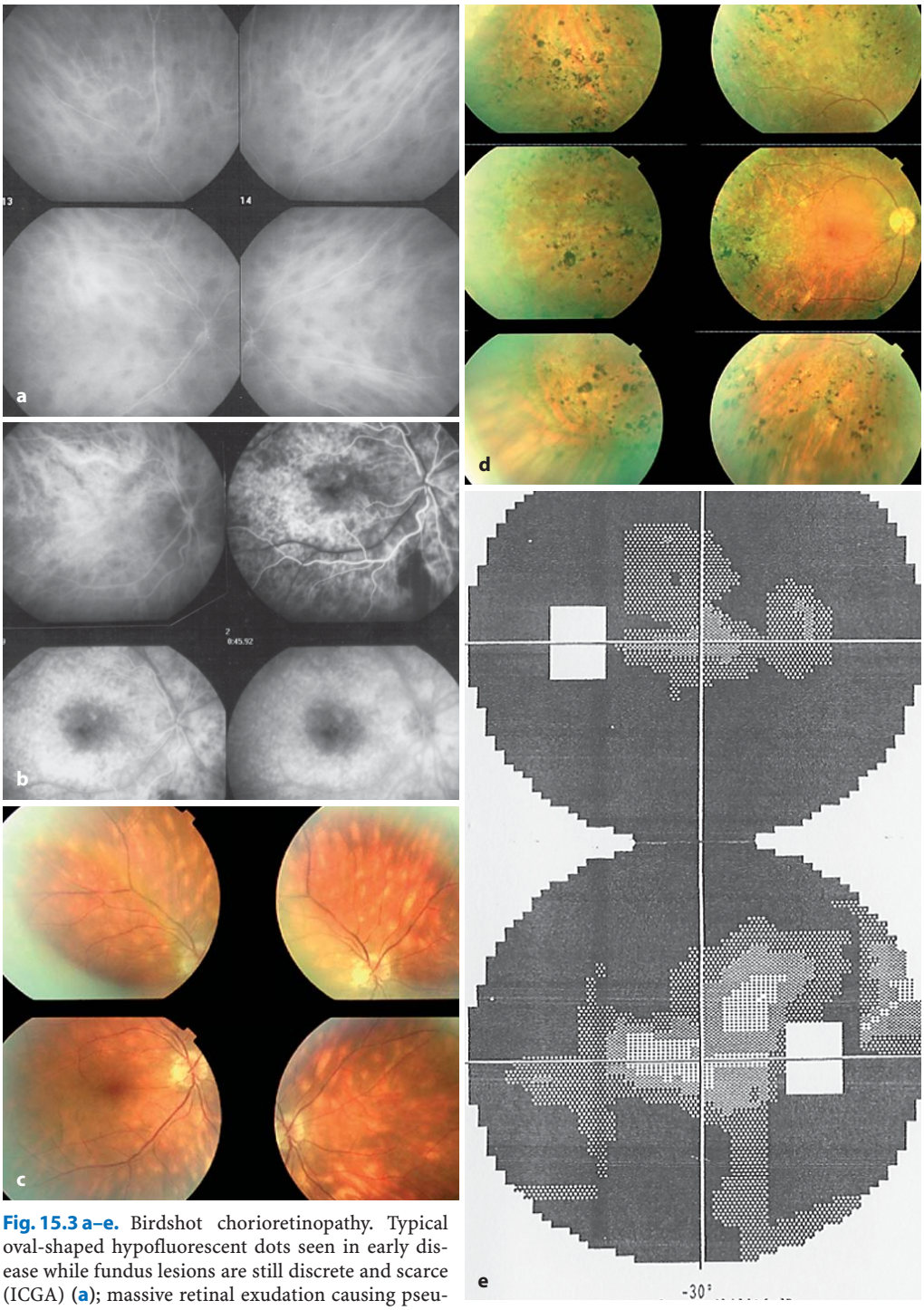

Fig. 15.3 a–e. Birdshot chorioretinopathy. Typical oval-shaped hypofluorescent dots seen in early disease while fundus lesions are still discrete and scarce (ICGA) (**a**); massive retinal exudation causing pseudo-delay of arteriovenous circulation as large veins are still not opacified at 45 s (**b**, *bottom right frame*). The ICG frame (**b**, *top left*) shows that arteriovenous transit is already complete at 19 s; typical oval-shaped cream-coloured lesions of late disease (**c**); pseudo-retinitis pigmentosa end stage disease (**d**); severe visual field loss of a birdshot patient with 1.5 vision at presentation (**e**)

15.2.1.3.2 Indocyanine Green Angiography

So far only the retinal involvement has been accessible to clinical and angiographic examination and analysed in detail. Since ICGA has been available, choroidal inflammatory involvement has been accessible to analysis and monitoring [36, 37]. ICGA represents a major improvement in the investigation of BC by showing the importance of choroidal disease [30]. In newly diagnosed disease, ICGA is very useful as it detects subclinical choroidal stromal disease, showing numerous dark dots, corresponding to stromal granuloma, that are present at the intermediate phase and persist or become isofluorescent in the late phase depending on the thickness of the granuloma (Fig. 15.3 a). In addition to visualization of the granuloma, ICGA also shows vasculitis of the larger choroidal vessels that have a fuzzy appearance in the intermediary phase of angiography and give rise to late diffuse choroidal hyperfluorescence [30].

15.2.1.3.3 Fluorescein Angiography

Fluorescein angiography accounts for the retinal involvement in BC. The main sign to search for is diffuse and massive leakage from small retinal vessels. The leakage is often such that there is not enough fluorescein to mark the large veins, which was erroneously interpreted as an arteriovenous circulatory delay [35] (Fig. 15.3 b). It is comprehensible that retinal function is impaired in cases with such a massive exudation from small retinal vessels. Additional FA signs include periphlebitis of larger retinal veins, disc hyperfluorescence and cystoid macular edema when present.

15.2.1.3.4 Visual Field Testing

We have recently shown that apart from central visual impairment due to macular edema, visual field changes are more frequently found than acknowledged in BC and have probably more deleterious effects on visual function than recognized so far [38]. These visual field changes probably are a consequence of retinal dysfunction rather than from choroidal or optic nerve disease and are seen in parallel to massive fluorescein exudation [35] (Fig. 15.3 e). In our hands computerized visual field testing is a routine follow-up examination and significant visual field changes are considered an indication to introduce therapy, despite full visual acuity. This attitude may explain why the rate of cystoid macular edema is relatively low in our centre [31].

15.2.1.3.5 Electroretinography and Pathophysiologic Implications

Full-field electroretinogram (ERG) becomes abnormal as disease progresses, indicating relentless retinal deterioration. The ERG shows a decrease of the rod a and b wave amplitudes with an increase in their implicit times [39]. Hirose and colleagues showed that in BC the neural layers of the retina were more diffusely and severely involved than the receptor-retinal pigment epithelium-choroid complex [40]. Similarly Priem and colleagues showed that the nature of the abnormalities suggested that dysfunction was caused by inner retinal disease. Moreover, in the first years of evolution, there has been little evidence indicating outer retinal dysfunction resulting from choroidal inflammation [41].

These reports and findings are in contrast to ERG findings reported in PICCPs, where outer retinal dysfunction is found and therefore distinguishes BC from PICCPs also electrophysiologically.

15.2.1.3.6 Treatment and Prognosis

Management of BC is empirical as is the case for many of the rare diseases where controlled studies cannot be performed. Birdshot chorioretinopathy should be observed only as long as no functional impairment is detected. In the absence of cystoid macular edema, the first functional parameter to deteriorate is the visual field. If this deterioration is progressing, action should be taken and therapy can be started us-

ing sub-Tenon's corticosteroid injections with a usual good response on visual fields. The use of periocular steroids usually allows the use of systemic therapy to be retarded. In the case of progression, systemic therapy has to be tried beginning with corticosteroids and subsequent association of immunosuppressants. We have good experience of using azathioprine (2.5–3 mg/day) as the first choice immunosuppressant, adding or substituting it with cyclosporine if necessary. Immunoglobulins have shown some effect but are expensive and difficult to administer [42]. The use of anti-TNF-α drugs is presently under investigation. The ICGA dark dots respond well to therapy and resolve, leaving behind depigmented areas that appear as oval cream-coloured fundus lesions. However, the impact of therapy on retinal involvement is less satisfactory, explaining the progression of functional loss despite therapy [31].

Summary for the Clinician

Birdshot Chorioretinopathy

- **Dual primary independent inflammation of the retina and the choroid. Retinal pigment epithelium remains intact between the two inflamed sectors**
- **Retinal involvement includes vasculitis of large and small vessels, cystoid macular edema (in about half of cases) and associated papillitis**
- **Choroidal involvement is a primary stromal choroiditis consisting of small even granulomas regularly distributed in the midperiphery**
- **Fundus findings: retinal vasculitis; oval yellow choroidal lesions faint and scarce at onset (granulomas), numerous and well visible in chronic treated disease (choroidal depigmentation); at end stage retinitis pigmentosa like atrophy**
- **ICGA: *at onset:* numerous, even, regularly distributed oval hypofluorescent dark dots present in the intermediate phase mostly becoming isofluorescent in the late phase (partial thickness granulomas). *Chronic, treated stage:* resolution of ICG hypofluorescent dark dots: resolution of granulomas without scarring leaving depigmented areas**
- **FA: generalized vasculitis of large and small vessels with profuse exudation, disc hyperfluorescence, and cystoid macular edema when present**
- **Visual field is the most important follow-up parameter. ERG shows inner retinal involvement and later inner and outer retinal dysfunction**
- **Evolution: choroidal disease responds to therapy; retinal involvement is slowly progressive despite treatment. Treatment (empirical): combination of corticosteroids and immunosuppressants**

15.2.2 Stromal Choroiditis as a Random Involvement of a Systemic Disease

This group of diseases involves the choroid by mere chance as one of the possible sites of inflammation, the choroid being the innocent host of an inflammatory or infectious process but not an elective target.

15.2.2.1 Sarcoidosis

Sarcoidosis is a multisystemic granulomatous disease of unknown aetiology. Lung involvement occurs most frequently and skin and eye lesions are next in frequency. Distribution is worldwide and prevalence of the disease differs according to racial group, being 10–20 times more frequent among blacks than whites. Sarcoidosis is characterized by the presence of non-caseating granuloma in involved organs. Sarcoidosis may affect any ocular tissue:

- Lacrymal gland enlargement
- Conjunctival nodules
- Corneal sarcoidosis, keratoconjunctivitis sicca
- Scleritis
- Anterior uveitis: acute or chronic
- Retinal vasculitis: periphlebitis (periarteritis)
- Retinal or vitreous granulomata

- Multifocal choroiditis or solitary choroid granuloma
- Papillitis
- Optic nerve granuloma

Uveitis is the most common and most serious form of ocular involvement in sarcoidosis and posterior segment inflammation occurs in up to one-third of patients with ocular sarcoidosis [43]. No specific test is available for sarcoidosis diagnosis, except typical histology on biopsy. Non-specific tests can help to orient the diagnosis. Chest X-ray may show hilar or mediastinal lymphadenopathy or interstitial fibrosis and may also help to exclude tuberculosis. Serum angiotensin converting enzyme (ACE) is usually elevated in active sarcoidosis as is serum lysosyme. Tuberculin test anergy is present in 50 % of cases. A frequent but often ignored feature is polyclonal immunoglobulin activation that can mislead when high titres to infectious agents are found but can also be used as a diagnostic indication [44]. Gallium scintigraphy shows increased uptake in salivary or lacrymal glands and in the mediastinum and liver. Bronchoalveolar lavage typically shows a lymphocytosis with an elevated CD4/CD8 ratio.

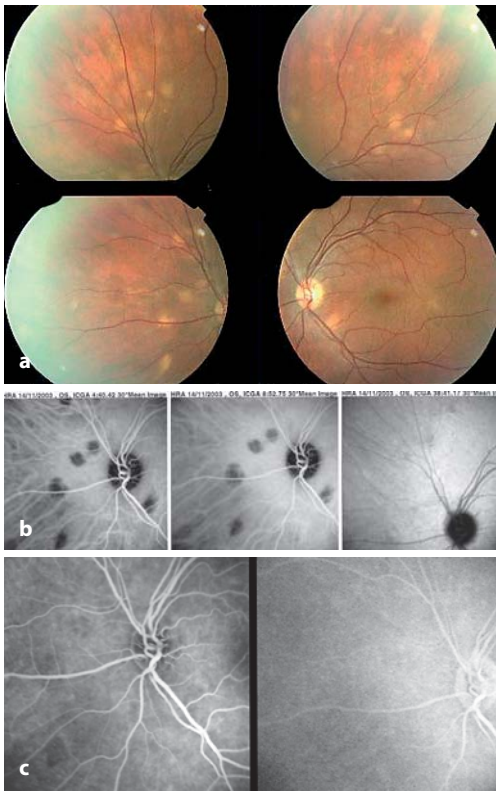

Fig. 15.4 a–c. Choroiditis in sarcoidosis. Unevenly sized randomly distributed fundus lesions (**a**) well seen in the intermediate angiographic phase (**b**, *middle and left frames*) and disappearing in the late phase (**b**, *right frame*), hardly visible on fluorescein angiography (**c**)

15.2.2.1.1 Symptoms and Clinical Findings

Symptoms are diverse and depend on the type of involvement. Patients can complain of blurred vision if cystoid macular edema or vitreitis is prominent or if significant anterior uveitis is present.

Anterior uveitis is often seen in association with choroiditis and retinitis. It is a granulomatous uveitis usually with large mutton-fat KPs, synechiae and iris nodules and infiltration.

Choroiditis and retinal inflammation can occur independently, both being possible random sites of involvement, or retinal involvement can be the consequence of underlying foci of choroiditis [45]. Retinal periphlebitis is the most common feature of posterior segment sarcoidosis, and vascular sheathing may persist in the characteristic "candle-wax" appearance. Multifocal choroiditis appears as multiple, pale-yellow, elevated lesions resembling those seen in birdshot chorioretinochoroidopathy; however, distribution is more at random and this is especially evident on ICGA (Fig. 15.4 a). A solitary choroidal granuloma is a rarer involvement of the choroid. Vitreous involvement is usually present as well as cystoid macular edema and papillitis.

15.2.2.1.2 Indocyanine Green Angiography

The signs seen on ICGA are not specific for sarcoidosis but can also be seen in other granulomatous involvements such as tuberculosis. Four principal features have been identified. The first and most common feature is hypofluorescent dark spots in the early and intermediate phases

of the angiogram. These spots either become isofluorescent or remain hypofluorescent in the late phase (Fig. 15.4b). The second feature is focal hyperfluorescent dots seen in the intermediate and late phases. The third feature is fuzzy choroidal vessels due to perivascular choroidal leakage in the intermediate phase. Finally, the last feature is a diffuse zonal hyperfluorescence representing choroidal staining in the late phase. The latter two features can resolve after systemic corticosteroid treatment [46].

15.2.2.1.3 Fluorescein Angiography

Fluorescein angiography is used to analyse the effects of inflammation at the level of the retina and retinal pigment epithelium. Depending on the type of involvement it can show disc hyperfluorescence, cystoid macular edema or retinal vasculitis as well as the combination of window effect and masking effect of chorioretinal atrophy of healed areas of granulomatous inflammation.

15.2.2.1.4 Therapy

Indication for therapy depends on severity of the lesions. Sarcoidosis, including posterior involvement, is usually well responsive to corticosteroids that can be given by periocular sub-Tenon's injections. If necessary, systemic steroids are usually efficient at controlling the disease and can be tapered over a period of 4–6 months.

In severe forms of disease or in the case of corticosteroid intolerance an immunosuppressive agent such as azathioprine can be added. Anti-TNF-α antibodies are presently being evaluated but will have to be reserved for severe cases.

Summary for the Clinician

Sarcoidosis

- **Multisystemic granulomatous disease of unknown aetiology**
- **May affect any ocular tissue but posterior segment inflammation in 30 % of the ocular involvement**
- **Fundus findings: choroiditis and retinal inflammation can occur together or separately. Retinal periphlebitis and characteristic vascular sheathing (candle-wax) are generally present. Cystoid macular edema and papillitis may be associated**
- **Investigations: chest X-ray or CT scan to detect hilar or mediastinal lymphadenopathy, skin test for anergy to tuberculin, serum angiotensin converting enzyme (ACE) and serum lysosyme elevation. Gallium scintigraphy to detect increased uptake signalling presence of granuloma**
- **ICGA: hypofluorescent dark dots present in the early and intermediate phases (granulomas). Focal hyperfluorescent pinpoints in the lesional areas in the late phase. Fuzzy choroidal vessels in the intermediate phase and diffuse hyperfluorescence due to choroidal staining in the late phase**
- **FA: may show retinal vasculitis, disc hyperfluorescence, and cystoid macular edema when present**
- **Treatment: corticosteroids. Indication and method of administration (sub-Tenon's injection, systemic) depend on severity of the lesions. Immunosuppressive agents may have to be added**

15.2.2.2 Tuberculous Choroiditis

After a gradual decrease of the incidence of tuberculosis since the 1950s, morbidity due to this infectious agent has increased again in the past decade [47]. Ocular involvement can occur without evidence of involvement of other organs [48, 49]. It is, however, difficult to isolate the infectious agent in ocular samples and in most cases we speak of presumed ocular tuberculosis [50]. As incidence was low in the recent past and tuberculosis is a reemerging disease, diagnosis is often performed with some delay [51].

Tuberculous uveitis has to be suspected when a granulomatous anterior uveitis or panuveitis or a chorioretinitis is associated with a hyperreactive delayed type hypersensitivity skin tuberculin test [52]. Tuberculous choroiditis is a chance localization of an entity that can affect

different organs. It can involve the choroid or the retina or both, or retinal inflammation can be secondary to choroidal granulomas [53, 52].

15.2.2.2.1 Symptoms and Clinical Findings

A large proportion of patients are treated for a recurrent granulomatous uveitis that flares up with a still relatively high steroid treatment level. In 25% of cases there may be an isolated chorioretinitis and in 75% chorioretinitis is associated with an anterior granulomatous uveitis. *On funduscopy,* in long-lasting cases there are multiple bilateral chorioretinal scars sometimes strongly pigmented (Fig. 15.5a) which may be associated with recent choroidal lesions presenting as yellow-grey deep discolorations, which are also found in new cases (Fig. 15.5b). The retina is usually also involved, showing vasculitis and infiltration, and the degree of involvement may be very different between the retina and the choroid. The disease is often bilateral and involvement is usually asymmetric and the distribution and size of lesions random and asymmetric. Since ICGA has become available, choroid involvement can be analysed precisely [55]. Choroiditis can occur alone or in association with retinal disease. So far it has only been possible to analyse retinal involvement or the consequences of choroidal disease on the retina. With ICGA subclinical choroidal lesions were detected in all patients analysed, showing that choroidal involvement is probably predominant [55].

15.2.2.2.2 Angiographic Characteristics

Dual fluorescein and ICG angiography should be performed to assess both retinal and choroidal disease. Fluorescein angiography (FA) is useful for detecting macular edema. It often shows disc hyperfluorescence with leakage, retinal vasculitis and choroidal lesions detected by the changes caused to the overlying retina, characterized by early hypofluorescence followed by progressive hyperfluorescence and leakage around the margins. It can also show secondary changes from choroidal involvement (Fig. 15.5c).

ICGA shows four main angiographic signs including: (1) hypofluorescent areas in the early and intermediate phases of angiography that remain hypofluorescent or become isofluorescent in the late angiographic phase representing full thickness or partial thickness lesions; they are of variable size and are randomly distributed (Fig. 15.5d); (2) choroidal fuzzy vessels that lose their sharp pattern indicating vasculitis of larger choroidal vessels that do not normally leak (Fig. 15.5d); (3) diffuse choroidal hyperfluorescence from leaking large choroidal vessels that adds to the physiologic background fluorescence from the choriocapillaris; and (4) hyperfluorescent pinpoints seen in the late angiographic phase (Fig. 15.5d). All lesions respond to specific antituberculous therapy combined with low doses of systemic corticosteroids for those patients that have no prediagnostic corticosteroids and/or immunosuppressants [55] (Fig. 15.5e).

15.2.2.2.3 Therapy and Prognosis

Specific antituberculous tri- or quadritherapy given for a minimum of 6–9 months allows the corticosteroid and/or immunosuppressant therapy to be progressively diminished and in some patients to be stopped. Specific therapy is also useful as a therapeutic trial in doubtful cases. In a recent study we showed that immunosuppressive therapy could be stopped in most cases after specific therapy was introduced, recurrences dropped significantly, visual function increased, inflammation subsided and intraocular pressure reduced [51].

Summary for the Clinician

Tuberculous Choroiditis

- **Reemerging multisystemic granulomatous infectious disease**
- **Ocular involvement present in 1% of cases of pulmonary tuberculosis**
- **Fundus findings: chorioretinitis presenting as yellow-grey deep discolorations, retinal infiltration, vasculitis, vitritis**
- **ICGA: four main signs: (1) hypofluorescent dark dots or areas; (2) fuzzy choroidal**

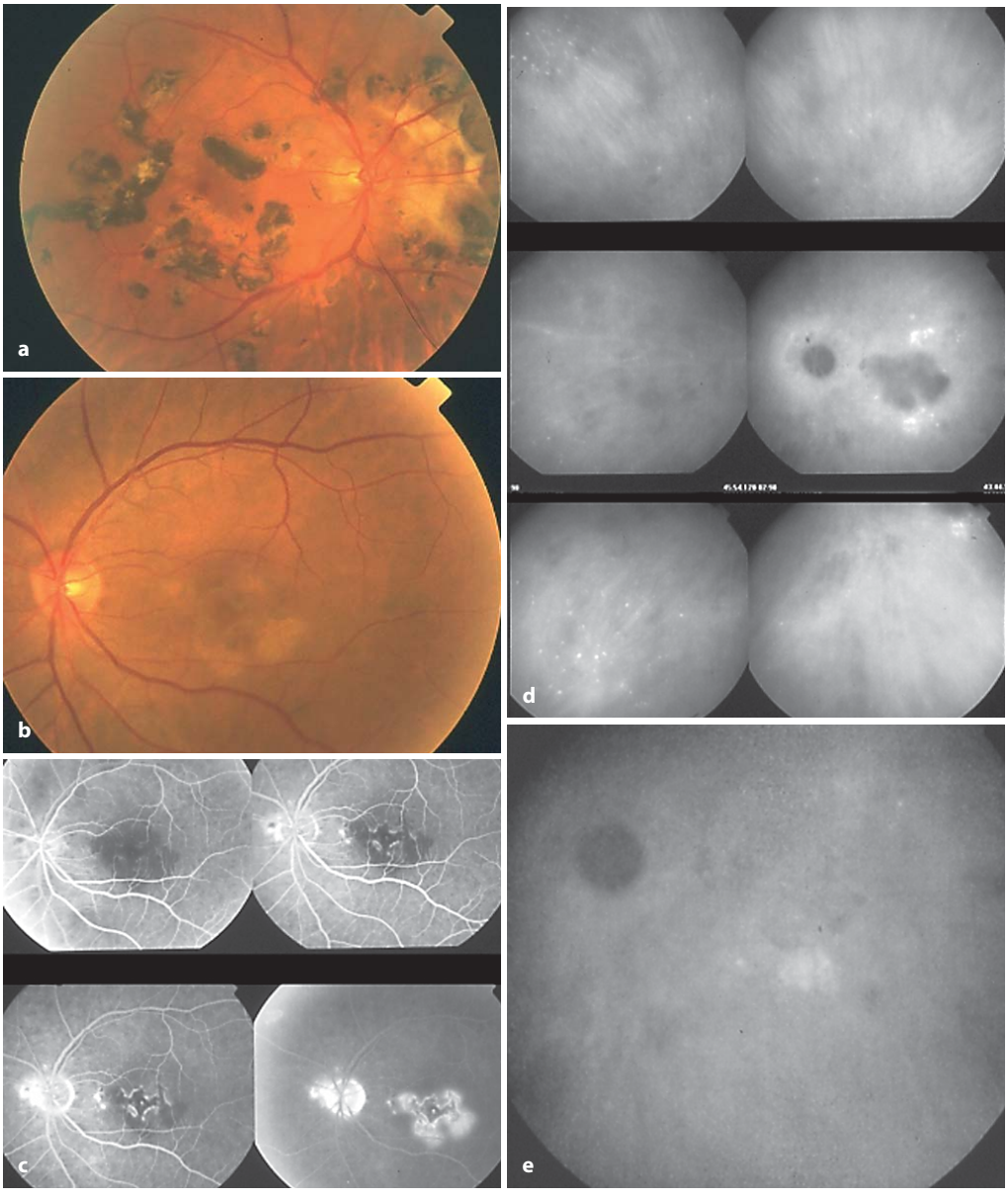

Fig. 15.5 a–e. Tuberculous choroiditis. Old lesions of previous episode in the right eye (**a**); new lesions in the macula of the left eye (**b**); FA showing central hyperfluorescent lesions with a background of choriocapillaris non-perfusion and late hyperfluorescence due to retinal leakage (**c**); ICGA in the acute stage shows secondary inflammatory choriocapillaropathy, hyperfluorescent pinpoints and diffuse randomly distributed hypofluorescent dots (**d**); after specific therapy the secondary inflammatory choriocapillaropathy has resolved, some residual hyperfluorescent pinpoints and a few hypofluorescent dots indicating scars (**e**)

vessels in intermediate phase; (3) diffuse choroidal hyperfluorescence; and (4) hyperfluorescent pinpoints in the late phase
- **FA: may show retinal vasculitis, disc hyperfluorescence, cystoid macular edema when present**
- **Treatment: specific antituberculous tri- or quadritherapy**

15.2.2.3 Syphilis

Syphilis is a chronic systemic infection sexually transmitted and caused by treponema pallidum. It is characterized by an incubation period averaging 3 weeks, followed by a primary lesion (chancre) associated with a regional lymphadenopathy. A secondary bacteraemic stage associated with generalized mucocutaneous lesions with a generalized lymphadenopathy then occurs followed by a latent period of subclinical infection lasting many years, and in about 30 % of untreated cases, a tertiary stage characterized by progressive destructive mucocutaneous, musculoskeletal, aortic, or central nervous system disease. Syphilis can also be transmitted transplacentally to the foetus, giving rise to congenital syphilis. Patients at risk for HIV are also at increased risk for other sexually transmitted diseases such as syphilis. Concomitant HIV infection may worsen the natural course of syphilis with unusual manifestations [56]. Ocular involvement can occur at any stage of syphilis and is estimated to be the cause of 1% of uveitides and optic neuritis [57]. Syphilis can mimic many different ocular disorders and should be suspected in any case of intraocular inflammation resistant to conventional therapy [58].

Symptoms of syphilitic uveitis include blurred vision, redness, photophobia, and floaters and cannot be clinically differentiated from other causes of uveitis. The course may be acute or chronic, involvement unilateral or bilateral. Posterior uveitis occurring during secondary syphilis can appear as multifocal or unifocal choroiditis, most often affecting the posterior pole and juxtapapillary area. Healed lesions appear as areas of chorioretinal atrophy associated with hyperpigmentation. Fluorescein angiography typically shows early diffuse or leopard-spot hypofluorescence of the involved area with late staining at the level of the retinal pigment epithelium. Ocular manifestations associated with syphilis can be summarized as follows:
- **Eyelid chancre, madarosis**
- **Conjunctivitis, conjunctival nodules**
- **Stromal keratitis**
- **Episcleritis, scleritis**
- **Iridocyclitis granulomatous or not**
- **Choroiditis, retinal vasculitis**
- **Optic neuritis, neuroretinitis**

Two types of serologic tests are available for syphilis diagnosis: non-treponemal tests such as the VDRL (Venereal Disease Research Laboratory) test, and treponemal tests such as the FTA-ABS (fluorescent treponemal antibody absorption) and TPHA (treponema pallidum haemagglutination) tests. The VDRL test is a non-specific reagin test useful in screening for active disease; it becomes positive after the development of the primary chancre and negative after adequate treatment. The FTA-ABS test is a specific test to detect anti-treponemal antibodies, allowing confirmation of previous or current infection; it remains positive even after treatment. The development of polymerase chain reaction (PCR) amplification for treponema pallidum will help to detect its presence in intraocular fluids [59]. Because central nervous system disease may occur during any stage of syphilis, cerebrospinal fluid (CSF) examination is recommended in any patient with syphilitic uveitis and can show leucocytosis, elevated protein levels and a reactive VDRL [59].

Penicillin remains the treatment of choice for syphillis. Patients with active syphilis and intraocular inflammation are treated as for neurosyphilis. Therapy for ocular syphilis consists of 10–14 days intravenous penicillin G, 18–24 MU daily [59].

Bejel or endemic syphilis or non-venereal syphilis is caused by treponema pallidum endemicum and occurs in East Africa, the Near East and Southeast Asia. The serologic tests for syphilis are also positive in bejel. Ocular involvement is comparable to syphilis and can include choroiditis, and treatment is similar to venereal ocular syphilis [60].

Summary for the Clinician

Syphilitic Choroiditis

- **Chronic systemic sexually transmitted infection due to treponema pallidum. Classically evolving in three stages**
- **Ocular involvement may occur at any stage**
- **Fundus findings: multifocal choroiditis in the posterior pole or juxtapapillary area. Healed lesions appear as chorioretinal atrophy associated with hyperpigmentation**
- **Investigations: serologic testing is mandatory in every non-resolved case of choroiditis. CSF analysis may be indicated to detect neurosyphilis. The development of PCR for treponema pallidum will help to detect its presence in ocular fluids and CSF. Therapy of choice is penicillin**

15.2.3 Other Infectious Choroiditides

Although immune mechanisms, possibly secondary to an infectious trigger, are suspected in a large proportion of choroiditis cases, direct infectious causes should always be kept in mind as a possibility as more and more emerging infectious agents are identified thanks to newly available technologies such as the polymerase chain reaction techniques. The infectious agents that can cause choroiditis are numerous. An exhaustive review of infectious choroiditis is beyond the scope of this practical review of choroiditis and only a few agents are given here (Table 15.2).

15.2.3.1 West Nile Virus Choroiditis

The West Nile virus (WNV) is one of the emerging infectious agents causing choroiditis and a good example of a pathology that may have been included in the "autoimmune" or "idiopathic" choroiditides because the infectious agent was not searched for. WNV is a single-stranded RNA flavivirus belonging to the Japanese encephalitis virus serocomplex that was first isolated in 1937 in the West Nile district of Uganda. It is transmitted by a mosquito vector, with wild birds serving as its reservoir and is distributed extensively throughout Africa, Asia, the Middle East, Europe, and North America. Incubation period ranges from 3 to 14 days. About 20 % of infected persons become symptomatic and present a flu-like illness developing into meningitis or encephalitis in 1 % of cases.

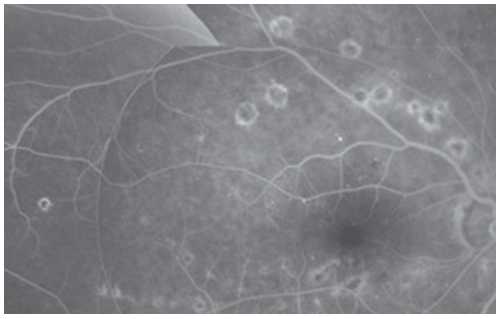

Fig. 15.6. West Nile virus choroiditis. Typical round pearl string scars shown by FA (courtesy M. Khairallah)

Almost 80 % of patients have posterior segment involvement, which is symptomatic in one-third of the patients. There can be a decrease of visual acuity, blurred vision and floaters. Posterior lesions consist of a typical multifocal bilateral choroiditis associated in all cases with a mild vitreous inflammatory reaction. The lesions, circular in shape, vary in number from less than 20 to more than 50 per eye. Chorioretinal lesions involve the midzone and/or periphery, more prominently in the temporal and the superonasal quadrants as well as the posterior pole. Lesion size ranges from 100 µm to 1,500 µm, with most of the lesions measuring 200–500 µm. Linear clustering of chorioretinal lesions gives the typical pearls on a string aspect of this choroiditis (Fig. 15.6). Active chorioretinal lesions appear as deep, creamy lesions on ophthalmoscopy, with early hypofluorescence and late staining on fluorescein angiography. Resolved chorioretinal lesions are partially atrophic and partially pigmented centrally with central hypofluorescence and peripheral hyperfluorescence (Fig. 15.5).

Associated retinal vascular changes include intraretinal haemorrhages, white-centered haemorrhages, focal vascular sheathing, and retinal

Table 15.2. Synoptic table of some infectious choroiditides

	Aetiology	Epidemiology	Systemic manifestations	Ocular manifestations	Diagnosis	Treatment
Toxocariasis [62, 63]	*Toxocara canis* (larva)	Child	Non-specific mild systemic flu-like illness	1. Retinal granuloma with radiating vitreous membrane 2. Chronic endophthalmitis	ELISA *Toxocara* test (vitreous) Eosinophilia	Systemic corticosteroid if active vitritis Vitrectomy
Choroidal pneumocystosis [64, 65]	*Pneumocystis carinii* (protozoan)	Defect in cell-mediated immune function (AIDS)	Can be early sign of disseminated *P. carinii* infection	Multiple yellow-white choroidal lesions scattered throughout posterior pole		Systemic trimethoprim-sulphamethoxazole or parenteral pentamidine
Coccidioido-mycosis [65, 66, 67]	*Coccidioides immitis* (dimorphic fungus)	Endemic in southwestern and far western USA	Acute self-limiting respiratory tract infection	Phlyctenular conjunctivitis Uveitis rare Focal chorioretinitis	Skin test Serology	Amphotericin B
Histoplasmosis [66, 67, 68, 69]	*Histoplasma capsulatum* (fungus)	Endemic in midwestern USA, central America, Asia, Italy, Turkey, Israel, Australia	Subclinical or mild flu-like reaction	Subretinal infiltrates with distinct borders, optic neuritis, uveitis. Endophthalmitis rare	Histoplasmin skin test (may reactivate ocular lesions)	Corticosteroids (maculopathy), laser photo coagulation (sub-retinal neovessels)
Candidiasis [68, 69]	*Candida albicans* (fungus)	Drug addict Compromised host (AIDS, malignancies, immunosuppres-sive therapy) Indwelling vascular catheter	Candidaemia	Creamy-white round, circumscribed chorioretinal lesions, vitreous inflammation	Culture from blood, urine, vitreous	Amphotericin B intravenous, intra-vitreal injection, fluconazole [69], vitrectomy

vascular leakage. The multifocal chorioretinitis usually has a self-limited favourable course without treatment and vision is conserved.

Unrecognized cases of West Nile virus choroiditis might be classified as multifocal choroiditis by clinicians not aware of this clinical entity. Thanks to the work of a Tunisian group that published the largest series so far on ocular involvement due to West Nile virus infection and performed a prospective study on the last zoonose in their country, the clinical picture has been well established [61].

References

1. Bouchenaki N, Cimino L, Auer C, Tran VT, Herbort CP (1985) Assessment and classification of choroidal vasculitis in posterior uveitis using indocyanine green angiography. Klin Monatsbl Augenheilkd 219:243–249
2. Chan CC, Ben Ezra D, Hsu SM, Palestine AG, Nussenblatt RB (1985) Granulomas in sympathetic ophthalmia and sarcoidosis. Arch Ophthalmol 103:198–202
3. Inomata H, Sakamoto T (1990) Immunohistochemical studies of Vogt-Koyanagi-Harada disease with sunset sky fundus. Curr Eye Res 95:35–40
4. Gaudio PA, Kaye DB, Brooks Crawford J (2002) Histopathology of birdshot retinochoroidopathy. Br J Ophthalmol 86:1439–144
5. Herbort CP, LeHoang P, Guex-Crosier Y (1998) Schematic interpretation of indocyanine green angiography in posterior uveitis using a standard angiographic protocol. Ophthalmology 105:432–440
6. Moorthy RS, Inimata H, Rao NA (1995) Vogt-Koyanagi-Harada Syndrome. Surv Ophthalmol 39: 265–292
7. Read RW, Rao NA, Cunningham ET (2000) Vogt-Koyanagi-Harada disease. Curr Opin Ophthalmol 11:437–442
8. Mimura Y (1980) Vogt-Koyanagi-Harada disease, vol 12. In: Uyama M (ed) Ganka Mook, Tokyo, pp 116–144
9. Murakami S, Inaba Y, Mochizuki M (1994) A nationwide survey on the occurrence of Vogt-Koyanagi-Harada disease in Japan. Jpn J Ophthalmol 38:208–213
10. Shindo Y, Ohno S, Yamamoto T, Nahamura S, Inoko H (1994) Complete association of the HLA-DRB1*04 and -DQB1*04 alleles with Vogt-Koyanagi-Harada's disease. Hum Immunol 39:169–176
11. Kim MH, Seong MC, Kwak NH, Yoo JS, Kim TG, Han H (2000) Association of HLA with Vogt-Koyanagi-Harada syndrome in Koreans. Am J Ophthalmol 129:173–177
12. Inomata H, Rao NA (2001) Depigmented atrophic lesions in sunset glow fundi of Vogt-Koyanagi-Harada disease. Am J Ophthalmol 131:607–614
13. Perry HD, Font RL (1977) Clinical and histopathologic observations in severe Vogt-Koyanagi-Harada syndrome. Am J Ophthalmol 83:242–254
14. Read RW, Holland GN, Rao NA, Tabbara KF, Ohno S, Arellanes-Garcia L, Pivetti-Pezzi P, Tessler HH, Usui M (2001) Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. Am J Ophthalmol 131:647–652
15. Read RW, Rao NA (2000) Utility of existing Vogt-Koyanagi-Harada syndrome diagnostic criteria at initial evaluation of the individual patient: a retrospective analysis. Ocular Immunol Inflamm 8:227–234
16. Bouchenaki N, Herbort CP (2001) The contribution of indocyanine green angiography to the appraisal and management of Vogt-Koyanagi-Harada disease. Ophthalmology 108:54–64
17. Rubsamen PE, Gass JD (1991) Vogt-Koyanagi-Harada syndrome. Arch Ophthalmol 109:682–687
18. Fujioka T, Fukuda M, Okinami S (1980) A statistic study of Vogt-Koyanagi-Harada syndrome. Acta Soc Ophthalmol Jpn 84:1979–1982
19. Bouchenaki N, Morisod L, Herbort CP (2000) Syndrome de Vogt-Koyanagi-Harada: importance de la rapidité du diagnostic et du traitement. Klin Monatsbl Augenheilkd 216:290–294
20. Sonoda S, Nakao K, Ohba N (1999) Extensive chorioretinal atrophy in Vogt-Koyanagi-Harada disease. Jpn J Ophthalmol 43:113–119
21. Bouchenaki N, Herbort CP (2000) Indocyanine green angiography (ICGA) in the assessment and follow-up of choroidal inflammation in active chronically evolving Vogt-Koyanagi-Harada disease. In: Dodds EM, Couto CA (eds) Uveitis in the third millenium. Elsevier, Amsterdam, pp 35–38
22. Lubin JR, Albert DM, Weinstein M (1980) Sixty-five years of sympathetic ophthalmia: a clinicopathological review of 105 cases (1913–1978). Ophthalmology 87:109–121
23. Rao NA, Robin J, Hartmann D (1983) The role of penetrating wound in the development of sympathetic ophthalmia: experimental observations. Arch Ophthalmol 101:102–104
24. Rao NA, Marak GE (1983) Sympathetic ophthalmia simulating Vogt-Koyanagi-Harada disease: a clinico-pathologic study of four cases. Jpn J Ophthalmol 27:506–511

25. Goto H, Rao NA (1990) Sympathetic ophthalmia and Vogt-Koyanagi-Harada syndrome. Int Ophthalmol Clin 30:279–285
26. Winter FC (1955) Sympathetic uveitis: a clinical and pathological study of the visual result. Am J Ophthalmol 39:340–347
27. Bernasconi O, Auer C, Zografos L, Herbort CP (1998) Indocyanine green findings in sympathetic ophthalmia. Graefes Arch Clin Exp Ophthalmol 236:350–353
28. Ryan SJ, Maumenee AE (1980) Birdshot retinochoroidopathy. Am J Ophthalmol 89:31–45
29. Gass JDM (1981) Vitiliginous chorioretinitis. Arch Ophthalmol 99:1778–1787
30. Fardeau C, Herbort CP, Kullman N, Quentel GG, LeHoang P (1999) Indocyanine green angiography in birdshot chorioretinopathy. Ophthalmology 106:1928–1934
31. Herbort CP, Probst K, Cimino L, Tran VT (2004) Differential inflammatory involvement in retina and choroid in birdshot chorioretinopathy. Klin Monatsbl Augenheilkd 221 (in press)
32. Oh KT, Christmas NJ, Folk JC (2002) Birdshot retinochoroiditis: long term follow-up of a chronically progressive disease. Am J Ophthalmol 133: 622–629
33. De Courten C, Herbort CP (1998) The potential role of computerized field testing for the appraisal and follow-up of birdshot chorioretinopathy. Arch Ophthalmol 116:1389–1391
34. LeHoang P, Ozdemir N, Benhamou A, et al. (1992) HLA-A29.2 subtype associated with birdshot retinochoroidopathy. Am J Ophthalmol 113:32–35
35. Guex-Crosier Y, Herbort CP (1997) Prolonged arterio-venous circulation time by fluorescein but not indocyanine green angiography in birdshot chorioretinopathy. Ocul Immunol Inflamm 5: 203–206
36. Herbort CP (1998) Posterior uveitis: new insights provided by indocyanine green angiography. Eye 12:757–759
37. Herbort CP, Bodaghi B, LeHoang P (2001) Angiographie au vert d'indocyanine au cours des maladies oculaires inflammatoires: principes, interprétation schématique, sémiologie et intérêt clinique. J Fr Ophthalmol 24:423–447
38. Cimino L, Tran VT, Herbort CP (2002) Importance of visual field testing in the functional evaluation and follow-up of birdshot chorioretinopathy. Ophthalmic Res 34 (S1):141
39. Fuerst DJ, Tesler HH, Fishman A, Yokoyama MM, Wyhinny GJ, Vygantas CM (1984) Birdshot retinochoroidopathy. Arch Ophthalmol 102:214–216
40. Hirose T, Katsumi O, Pruett RC, Sakaue H, Mehta M (1991) Retinal function in birdshot retinochoroidopathy. Acta Ophthalmol (Copenh) 69: 327–337
41. Priem H, De Rouck A, De Laey JJ, Bird AC (1988) Electrophysiologic studies in birdshot chorioretinopathy. Am J Ophthalmol 106:430–436
42. LeHoang P, Cassoux N, George F, Kullmann N, Kazatchkine MD (2000) Intravenous immunoglobulin (IVIg) for the treatment of birdshot retinochoroidopathy. Ocular Immunol Inflamm 8:49–57
43. Spalton DJ, Sanders MD (1981) Fundus changes in histologically-confirmed sarcoidosis. Br J Ophthalmol 65:348–352
44. Berthoud JF, Keller A, Herbort CP (1994) Polyclonal antibody rise in sarcoidoisis. Klin Monatsbl Augenheilkd 204:323–329
45. Herbort CP (2000) Precise monitoring and differentiation of inflammatory events by indocyanine green angiography in a case of recurrent posterior sarcoid uveitis. Ocular Immunol Inflamm 8:303–306
46. Wolfensberger TJ, Herbort CP (1999) Indocyanine green angiographic features in ocular sarcoidosis. Ophthalmology 106:285–289
47. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC (1999) Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country: WHO Global Surveillance and Monitoring Project. JAMA 282:677–686
48. Sarvananthan N, Wiselka M, Bibby K (1998) Intraocular tuberculosis without detectable systemic infection. Arch Ophthalmol 116:1386–1388
49. Bodaghi B, LeHoang P (2000) Ocular tuberculosis. Curr Opin Ophthalmol 11:443–448
50. Gupta V, Arora S, Gupta A, et al. (1998) Management of presumed intraocular tuberculosis: possible role of the polymerase chain reaction. Acta Ophthalmol Scand 76:679–682
51. Cimino L, Tran VT, Auer C, Herbort CP (2001) Presumed tuberculous uveitis: characteristic signs and evolution. Ophthalmic Res 33(S1):170
52. Morimura Y, Okada A, Kawahara S, et al. (2002) Tuberculin skin testing in uveitis patients and treatment of presumed intraocular tuberculosis in Japan. Ophthalmology 109:851–857
53. Saini JS, Mukherjee AK, Nadkarni N (1986) Primary tuberculosis of the retina. Br J Ophthalmol 70:533–535
54. Mansour AM, Haymond R (1990) Choroidal tuberculomas without evidence of extraocular tuberculosis. Graefes Archiv Clin Exp Ophthalmol 228:382–385

55. Wolfensberger TJ, Piguet B, Herbort CP (1999) Indocyanine green angiographic features in tuberculous chorioretinitis. Am J Ophthalmol 127:350–353
56. Kuo IC, Kapusta MA, Rao NA (1998) Vitritis as the primary manifestation of ocular syphilis in patients with HIV infection. Am J Ophthalmol 125:306–311
57. Deschenes J, Seamone C, Baines M (1992) Acquired ocular syphilis: diagnosis and treatment. Ann Ophthalmol 24:134–138
58. Villanueva AV, Sahouri MJ, Omerod LD, Reyes MP (2000) Posterior uveitis in patients with positive serology for syphilis. Clin Infect Dis 30:479–485
59. Aldave AJ, King JA, Cunningham ET (2001) Ocular syphilis. Curr Opin Ophthalmol 12:433–441
60. Tabbara KF, Al Kaff AS, Fadel T (1989) Ocular manifestations of Bejel. Ophthalmology 96:1087–1091
61. Khairallah M, Ben Yahia S, Ladjimi A, Zeghidi H, Ben Romdhane F, Besbes L, Zaouali S, Riadh Messaoud R (2004) Chorioretinal involvement in patients with West Nile virus infection. Ophthalmology 111 (in press)
62. Tran VT, Lumbroso L, LeHoang P, Herbort CP (1999) Ultrasound biomicroscopy (UBM) in peripheral retinovitreal toxocariasis. Am J Ophthalmol 127:607–609
63. Yokoi K, Goto H, Sakai J, Usui M (2003) Clinical features of ocular toxocariasis in Japan. Ocul Immunol Inflamm 11:269–275
64. Shami MJ, Freeman W, Friedberg D, Sideres E, Listhaus A, Ai E (1991) A multicenter study of pneumocystis choroidopathy. Am J Ophthalmol 112:15–22
65. Rodenbiker HT, Ganley JP (1980) Ocular coccidiomycosis. Surv Ophthalmol 24:263–290
66. McMillan TA, Lashkari K (1996) Ocular histoplasmosis. Int Ophthalmol Clin 36:179–186
67. Spencer WH, Chan CC, Shen de F, Rao NA (2003) Detection of histoplasma capsulatum DNA in lesions of chronic ocular histoplasmosis syndrome. Arch Ophthalmol 121:1551–1555
68. Donahue SP, Greven CM, Zuravleff JJ, Eller AW, Nguyen MH, Peaock JE, Wagener MW, Yu VL (1994) Intraocular candidiasis in patients with candidemia. Clinical implications derived from a prospective multicenter study. Ophthalmology 101:1302–1309
69. Guex-Crosier Y, Herbort CP (1993) Postpartum *Candida* endophthalmitis treated with fluconazole. Ophthalmologica 206:214–215

Immunomodulatory Therapy in Uveitis

16

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Core Messages

- Uveitis patients with severe sight threatening disease are conventionally treated with anti-inflammatory and immunosuppressive therapy
- Immunosuppressive therapies include antimetabolites, antibiotics and calcineurin inhibitors; they all have generalized effects on the organism and are burdened with various side effects
- "Biologicals" target the autoaggressive immune response more specifically, with reduced generalized, but in some cases severe, side effects
- Autoantigen-specific therapies are under investigation (oral tolerance induction), which are so far the most specific and least side effect-burdened approaches

16.1 Introduction

Since uveitis is a disease that potentially destroys intraocular tissues, immediate anti-inflammatory and causal treatment is needed in many cases. If uveitis is autoimmune mediated, immunosuppressive or immunomodulatory therapy is needed in those patients who do not sufficiently respond to corticosteroids. The use of corticosteroids is extensively discussed elsewhere in this issue.

Autoimmune uveitis is mediated by T-helper cells, presumably of the Th1 type, characterized by secretion of interleukin-2 (IL-2), interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α). These T cells recognize ocular autoantigens and undergo reactivation within the eye, which is followed by secretion of cytokines and chemokines attracting inflammatory cells such as macrophages and granulocytes. These inflammatory cells have the capacity to destroy the delicate structures of the eye and are primarily targeted by anti-inflammatory therapies.

The inflammation as well as relapses are orchestrated by T cells, which are an important target of immunosuppressive strategies. These therapies, however, also suppress necessary and desired immune responses to infections and tumours; therefore, more disease-specific therapies are preferred over generalized immunosuppression. Based on the increasing knowledge of the immune mechanisms that underlie autoimmune diseases, a new group of "biologicals" has been generated. These are immunologically active proteins focussing on specific cells, receptors or ligands. They target those immune responses that are involved in uveitis, either by blocking inflammatory cytokines (anti-TNF therapies such as etanercept, infliximab or adalimumab), by affecting T-helper cells (anti-IL2 receptor) or suppressing the autoantigen-specific immune response by induction of mucosal tolerance (oral tolerance).

In the following, new developments in immunosuppressive and immunomodulatory therapies will be described with respect to indication, immune mechanisms, effectiveness and side effects (Table 16.1, Fig. 16.1). It is important to keep in mind that the use of most of the therapies described in this chapter is off-label, with the exception of cyclosporine. Nevertheless, there is a growing body of literature supporting the use of these substances for the treatment of uveitis.

Table 11.1. Dose, route and major adverse events of specific drugs

Class	Drug	Dose and route	Major adverse events
Cytotoxic drugs	Azathioprine	1–3 mg/kg/day PO	Bone marrow suppression, leucopenia, secondary infection, nausea (especially in TPMT deficient patients)
	Methotrexate	0.15 mg/kg/once weekly PO, i.m.	Bone marrow suppression, leucopenia, hepatotoxicity, nausea, diarrhoea, ulcerative stomatitis, hair loss. Not indicated in patients with renal insufficiency
	Mycophenolate mofetil	1,000 mg, 2 times daily, PO	Bone marrow suppression, leucopenia, nephrotoxicity, secondary infections, gastritis, nausea, diarrhoea
	Cyclophosphamide	Long-term oral: 1–2 (–4) mg/kg/day PO; intravenous bolus: 15–20 mg/kg (or 1 g/m^2 body surface)	Haemorrhagic cystitis, myelosuppression, secondary malignancies, secondary infections, hair loss, transient blurring of vision, infertility, teratogenicity
	Chlorambucil	12–18 mg/day (0.1–0.2 mg/kg/day), PO	Myelosuppression, hepatotoxicity, gonadal dysfunction, secondary malignancies
Antibiotics	Sulphasalazine	100–2,000 mg/day, PO	Exanthema, nausea, vomiting, headache, oligospermia
Calcineurin inhibitors	Cyclosporine A	2.5–7 mg/kg/day, PO	Nephrotoxicity, hypertension, nausea, hypertrichosis, gingival hyperplasia, hepatotoxicity, paraesthesia, headache
	Tacrolimus	0.1–0.25 mg/kg/day, PO	Nephrotoxicity, hypertension, neurotoxicity, hepatotoxicity, diabetes mellitus
Immunomodulatory agents	Thalidomide	100–300 mg/day (paediatric: 5–10 mg/kg/day), PO	Teratogenicity, somnolence, dizziness, constipation, headache, weight gain, rash, dry mouth
	Etanercept	25 mg two times weekly, sc	Local reaction at the injection site, (severe) secondary infections, respiratory infections, pancytopenia
	Infliximab	3–5 mg/kg repeated after 2 and 6 weeks, then every 8 weeks, i.v.	Infusion reaction, (severe) secondary infections, respiratory infections
	Interferon-α2a	6 mio IU/day, decreasing to 2–3 mio IU three times weekly, sc	Flu-like symptoms, autoantibodies, thyroiditis, SLE, haemolytic anaemia
	Daclizumab	2 mg/kg, 2× in 2 weeks, followed by 1 mg/kg every 4 weeks, i.v.	Granulomatous dermatitis, GI tract irritation, neuropathies, infections

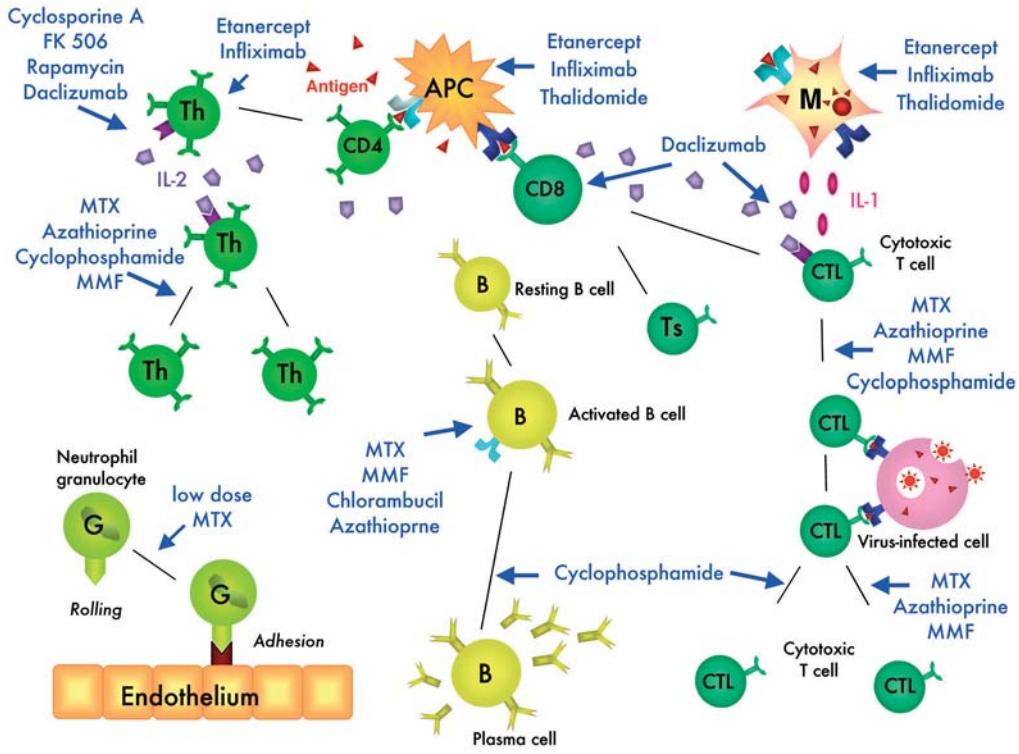

Fig. 16.1. Targets of immunosuppressive and immunomodulatory therapeutics. The network of antigen presenting cells (*APC*), macrophages (*M*), T-helper cells (*Th*), T-suppressor cells (*Ts*) and cytotoxic T lymphocytes (*CTL*) as well as B-cells (*B*) maturation and adhesion of granulocytes (*G*) to endothelial cells is shown. *Black lines* represent the track of the cells by maturation, proliferation or differentiation. MHC class II antigens on APC and B cells are shown in *light blue,* MHC class I antigens in *dark blue*. T cells bear T-cell receptors; B cells immunoglobulin receptors (antibodies) (*red circles* viruses, *red triangles* antigen peptides)

The drugs discussed here can be grouped according to their basic mode of action. Cytotoxic as well as some antibiotic agents have immunosuppressive effects, which warrant their use for the treatment of uveitis. Whereas cytotoxic drugs mainly interfere with DNA replication and transcription, antibiotics impede the cellular metabolism with respect to cell activation and protein synthesis.

16.2 Cytotoxic Drugs

Cytotoxic drugs like azathioprine and methotrexate have been used extensively for the treatment of autoimmune uveitis. Cyclophosphamide and chlorambucil have not gained such a broad acceptance due to the fear of severe adverse events, of which the most important ones are secondary malignancies. Nevertheless, there is an increasing body of literature reporting on their safe use.

16.2.1 Azathioprine

Azathioprine is a prodrug metabolized to its active form 6-mercaptopurine. It is converted to thioinosine-5-phosphate, a purine analogue. This is used instead of purine in the synthesis of the nucleobases adenine and guanine, thereby interfering with DNA synthesis. Since azathioprine inhibits cell division, its primary therapeutic targets are rapidly dividing cells like leucocytes. It inhibits T-cell and, to a lesser extent, B-cell functions. There is also an effect on the development of monocytes. Azathioprine is given orally at a single or divided dose of 2–3 mg/kg daily. Due to the inhibition of cell proliferation, the major side effects are a consequence of bone marrow suppression resulting in leucopenia and thrombocytopenia. Therefore blood cell counts are the most important aspect of monitoring therapy; leucocyte counts should be above 3,000 cells/µl and thrombocytes above 100,000 cells/µl. Especially patients with a defective thiopurine-methyl-transferase (TPMT) activity will develop signs of bone marrow suppression very early in the treatment. Nausea, vomiting and diarrhoea are signs of GI tract alterations, which should prompt the discontinuation of therapy. Concerns about induction of malignancies like leukaemia and lymphoma exist, since conflicting reports have prevented final evaluation of this question [67].

During the last few years, larger studies reporting on the effect of azathioprine in uveitis patients have not been published. It is used frequently as a steroid-sparing agent in patients with multifocal choroiditis with panuveitis, pars planitis and tubulointerstitial nephritis with uveitis [22, 31, 45]. The therapeutic potential of azathioprine in combination with other immunosuppressive agents is of interest, although the long-term use for treating severe uveitis as in Behçet's disease [33] or serpiginous choroiditis [1] is not always efficacious in controlling inflammation.

16.2.2 Methotrexate

Low-dose methotrexate is now widely used in the management of chronic or relapsing uveitis with the major goal of reducing concomitant steroid treatment. It blocks dihydrofolate reductase, preventing the conversion of dihydrofolate to tetrahydrofolate, an essential metabolite of purine nucleotide synthesis. In addition, thymidylate synthesis is also inhibited. Both factors contribute to the inhibition of DNA replication and cell division. Therefore methotrexate inhibits rapidly dividing cells like immune cells, but also mucosal cells of the GI tract and bone marrow cells. The immunosuppressive effect is mediated by inhibiting T and B cells, especially during antigen recognition. Low-dose methotrexate has a stronger effect on the immune response than on other tissues. This has led to low-dose, long-term treatment of inflammatory rheumatic and ocular diseases [65].

A low-dose regimen with usually 7.5–25 mg is given orally once a week. Depending on the therapeutic response, which may take 6–10 weeks to become obvious, the dose can be increased to 50 mg in single cases. In children, the dose may be lower. Gastrointestinal side effects can be reduced by i.m. injection.

Side effects include suppression of bone marrow with reduced cell counts in peripheral blood. A preventive measure is the administration of leucovorin (folinic acid), which bypasses some of the cytostatic effects of methotrexate on cell division, but also abrogates the therapeutic immunosuppressive effect. Other organs that may rarely be affected are the liver, on which methotrexate has a toxic effect resulting in cirrhosis, and the lung, developing pneumonitis and fibrosis. More often methotrexate causes alterations of the GI tract like nausea, mucositis and diarrhoea, which resolve after dose reduction or cessation of therapy. Methotrexate should not be given to patients with renal insufficiency.

Recently, Kaplan and colleagues reported a cohort of 39 patients, who were treated with methotrexate [34]. Full or partial control of in-

flammation was achieved in 23, and 10 patients finally were taken off methotrexate due to complete, long-lasting remission. Due to side effects, treatment was discontinued in ten patients. Another retrospective study of 160 patients found that inflammation was controlled in 76.2% of patients, while a steroid-sparing effect was obtained in 56% [62]. Side effects requiring discontinuation of medication occurred in 18% of patients, while 8.1% experienced potentially serious adverse reactions. In a series of 11 patients, Bom et al. found that the addition of methotrexate to other regimens, including corticosteroids, allowed control of the inflammation with a reduction of the corticosteroid dose in more than 50% of the cases and a decreased number of disease relapses in 45% [8]. In some patients with reactive arthritis and associated uveitis, systemic immunosuppression is indicated [37]. Here, like in paediatric patients with uveitis, low-dose methotrexate has steroid sparing effects, is required for the preservation of visual acuity and is generally well tolerated [69]. These studies indicate that methotrexate is safer than other cytotoxic drugs and is often well tolerated. It seems to have an important therapeutic role in various types of uveitis [71].

16.2.3 Mycophenolate Mofetil

Like methotrexate, mycophenolate mofetil (MMF) interferes with purine synthesis. Both drugs selectively and reversibly inhibit the enzyme inosine monophosphate dehydrogenase, which is important for guanosine and purine synthesis. With the exception of lymphocytes, most cells in the body can use salvage pathways to circumvent the inhibition of the de novo synthesis of purine. Since T and B cells depend exclusively on the de novo synthesis pathway, MTX and MMF impede cell growth and proliferation, resulting in a reduced activation status [36], decreased responses to antigenic stimulation and reduced antibody production.

The typical dose of MMF is 1,000 mg orally twice a day. Side effects include nausea and GI tract discomfort especially during the first weeks of therapy. This can be reduced if a low dose is given initially and increased to the final dose within 2–4 weeks. Leucopenia, renal and liver toxicity are rare, but appropriate monitoring should be installed.

Patients with all types of uveitis have been treated with MMF, either as a steroid-sparing agent or as a rescue therapy in otherwise non-responsive inflammation. Downmodulation of inflammation or prevention of relapses was reported in eight of ten patients [82]. In a recent study, control of ocular inflammation with MMF as monotherapy was achieved in 65% of patients, and a steroid-sparing effect in 54% of patients [5]. In both studies, MMF was considered safe. The concomitant application of MMF with cyclosporine promises additive effects, since both inhibit T cells, although the modes of action of the two drugs are different. Therefore, the combination therapy of MMF with other immunosuppressive regimens, predominantly with cyclosporine, led to a relief of symptoms and the ability to reduce the dose of prednisone in most patients. Ten of 11 patients showed a favourable response to MMF, with few side effects noted [41]. Another study supported this finding [25]. Here a marked resolution of ocular inflammatory activity occurred in 13 of 18 patients. Corticosteroids were discontinued in four patients and the steroid dose could be reduced in 14 patients following MMF therapy.

16.2.4 Leflunomide

Leflunomide is another cytotoxic agent that is widely used for the treatment of rheumatoid arthritis, but there are no reports on its use for ophthalmic inflammatory conditions.

16.2.5 Cyclophosphamide

Cyclophosphamide is a member of the nitrogen mustard agent family. Most of the orally ingested drug is resorbed (75%) and readily distributed throughout the body. In the liver cyclophosphamide is metabolized into the cytotoxic agents phosphoramide mustard and hydroxy-

cyclophosphamide [9]. These metabolites crosslink DNA, RNA and cellular proteins, thus ultimately leading to cytotoxicity. Although primarily secreted unaltered through the kidney, the metabolite acrolein is considered to be involved in the development of bladder toxicity [10]. Therefore mesna (sodium 2-mercaptoethane sulphonate) should be given to reduce the risk of haemorrhagic cystitis [9]. Cyclophosphamide inhibits T- and B-cell functions, resulting in reduced delayed type hypersensitivity (DTH) reaction as well as reduced antibody responses [72].

Treatment indication for cyclophosphamide is the inflammatory involvement of retina and choroid in Behçet's disease. Cyclophosphamide has been shown to be effective and to be superior to corticosteroids or chlorambucil [33 60].

Serpiginous choroiditis is a sight-threatening disease when choriodal lesions develop in or close to the macula. Treatment with steroids is effective in controlling active inflammation and may reduce lesion size, but there is evidence that steroids fail to prevent relapses [12]; moreover, even cyclosporine may not completely avoid recurrences (summarized in [2]). Therefore more aggressive immunosuppression with low-dose cyclophosphamide (50–200 mg/day) for up to 53 months has been employed successfully for the prevention of relapses [3]. Current recommendations limit the duration of therapy to 3–6 months due to the increasing risk of developing neoplasia and bone marrow toxicity. Even after cessation of treatment, some of these patients did not experience recurrences.

The recommended use of cyclophosphamide is the *oral treatment* with 1–2 mg/kg/day, which may be increased to 3–4 mg/kg/day for several weeks in selected, severe cases only. Dose must be adjusted to renal and ocular function with close monitoring of leucopenia. For long-term treatment the dose is adapted to the therapeutic response as well as the leucocyte count, which should be maintained above 3,500 cells/µl. The patients are ordered to take their dose in the morning and to drink 2–3 l of beverage daily to maintain frequent voiding. The *intravenous administration* is indicated in cases with severe inflammation, like patients with occlusive retinal vasculitis associated with Behçet's disease where a fast onset of therapeutic effect is important to preserve visual acuity. In addition, it reduces the exposure of acrolein to the bladder and induces only transient leucopenia. Every 3–4 weeks a bolus of 15–20 mg/kg (or 1 g/m^2 body surface) is given, with concomitant application of mesna to protect the bladder from toxicity.

16.2.6 Chlorambucil

Like cyclophosphamide, chlorambucil is an alkylating nitrogen mustard derivative. It interferes with DNA replication, transcription and RNA translation, resulting in cytotoxicity. Chlorambucil is readily resorbed after oral administration and metabolized in the liver to the active substance phenylacetic acid mustard. The major excretion route of this and other metabolites is through the kidney. Chlorambucil is used for the treatment of malignancies as well as autoimmune diseases with vasculitic complications [13]; its immunosuppressive effect is primarily mediated through inhibition of B cells.

The dose of chlorambucil is titrated according to the therapeutic response and the leucocyte count, which should be maintained above 3,000–3,500 cells/µl. The maximal dose should not exceed 12–18 mg/day (0.1–0.2 mg/kg daily). Haematologic toxicity is the most prominent side effect. Myelosuppression is dose dependent and may be profound at doses above 10 mg/day and, although reversible, may persist for months after discontinuation of chlorambucil. Haematologic and liver function tests should be performed regularly. Higher doses (10–30 mg/day) significantly increase the incidence of gonadal dysfunction like amenorrhoea, azoospermia, testicular atrophy and erectile dysfunction [23]. Malignancies seem to occur only at higher doses given for extended periods.

Chlorambucil is widely used for the treatment of Behçet's disease. Long-standing remissions can be induced and there is evidence that it is superior to low-dose cyclosporine (5–7 mg/kg/day) [48]. In addition to Behçet's disease, Goldstein et al. and Miserocchi et al. reported the use of chlorambucil for the treatment of a variety of other forms of therapy for refractive and

sight-threatening uveitis [23, 47]. The average treatment was 20 mg daily for 16 weeks or 8 mg daily for 12 months, respectively. Both case series describe long-term remissions even after cessation of chlorambucil therapy in patients who were resistant to other treatment modalities.

16.3 Antibiotics

16.3.1 Sulphasalazine

Sulphasalazine is a prodrug that is metabolized by commensal bacteria in the colon or in the liver into two products: 5-aminosalicylic acid (5-ASA) and sulphapyridine. There is some controversy as to which of these two products is responsible for the activity of sulphasalazine. Whereas it is known that 5-ASA is therapeutically effective, it is not clear whether sulphapyridine adds any further benefit. In the colon, the products created by the breakdown of sulphasalazine work as anti-inflammatory agents for treating inflammation of the colon. The beneficial effect of sulphasalazine is believed to be due to a local effect on the bowel, although there may also be a beneficial systemic immunosuppressive effect as well. Following oral administration, 33 % of the sulphasalazine, all of the sulphapyridine and about 33 % of the 5-ASA are resorbed.

The dose of sulphasalazine ranges from 1,000 to 2,000 mg/day, divided into two doses. Frequently occurring side effects include GI tract disturbances, nausea, vomiting, gastric distress and headache. Sulphasalazine can induce myelosuppression. Photosensitivity and allergic reactions may induce rash. Typically, sulphasalazine is indicated in patients with spondylarthropathies as a disease-modifying or steroid-sparing drug. For the treatment of uveitis, sulphasalazine is used as a preventive measure to maintain remissions of anterior uveitis. In a prospective 3-year follow-up of patients it was shown that sulphasalazine significantly reduced the number of relapses and the severity of anterior uveitis associated with ankylosing spondylitis [7]. Another retrospective study demonstrated that sulphasalazine treatment reduced the number of flares over a 1-year period in patients with recurrent acute anterior uveitis [49].

16.4 Calcineurin Inhibitors

The inhibition of calcineurin in the intracellular signalling pathway interferes with DNA transcription and thus leads to a decreased function of immune cells. The calcineurin inhibitors are potent immunosuppressive drugs acting predominantly through the inhibition of T-cell activation. While FK506 is 50–100 times more potent than cyclosporin A, it still shares its toxicity and many of the side effects. CsA binds cyclophilins, whereas FK506 and rapamycin bind so-called "FK506-binding proteins" (FKBPs). Cyclophilins and FKBPs are ubiquitous, abundant, and are active in multiple cellular compartments.

16.4.1 Cyclosporine A (CsA)

Cyclosporine inhibits T cells by interfering with intracellular signalling pathways. After antigen recognition of the T cell, the T-cell receptor/CD3 complex on the cell surface is activated and induces a cascade of intracellular signalling pathways, finally upregulating transcription of inflammatory cytokines like IL-2, -3, -4, IFN-ɣ and expression of IL-2 receptor [43]. Cyclosporine as well as tacrolimus inhibit this signalling and therefore inhibit activation of T cells after antigen recognition. Due to their mode of action these drugs act on the afferent, antigen-specific arm of the immune response and only indirectly on the efferent effector mechanisms, explaining the late onset of immunosuppression in autoimmune diseases after initiation of treatment. Recently it was shown for uveitis patients that cyclosporine is capable of downregulating a pathogenic Th1-type immune response with correlation to clinical disease activity [20]. Since the first description of the use of cyclosporine in uveitis patients, the doses have been reduced markedly in order to avoid side effects

[52]. The recommended dose is in the range of 2.5–7 mg/kg/day, which is divided into two daily doses. Side effects are numerous and dose dependent. The most frequent and worrisome are hypertension and nephrotoxicity [30]. Therefore, a close monitoring of blood pressure and kidney function is important. During cyclosporine treatment creatinine should not increase more than 30 % compared to pretreatment levels. Other frequent side effects include hypertrichosis, gingival hyperplasia, GI tract irritation, liver toxicity and headaches.

Although the use of cyclosporine was advocated in Behçet's disease with ocular involvement [6], recent investigations of the long-term effect draw the conclusion that it is not the ideal therapeutic agent, because it cannot completely eliminate ocular Behçet's disease. However, it is currently one of the most effective and efficient drugs for controlling uveitis and its complications until better treatment modalities are developed [55]. Cyclosporine is often used in combination with steroids, a regimen that is also effective in uveitis associated with tubulointerstitial nephritis [29]. In combination with other immunosuppressive agents, cyclosporine is very useful in suppressing disease while side effects can be minimized [33, 40].

16.4.2 Tacrolimus (FK506)

FK506 is a metabolic product of the fungus *Streptomyces tsukabaensis*. Calcineurin, the protein which function is inhibited by FK506, plays a role in the regulation of a wide variety of transcription factors. Through inhibiting the efficacy of calcineurin, FK506 not only suppresses the immune system but also causes the side effects that are linked to FK506's use. Both FK506 and rapamycin modulate IL-2 and GM-CSF gene expression at the transcriptional as well as the post-transcriptional level [28]. The oral dose of tacrolimus is in the range of 0.1–0.25 mg/kg/day, divided into two doses. Clinically relevant FK506 side effects are nephrotoxicity, neurotoxicity, diabetes mellitus and hypertension. Most of these side effects are more closely related to FK506 blood levels than dosage; therefore therapeutic drug monitoring (TDM) of FK506 levels is a prerequisite for therapy [57].

Tacrolimus has only been applied to a small group of uveitis patients. Uchio demonstrated that the therapeutic effect is correlated with higher levels of sICAM-1 in patients with Behçet's disease [78]. In another study, patients with sight-threatening uveitis refractory to cyclosporine were treated with tacrolimus. The posterior uveitis remained controlled in all patients while they were taking tacrolimus. Five of the six patients showed improvement in visual acuity while side effects were less troublesome compared to cyclosporine [68].

16.4.3 Sirolimus (Rapamycin)

Rapamycin is a peptide that was isolated from the bacteria strain *Streptomyces hygroscopicus*. Rapamycin is believed to block the immune response by causing programmed cell death (apoptosis) in T cells.

Side effects from the use in transplantation have been reported for rapamycin, such as raised lipid and cholesterol levels, hypertension, anaemia, diarrhoea, rash, thrombocytopenia, decreases in platelets and haemoglobin; however, these are generally less serious. Rapamycin effectively inhibits wound healing and should therefore not be applied after surgery. Positive effects on experimental animal models of autoimmune uveitis were described, but studies on the effect of sirolimus in uveitis patients have not yet been published.

16.5 Immunomodulatory Substances

16.5.1 Thalidomide

Thalidomide, or alpha-(*N*-phthalimido)-glutarimide, is a glutamic acid derivative. In the fifties and sixties of the last century, the drug was used for its sedative and antiemetic effects. Thalidomide was used by pregnant women to treat morning sickness and nausea, for it was

promoted as being non-toxic with no side effects [56]. Later, thalidomide became most notorious for its dramatic side effects, with teratogenicity as the most worrisome and serious one. The teratogenic effects include limb, ear and eye abnormalities and defects of internal organs. A single dose of thalidomide is believed to be teratogenic [77]. Another serious and potentially not reversible side effect, not even after withdrawal of the drug, is peripheral neuropathy causing symmetrical, painful paraesthesias of hands and feet accompanied by sensory loss.

The mechanism of action is still unclear. It is known that it downregulates TNF-α in conditions with increased TNF-α production [38]. TNF-α is secreted by macrophages and T-helper lymphocytes upon activation and is thus found in many inflammatory diseases such as uveitis. However, it is not a simple inhibitor of TNF-α: it can switch off the overproduction of TNF, but does not inhibit the basal level needed for normal cellular function. It also inhibits angiogenesis, which is essential for many physiologic and pathologic pathways. Other effects of thalidomide on the immune system include inhibition of leukocyte chemotaxis, decrease in density of adhesion molecules, inhibition of lymphocyte proliferative responses, change in the levels of different cytokines and reduction of phagocytosis, thus preventing the invasion of the eye by inflammatory cells [56].

Due to its severe side effects and potent teratogenicity, thalidomide treatment is restricted to severe cases only and is then used in higher doses than formerly as a sedative drug. The dose varies according to the indication for use, but usually 100–300 mg is given per day, divided into two doses. There were no larger trials with thalidomide performed for uveitis; only some reports on treatment of Behçet's disease patients. It has been used successfully with a complete response rate of 6–16% versus none of placebo treated patients [27]. Thalidomide has also been used in a small group of paediatric patients with doses ranging from 5 to 10 mg/kg [35]. With this treatment regimen adverse effects like somnolence, dizziness, constipation, headache, weight gain, rashes and a dry mouth are milder. Taking the drug at bedtime helps to minimize the likelihood of morning hangovers.

16.5.2 Anti-TNF-α Treatment

Tumour necrosis factor-α (TNF-α) is a proinflammatory cytokine, which is released primarily by activated monocytes and macrophages. It initiates the secretion of a cascade of other cytokines in many different cell types. Monocytes are activated to secrete IL-1 and -6, B cells produce antibodies and T cells IL-2, IFN-γ and other cytokines (Fig. 16.2). In endothelial cells TNF-α upregulates different cytokines, adhesion molecules and inducible nitric oxide synthetase (iNOS). TNF-α also has effects on many non-immune cells. In the brain it induces fever and sleep, and in osteoblasts, fibroblast and my-

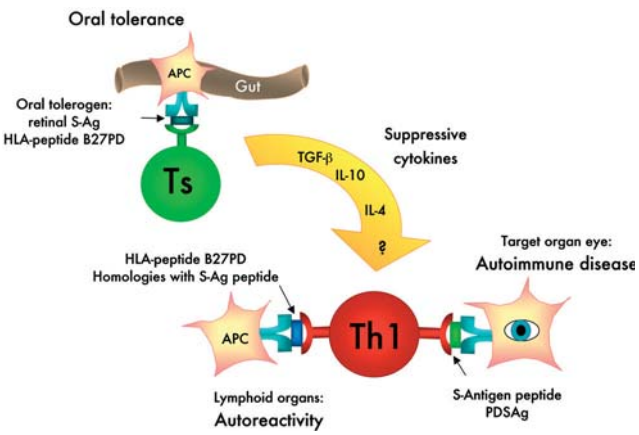

Fig. 16.2. Cytokine network. Interactions between T-helper cell (*Th*), B-cell (*B*) and macrophage (*M*) as a representative for antigen presenting or inflammatory cell, respectively. Effects on tissue cells are shown, such as upregulation of inducible nitric oxide synthetase (*iNOS*) and adhesion molecules by endothelia or enhanced production of tissue destructive proteases, all mediated by TNF-α. The *violet arrow* indicates the ability of T-helper cells to self-activate by IL-2 secreted by themselves binding to their own IL-2 receptor (*IL-2R*)

ocytes the production of proteases, which will lead to tissue destruction [15]. Besides the proinflammatory activity of TNF-α these latter effects are responsible for the destruction of bone and connective tissue in rheumatic diseases. In contrast, low concentrations of TNF-α have a neuroprotective function, inducing remyelination in the central and peripheral nervous system [4]. This might be the reason for the demyelinating disease with MS-like symptoms or optic neuritis described as an adverse event in some patients treated with anti-TNF-α [18, 66] .

Nevertheless, therapeutics with inhibitory activity on TNF-α have been successfully applied in rheumatic disorders; however, the reports about effects on intraocular inflammatory diseases are controversial.

During the past several years two substances have been used. Infliximab is a partially humanized monoclonal antibody neutralizing TNF-α. A new completely humanized monoclonal antibody is now available, but there are at present no reports about its effect in uveitis patients. Etanercept is a construct consisting of a TNF-α-receptor 2 and a human IgG heavy chain, which acts as a receptor agonist binding both TNF-α and TNF-β. A new fusion protein with a TNF receptor is currently under investigation for human use. Initial reports indicate a therapeutic effect in uveitis patients [24]. Signalling through TNF receptor 2 is necessary for the induction of anterior chamber associated immune deviation (ACAID), an important feature for maintaining the immune privilege of the eye. This was nicely demonstrated in a mouse model by Masli et al. [44] and might explain the occurrence of uveitis in patients treated with etanercept for ankylosing spondylitis [58].

A major concern with the use of anti-TNF-α therapies is the development of autoantibodies in some patients even leading to symptoms of systemic lupus erythematodes.

16.5.2.1 Etanercept

Etanercept binds TNF-α as well as TNF-β, preventing the interaction with the natural receptor on cell surfaces. Due to its long half-life of 98–300 h, 25 mg of etanercept is applied subcutaneously two times a week only. Side effects include a local reaction at the injection site, which usually does not require special care. Other unwanted effects are a consequence of its anti-inflammatory and immunosuppressive activity, which interferes with the host's defence against infections. These include respiratory infections, the reactivation of tuberculosis and sepsis, from which several of these patients have died. Since patients with rheumatoid diseases have an increased risk of infections due to their disease, close monitoring is mandatory.

In patients with chronic or relapsing uveitis, etanercept was used with the aim of preventing relapses after disease has been controlled by methotrexate [19]. With regard to the frequency of relapses and the final visual acuity, the authors did not find any significant difference between the treatment and placebo groups. Fortunately, no patient suffered from any irreversible long-term morbidity or mortality.

Reports about the efficacy of etanercept in children with treatment-resistant uveitis with or without underlying juvenile chronic arthritis are conflicting. Reiff et al. reported alleviation of uveitis in 10 of 16 eyes and prevention of relapses in most children. Even after long-term follow-up the authors noted a prolonged therapeutic effect [59]. On the other hand, Smith et al. reported the results of a small, double-blind and placebo-controlled trial of 12 children with paediatric uveitis treated with etanercept [70]. They did not find any therapeutic effect in either group, questioning the efficacy of etanercept in these patients.

16.5.2.2 Infliximab

The monoclonal antibody infliximab is injected intravenously. The usual dose is 3–5 mg/kg body weight; escalation to 10 mg/kg is used in single cases. Infusions are repeated after 2 and 6 weeks and then every 8 weeks. Side effects during infusion include dizziness and headaches. Allergic reactions to infliximab seem to be rare. Similar to etanercept infliximab induces immunosuppression and thus inhibition of defence from infections. Viral and respiratory infections as well as tuberculosis [42] occur frequently. Thus, be-

fore initiation of therapy tuberculosis has to be excluded by chest X-ray and tuberculin skin testing. In the case of positive results a prophylactic INH(isoniazid) treatment is mandatory. Recently, infliximab has been associated with higher incidences of mortality and hospitalization of patients with moderate to severe congestive heart failure [46]. Optic neuritis may develop [18], but it seems that infliximab may also exert a direct toxic effect [74].

Infliximab has been used for the treatment of many different uveitis entities. In most of these studies patients with chronic or relapsing uveitis were included who did not respond sufficiently to conventional therapy. Anterior uveitis associated with HLA-B27 seems to respond quickly to monotherapy with infliximab. Some of the patients experienced relapses after a median period of 5 months, which might reflect the natural course of the disease [16, 21]. Due to the severity of uveitis in Behçet's disease, several groups have used infliximab and published their results. A single infusion of infliximab rapidly induces a remission within 1–2 days and complete remission within 2 weeks [32, 64]. Retreatment of patients with relapses was successful, despite development of ocular and systemic tuberculosis in one patient, who responded to antituberculous treatment. The use of infliximab in children with Behçet's uveitis is limited, but shows positive effects [63].

Infliximab has been used more often than etanercept for the treatment of uveitis. Until now, there has been no study directly comparing these two drugs. Only one single report of a patient whose Behçet's disease did not respond to etanercept but subsequently to a single infusion with infliximab indicates that the monoclonal antibody might be superior to etanercept [17].

16.5.3 Interferon-α

Interferon-α and interferon-β (IFN-α, -β) are type 1 interferons, induced by viral infections and tumours or foreign cells. IFN- α subtypes are preferentially produced by monocytes/macrophages, but mainly by plasmacytoid dendritic cells (PDC) during viral infections, triggered by DNA with viral or bacterial CpG-motifs. IFN-α was thus primarily used for the treatment of chronic hepatitis B and C.

The mechanism of action of recombinant IFN-α2a treatment is not yet fully understood. The effect may include a modulation of the immune system. NK (natural killer) cells and NKT cells, a cell population bearing NK receptors as well as T-cell receptors (of restricted variability), are stimulated with IFN-α. The original hypothesis was based on reports that the NK/NKT cell activity is impaired and their number decreased in several autoimmune animal models and human diabetes [81]. NKT cells have an important regulatory function in the innate as well as the adaptive immune response [73]. The deficiency of NK cells could be corrected with IFN-α treatment. Later, IFN-α was described as an inductor of circulating IL-1 receptor antagonists. In this case, induction of an anti-inflammatory status was suggested through modulation of the IL-1/IL-1 receptor antagonist balance. Recent results suggest that host immunity is an important factor in the response to interferon therapy [61].

Side effects of IFN-α therapies are the development of anti-thyroid antibodies, sometimes leading to thyroiditis, and anti-DNA antibodies. Since increased IFN-α and anti-DNA antibodies are also found in patients with lupus erythematodes, it is a major concern that IFN-α treatment even has the potential to induce SLE. Most patients experience flu-like symptoms, which resolve spontaneously.

In an uncontrolled prospective study, 50 patients with Behçet's disease and sight-threatening uveitis were treated with a daily subcutaneous dose of 6 million units recombinant human IFN-α. Forty-six patients responded well with increasing visual acuity and regressing intraocular inflammation. The overall activity of Behçet's disease was reduced to 50 %, and after a mean observation period of 3 years 20 patients were able to discontinue treatment and were in remission for 7–58 months. The remaining patients were able to reduce their dose of IFN-α to 3 million units three times a week [39].

16.5.4 Daclizumab

T cells upregulate their receptor for IL-2 (IL-2R) upon activation. Targeting the α-chain of the high-affinity IL-2R will thus affect only activated T cells, the population that maintains the autoaggressive immune response, while leaving the pool of memory and naïve T cells untouched. In rodent experimental autoimmune uveitis the autoaggressive Th1 cells express large numbers of IL-2R [11]. In a non-human primate model, targeting IL-2 receptors could effectively downregulate experimentally induced intraocular inflammation [26], offering the rationale for treating the first patients in a non-randomized open-label pilot study [50]. Ten uveitis patients were successfully treated with the humanized antibody specific for the IL-2 receptor (daclizumab); 1 mg/kg bodyweight was infused in 2-week intervals. After 24 weeks the intervals between the infusions were increased to 4 weeks. Within the 1st year patients did not need any other immunosuppressive or anti-inflammatory therapy besides daclizumab.

Meanwhile seven of these patients were followed for more than 4 years. During the 3 years follow-up subcutaneous application of therapeutic antibody was tested (2 mg/kg body weight, two applications within the first 2 weeks, followed by 4 weekly maintenance treatments with 1 mg/kg body weight). In all patients treated s.c. the concomitant immunosuppressive therapy could be reduced, while visual acuity remained stable. Side effects within the 1st year of daclizumab therapy were granulomatous dermatitis in two patients, whereas during the following 3 years of treatment more side effects appeared, ranging from minor infections to renal cell carcinoma in one patient [53].

16.6 Oral Tolerance Induction

The various treatment strategies discussed above have one major feature in common: they generally suppress the immune system, but not only the autoaggressive immune response. Furthermore, the pharmaceutical and even some biological agents are burdened with severe side effects, which might even accumulate with duration of treatment. Although most of the side effects are dose dependent and can be reduced by combining different therapeutic agents, the side effects will limit efficiency of therapy in most patients, often resulting in loss of visual acuity for the sake of the patients' safety. It is therefore important to develop highly specific therapies, such as the induction of antigen specific mucosal tolerance. In this case the antigen which is attacked by the immune system is applied orally, and in that manner induces regulatory cells downregulating the autoaggressive immune response.

This mechanism of mucosal tolerance is usually effective for nutritional proteins, preventing adverse reactions that potentially lead to food allergies. This tolerance is mediated by suppressor cells specific for the respective antigen; however, the exact mechanisms of this suppression are not yet fully elucidated. It is assumed that suppressor T cells recognize the respective antigen and secrete suppressive cytokines, such as TGF-β, IL-10 (Th3, Tr type) or cytokines belonging to the respective antagonistic Th type of the immune response [79] (Fig. 16.3). To date various antigens have been orally applied to uveitis patients [51, 54, 75].

16.6.1 Retinal Autoantigens as Tolerogens

In the first pilot study of oral tolerance induction two patients with chronic intermediate and Behçet's uveitis, respectively, both requiring permanent immunosuppressive therapy, were orally treated with retinal S-Ag [54]. These patients received 30 mg of purified bovine retinal S-Ag, starting with 3 times a week initially. Later the intervals of oral antigen administration

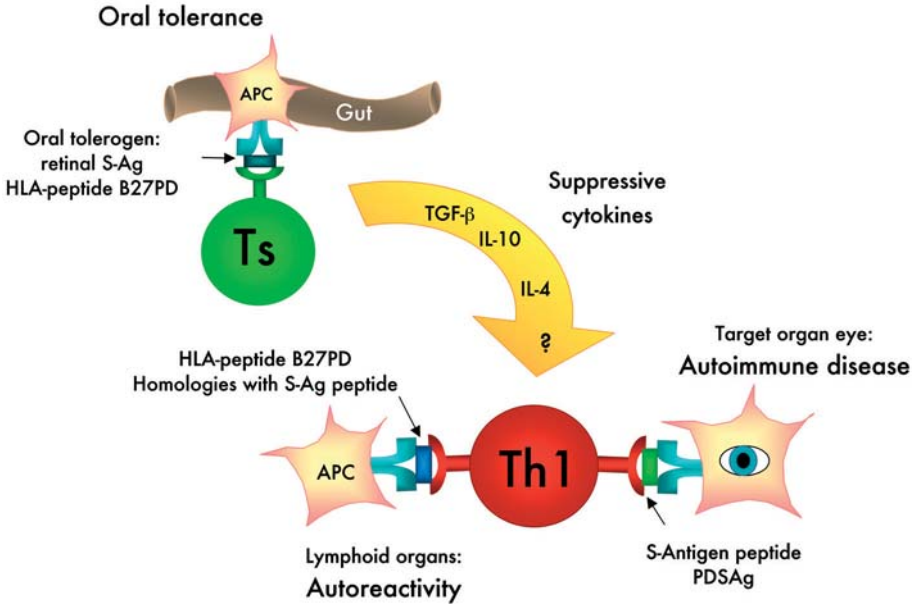

Fig. 16.3. Oral tolerance. Retinal autoantigen protein (*S-Ag*) or a peptide (*B27PD*) derived from HLA-B antigens and imitating an immunogenic peptide from S-Ag are applied via the gastrointestinal mucosa. Presented by antigen presenting cells (*APC*) in the gut they will activate specific suppressor cells (*Ts*), which induce tolerance in a still unknown way, probably by secretion of suppressive cytokines. This induction of tolerance is achieved by impeding the action of autoaggressive T-helper 1 (*Th1*) cells, which are crossreactive between the HLA-peptide B27PD and the peptide from retinal S-antigen

were extended and finally the patients were taken off oral S-Ag. In the 41 months follow-up the disease activity decreased and as a positive effect of treatment with oral S-Ag, the conventional medication could be reduced.

Later a prospective, randomized, double-blinded clinical phase I/II trial followed. Four groups of patients received either placebo, purified bovine retinal S-Ag, retinal extract enriched with S-Ag or bovine retinal extract [51] to ensure that tolerance was induced to all possible antigens, which might play a role in the course of uveitis [14]. The read out in this study was the time from study entry to tapering off immunosuppressive therapy, and in the follow-up the time until the next relapse occurred. With respect to both parameters, the patient group treated with purified oral S-Ag showed positive results compared to placebo controls or the other treatment groups. Unfortunately, the results did not reach statistical significance possibly due to a too small sample size.

16.6.2 HLA-peptide B27PD as Oral Tolerogen

The 14-mer peptide B27PD (ALNEDLSSWTAADT) has been shown to be highly effective for the treatment of experimental autoimmune uveitis in rats [80]. Therefore a prospective uncontrolled open trial for nine patients with chronic anterior, intermediate or posterior uveitis was initiated, using the encapsulated peptide for oral treatment [75]. All patients were on long-lasting conventional immunosuppressive therapy and either suffered from severe side effects or were unresponsive to this treatment. The patients received 4 mg of the peptide three times a week during the first 12 weeks and were then followed for another 9 months. The amount of concomitant immunosuppressive treatment was limited to 20 mg of prednisone or equivalent during the 12 weeks of tolerance induction, and during the

whole study period conventional therapy was adjusted to the patients' disease activity.

In all patients, visual acuity and/or intraocular inflammation improved during the peptide treatment. This allowed reducing corticosteroid therapy in all patients within 2–6 weeks, resulting in an average steroid dose reduction from 10.4 mg (in the year prior to study entry) to 3.1 mg daily within the year after study entry. At the same time visual acuity increased slightly. Extensive in vitro testing of peripheral blood lymphocytes revealed that immune responses to mitogens (PHA, phytohaemagglutinin) and recall antigens (tetanus toxoid, PPD (purified protein derivative of M. tuberculosis) were not altered by peptide treatment, indicating that tolerance induction does not cause a generalized immunosuppression.

During the follow-up of 4 years, four patients were successfully retreated with oral peptide. The average visual acuity of all patients remained stable with reduced concomitant corticosteroid therapy [76].

Summary for the Clinician

- **Uveitis patients with sight threatening chronic or remitting disease require immunosuppressive therapy if corticosteroids alone show unsatisfactory therapeutic effects or intolerable side effects**
- **Immunosuppressive therapy with cytotoxic drugs (azathioprine, methotrexate, mycophenolate mofetil, cyclophosphamide or chlorambucil), antibiotics (sulphasalazine) or calcineurin inhibitors (cyclosporine A, tacrolimus) is usually combined with corticosteroids in order to save on the therapeutic dose of either drug and thus to reduce side effects. The therapeutic effect of conventional immunosuppressive agents may be delayed by several weeks or up to 3 months**
- **New immunomodulatory therapies such as thalidomide, and biologicals targeting cytokines (anti-TNF-α: infliximab or TNF-αR-IgG: etanercept), cytokine receptors on immune cells (anti-IL-2Receptor: daclizumab) or the direct application of cytokines (IFN-α) are more specific in their mode of action. They have the potential to quickly induce remission in uveitis patients, even in those with certain underlying diseases (e.g. Behçet's disease). However, due to limited experience possible side effects are still not known**
- **Immunological therapies with the capacity to reinduce tolerance to autoantigens without affecting other immune responses, like oral tolerance induction, are under investigation and have the potential to be void of adverse events**

References

1. Akpek EK, Baltatzis S, Yang J, et al. (2001) Long-term immunosuppressive treatment of serpiginous choroiditis. Ocul Immunol Inflamm 9:153–167
2. Akpek EK, Ilhan Sarac O (2003) New treatments for serpiginous choroiditis. Curr Opin Ophthalmol 14:128–131
3. Akpek EK, Jabs DA, Tessler HH, et al. (2002) Successful treatment of serpiginous choroiditis with alkylating agents. Ophthalmology 109:1506–1513
4. Arnett HA, Mason J, Marino M, et al. (2001) TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. Nat Neurosci 4: 1116–1122
5. Baltatzis S, Tufail F, Yu EN, et al. (2003) Mycophenolate mofetil as an immunomodulatory agent in the treatment of chronic ocular inflammatory disorders. Ophthalmology 110:1061–1065
6. BenEzra D, Cohen E, Chajek T, et al. (1988) Evaluation of conventional therapy versus cyclosporine A in Behcet's syndrome. Transplant Proc 20:136–143
7. Benitez Del Castillo JM, Garcia Sanchez J, Iradier T, et al. (2000) Sulfasalazine in the prevention of anterior uveitis associated with ankylosing spondylitis. Eye 14:340–343
8. Bom S, Zamiri P, Lightman S (2001) Use of methotrexate in the management of sight-threatening uveitis. Ocul Immunol Inflamm 9:35–40
9. Brock N (1989) Oxazaphosphorine cytostatics: past-present-future. Seventh Cain Memorial Award lecture. Cancer Res 49:1–7
10. Cannon J, Linke CA, Cos LR (1991) Cyclophosphamide-associated carcinoma of urothelium: modalities for prevention. Urology 38:413–416
11. Caspi RR (2002) Th1 and Th2 responses in pathogenesis and regulation of experimental autoimmune uveoretinitis. Int Rev Immunol 21:197–208
12. Christmas NJ, Oh KT, Oh DM, et al. (2002) Long-term follow-up of patients with serpiginous choroiditis. Retina 22:550–556

13. Clements PJ, Davis J (1986) Cytotoxic drugs: their clinical application to the rheumatic diseases. Semin Arthritis Rheum 15:231–254
14. Deeg CA, Thurau SR, Gerhards H, et al. (2002) Uveitis in horses induced by interphotoreceptor retinoid-binding protein is similar to the spontaneous disease. Eur J Immunol 32:2598–2606
15. Eigler A, Sinha B, Hartmann G, et al. (1997) Taming TNF: strategies to restrain this proinflammatory cytokine. Immunol Today 18:487–492
16. El Shabrawi Y, Hermann J (2002) Anti-tumor necrosis factor-alpha therapy with infliximab as an alternative to corticosteroids in the treatment of human leukocyte antigen B27-associated acute anterior uveitis. Ophthalmology 109:2342–2346
17. Estrach C, Mpofu S, Moots RJ (2002) Behcet's syndrome: response to infliximab after failure of etanercept. Rheumatology (Oxford) 41:1213–1214
18. Foroozan R, Buono LM, Sergott RC, et al. (2002) Retrobulbar optic neuritis associated with infliximab. Arch Ophthalmol 120:985–987
19. Foster CS, Tufail F, Waheed NK, et al. (2003) Efficacy of etanercept in preventing relapse of uveitis controlled by methotrexate. Arch Ophthalmol 121:437–440
20. Frassanito MA, Dammacco R, Fusaro T, et al. (2003) Combined cyclosporin-A/prednisone therapy of patients with active uveitis suppresses IFN-gamma production and the function of dendritic cells. Clin Exp Immunol 133:233–239
21. Fries W, Giofre MR, Catanoso M, et al. (2002) Treatment of acute uveitis associated with Crohn's disease and sacroileitis with infliximab. Am J Gastroenterol 97:499–500
22. Gion N, Stavrou P, Foster CS (2000) Immunomodulatory therapy for chronic tubulointerstitial nephritis-associated uveitis. Am J Ophthalmol 129:764–768
23. Goldstein DA, Fontanilla FA, Kaul S, et al. (2002) Long-term follow-up of patients treated with short-term high-dose chlorambucil for sight-threatening ocular inflammation. Ophthalmology 109:370–377
24. Greiner K, Murphy CC, Willermain F, et al. (2004) Anti-TNFalpha therapy modulates the phenotype of peripheral blood CD4+ T cells in patients with posterior segment intraocular inflammation. Invest Ophthalmol Vis Sci 45:170–176
25. Greiner K, Varikkara M, Santiago C, et al. (2002) Efficiency of mycophenolate mofetil in the treatment of intermediate and posterior uveitis. Ophthalmologe 99:691–694
26. Guex Crosier Y, Raber J, Chan CC, et al. (1997) Humanized antibodies against the alpha-chain of the IL-2 receptor and against the beta-chain shared by the IL-2 and IL-15 receptors in a monkey uveitis model of autoimmune diseases. J Immunol 158:452–458
27. Hamuryudan V, Mat C, Saip S, et al. (1998) Thalidomide in the treatment of the mucocutaneous lesions of the Behcet syndrome. A randomized, double-blind, placebo-controlled trial. Ann Intern Med 128:443–450
28. Hanke JH, Nichols LN, Coon ME (1992) FK506 and rapamycin selectively enhance degradation of IL-2 and GM-CSF mRNA. Lymphokine Cytokine Res 11:221–231
29. Hudde T, Heinz C, Neudorf U, et al. (2002) Tubulointerstitial nephritis and uveitis (TINU syndrome) – comorbidity and complications in four patients. Klin Monatsbl Augenheilkd 219:528–532
30. Isnard Bagnis C, Tezenas du Montcel S, Beaufils H, et al. (2002) Long-term renal effects of low-dose cyclosporine in uveitis-treated patients: follow-up study. J Am Soc Nephrol 13:2962–2968
31. Jordan JF, Walter P, Ayertey HD, et al. (2003) Intermediate uveitis in childhood preceding the diagnosis of multiple sclerosis: a 13-year follow-up. Am J Ophthalmol 135:885–886
32. Joseph A, Raj D, Dua HS, et al. (2003) Infliximab in the treatment of refractory posterior uveitis. Ophthalmology 110:1449–1453
33. Kaklamani VG, Kaklamanis PG (2001) Treatment of Behcet's disease – an update. Semin Arthritis Rheum 30:299–312
34. Kaplan Messas A, Barkana Y, Avni I, et al. (2003) Methotrexate as a first-line corticosteroid-sparing therapy in a cohort of uveitis and scleritis. Ocul Immunol Inflamm 11:131–139
35. Kari JA, Shah V, Dillon MJ (2001) Behcet's disease in UK children: clinical features and treatment including thalidomide. Rheumatology (Oxford) 40:933–938
36. Kilmartin DJ, Fletcher ZJ, Almeida JA, et al. (2001) CD69 expression on peripheral CD4+ T cells parallels disease activity and is reduced by mycophenolate mofetil therapy in uveitis. Invest Ophthalmol Vis Sci 42:1285–1292
37. Kiss S, Letko E, Qamruddin S, et al. (2003) Long-term progression, prognosis, and treatment of patients with recurrent ocular manifestations of Reiter's syndrome. Ophthalmology 110:1764–1769
38. Klausner JD, Freedman VH, Kaplan G (1996) Thalidomide as an anti-TNF-alpha inhibitor: implications for clinical use. Clin Immunol Immunopathol 81:219–223
39. Kotter I, Zierhut M, Eckstein AK, et al. (2003) Human recombinant interferon alfa-2a for the treatment of Behcet's disease with sight threatening posterior or panuveitis. Br J Ophthalmol 87:423–431
40. Kulkarni P (2001) Review: uveitis and immunosuppressive drugs. J Ocul Pharmacol Ther 17:181–187

41. Larkin G, Lightman S (1999) Mycophenolate mofetil. A useful immunosuppressive in inflammatory eye disease. Ophthalmology 106:370–374
42. Lim WS, Powell RJ, Johnston ID (2002) Tuberculosis and treatment with infliximab. N Engl J Med 346:623–626
43. Liu J (1993) FK506 and cyclosporin, molecular probes for studying intracellular signal transduction. Immunol Today 14:290–295
44. Masli S, Turpie B, Streilein JW (2003) By altering TNFR2:TNFR1 expression, TGFb prevents ACAID-inducing antigen presenting cells from secreting IL-12. Invest Ophthalmol Vis Sci E-Abstract 44:990
45. Michel S-S, Ekong A, Baltatzis S, et al. (2002) Multifocal choroiditis and panuveitis: immunomodulatory therapy. Ophthalmology 109:378–383
46. Mikuls TR, Moreland LW (2003) Benefit-risk assessment of infliximab in the treatment of rheumatoid arthritis. Drug Saf 26:23–32
47. Miserocchi E, Baltatzis S, Ekong A, et al. (2002) Efficacy and safety of chlorambucil in intractable noninfectious uveitis: the Massachusetts Eye and Ear Infirmary experience. Ophthalmology 109: 137–142
48. Mudun BA, Ergen A, Ipcioglu SU, et al. (2001) Short-term chlorambucil for refractory uveitis in Behcet's disease. Ocul Immunol Inflamm 9:219–229
49. Munoz Fernandez S, Hidalgo V, Fernandez Melon J, et al. (2003) Sulfasalazine reduces the number of flares of acute anterior uveitis over a one-year period. J Rheumatol 30:1277–1279
50. Nussenblatt RB, Fortin E, Schiffman R, et al. (1999) Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: a phase I/II clinical trial. Proc Natl Acad Sci U S A 96:7462–7466
51. Nussenblatt RB, Gery I, Weiner HL, et al. (1997) Treatment of uveitis by oral administration of retinal antigens: results of a phase I/II randomized masked trial. Am J Ophthalmol 123:583–592
52. Nussenblatt RB, Palestine AG, Rook AH, et al. (1983) Treatment of intraocular inflammatory disease with cyclosporin A. Lancet 2:235–238
53. Nussenblatt RB, Thompson DJ, Li Z, et al. (2003) Humanized anti-interleukin-2 (IL-2) receptor alpha therapy: long-term results in uveitis patients and preliminary safety and activity data for establishing parameters for subcutaneous administration. J Autoimmun 21:283–293
54. Nussenblatt RB, Whitcup SM, de Smet MD, et al. (1996) Intraocular inflammatory disease (uveitis) and the use of oral tolerance: a status report. Ann N Y Acad Sci 778:325–337
55. Ozdal PC, Ortac S, Taskintuna I, et al. (2002) Long-term therapy with low dose cyclosporin A in ocular Behcet's disease. Doc Ophthalmol 105: 301–312
56. Patil CR, Bhise SB (2003) Re-emergence of thalidomide. Ind J Pharmacol 35:204–212
57. Qi S, Xu D, Peng J, et al. (2000) Effect of tacrolimus (FK506) and sirolimus (rapamycin) mono- and combination therapy in prolongation of renal allograft survival in the monkey. Transplantation 69:1275–1283
58. Reddy AR, Backhouse OC (2003) Does etanercept induce uveitis? Br J Ophthalmol 87:925
59. Reiff A (2003) Long-term outcome of etanercept therapy in children with treatment-refractory uveitis. Arthritis Rheum 48:2079–2080
60. Saenz A, Ausejo M, Shea B, et al. (2000) Pharmacotherapy for Behcet's syndrome. Cochrane Database Syst Rev 2
61. Saito H, Ebinuma H, Satoh I, et al. (2000) Immunological and virological predictors of outcome during interferon-alpha therapy of chronic hepatitis C. J Viral Hepat 7:64–74
62. Samson CM, Waheed N, Baltatzis S, et al. (2001) Methotrexate therapy for chronic noninfectious uveitis: analysis of a case series of 160 patients. Ophthalmology 108:1134–1139
63. Saulsbury FT, Mann JA (2003) Treatment with infliximab for a child with Behcet's disease. Arthritis Rheum 49:599–600
64. Sfikakis PP (2002) Behcet's disease: a new target for anti-tumour necrosis factor treatment. Ann Rheum Dis 61 Suppl 2:ii51–53
65. Shah SS, Lowder CY, Schmitt MA, et al. (1992) Low-dose methotrexate therapy for ocular inflammatory disease. Ophthalmology 99:1419–1423
66. Sicotte NL, Voskuhl RR (2001) Onset of multiple sclerosis associated with anti-TNF therapy. Neurology 57:1885–1888
67. Silman AJ, Petrie J, Hazleman B, et al. (1988) Lymphoproliferative cancer and other malignancy in patients with rheumatoid arthritis treated with azathioprine: a 20 year follow up study. Ann Rheum Dis 47:988–992
68. Sloper CM, Powell RJ, Dua HS (1999) Tacrolimus (FK506) in the treatment of posterior uveitis refractory to cyclosporine. Ophthalmology 106: 723–728
69. Smith J-R (2002) Management of uveitis in pediatric patients: special considerations. Paediatr Drugs 4:183–189
70. Smith JA, Smith S, Whitcup SM, et al. (2002) The treatment of JRA-associated uveitis with etanercept. Arthritis Rheum 46:S482
71. Stawell R (2003) Methotrexate in inflammatory eye disease. Ocul Immunol Inflamm 11:79–82

72. Stockman GD, Heim LR, South MA, et al. (1973) Differential effects of cyclophosphamide on the B and T cell compartments of adult mice. J Immunol 110:277–282
73. Taniguchi M, Harada M, Kojo S, et al. (2003) The regulatory role of Valpha14 NKT cells in innate and acquired immune response. Annu Rev Immunol 21:483–513
74. ten Tusscher MP, Jacobs PJ, Busch MJ, et al. (2003) Bilateral anterior toxic optic neuropathy and the use of infliximab. BMJ 326:579
75. Thurau SR, Diedrichs-Möhring M, Fricke H, et al. (1999) Oral tolerance with an HLA-peptide mimicking retinal autoantigen as a treatment of autoimmune uveitis. Immunol Lett 68:205–212
76. Thurau SR, Wildner G (2003) An HLA-peptide mimics organ-specific antigen in autoimmune uveitis: its role in pathogenesis and therapeutic induction of oral tolerance. Autoimm Rev 2:171–176
77. Tseng S, Pak G, Washenik K, et al. (1996) Rediscovering thalidomide: a review of its mechanism of action, side effects, and potential uses. J Am Acad Dermatol 35:969–979
78. Uchio E, Matsumoto T, Tanaka SI, et al. (1999) Soluble intercellular adhesion molecule-1 (ICAM-1), CD4, CD8 and interleukin-2 receptor in patients with Behcet's disease and Vogt-Koyanagi-Harada's disease. Clin Exp Rheumatol 17:179–184
79. Weiner HL (2001) Oral tolerance: immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. Microbes Infect 3:947–954
80. Wildner G, Thurau SR (1994) Cross-reactivity between an HLA-B27-derived peptide and a retinal autoantigen peptide: a clue to major histocompatibility complex association with autoimmune disease. Eur J Immunol 24:2579–2585
81. Wilson SB, Kent SC, Patton KT, et al. (1998) Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. Nature 391:177–181
82. Zierhut M, Stubiger N, Aboalchamat W, et al. (2001) Immunosuppressive therapy with mycophenolate mofetil (CellCept) in treatment of uveitis. Ophthalmologe 98:647–651

Vitrectomy in Uveitis

17

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Core Messages

- Pars plana vitrectomy (PPV) is performed to remove vitreous opacities in uveitis patients
- There are a large number of diagnostic and therapeutic indications for removal of vitreous opacities. A decision to perform PPV in an individual patient requires consideration of potential benefits and risks that are incompletely defined in the medical literature
- Uveitis may initiate a particular spectrum of complications after PPV, and therefore adequate control of intraocular inflammation in the preoperative and postoperative periods is mandatory. Combined management by uveitis and retina specialists is often required

17.1 Introduction

Over the last 25 years 60 papers have been published dealing with therapeutic vitrectomy in uveitis (overview in [1]). So far no prospective, controlled clinical trials have been performed to precisely evaluate the role of pars plana vitrectomy (PPV) as a single or combined surgical procedure in patients with intraocular inflammation. Inconsistent data exist regarding prognostic determinants and the role of perioperative immunosuppressive therapy. It is the purpose of this review to present available information from the medical literature and summarize our current recommendations concerning vitrectomy in uveitis.

17.2 General Approach to PPV in Uveitis

17.2.1 Patient Selection

Patients are selected for PPV for either diagnostic or therapeutic purposes. Often, both indications are present if it is felt that the patient will potentially benefit from removal of vitreous opacities. Non-elective indications include the management of uveitic complications, such as retinal detachment. Elective indications, such as improvement of vision by reduction of cystoid macular oedema or PPV as an alternative to further systemic immunomodulatory treatment, are difficult to define in the absence of clinical trials data. In general, intermediate uveitis with persistent, visually significant vitreous opacities and medically controlled macular oedema is probably the clearest indication for PPV.

17.2.2 Timing of Surgery

Whereas cataract surgery in uveitis is ordinarily performed only after complete quiescence of the uveitis for 3–6 months, PPV may under certain circumstances be performed in active disease. Manipulation of the posterior segment through small incisions is probably not as traumatic as the larger incisions and manipulation of iris performed in anterior segment surgery. However, the maximum amount of inflammation that can be tolerated without compromising surgical results is not clear. For non-urgent elective procedures, quiescence of anterior seg-

ment inflammation is advisable. Inflammatory choroidal effusion or exudative retinal detachments are commonly considered contraindications. Active pars plana exudation may increase the risk of retinal injury from the pars plana sclerotomy sites.

17.2.3 Technical Considerations

Standard three-port vitrectomy with 20-gauge instrumentation is ordinarily performed. New 25-gauge instrumentation may be a good alternative, especially diagnostic vitrectomies or other limited core vitrectomies to clear vitreous opacities [2].

PPV in uveitis patients often involves additional vitreoretinal procedures such as pars plana lensectomy, membrane peeling, detachment repair, retinal biopsy and occasionally retinotomy or retinectomy, or endolaser photocoagulation with attendant needs for specialized instrumentation and skills (see Sect. 17.4, "Combined Surgery", below.)

17.2.4 Diagnostic Procedures

Diagnostic testing of vitreous specimens needs to be carefully planned prior to surgery. Separate specimens need to be collected in appropriate transport containers for each diagnostic test. In general, undiluted vitreous is preferred for cytology, viral culture, polymerase chain reaction (PCR), and antibody determinations. Diluted vitreous wash can be used for flow cytometry and DNA gene rearrangement studies and filtered for bacterial and fungal cultures.

Vitreous cells and bacterial or fungal cultures are transported at room temperature. Viral cultures, PCR and antibodies are refrigerated or frozen, depending upon the time until the assay can be performed. Coordination with the laboratory prior to PPV is desirable to avoid damage to the specimen.

17.3 Perioperative Management

17.3.1 Assessment of Other Ocular Disease

17.3.1.1 Retinal Detachment

Small pupils from posterior synechiae, cataract, and vitreous opacities can interfere with the preoperative evaluation of the retina, making ultrasonography necessary prior to PPV. Ultrasound is useful in eyes with opaque media to assess the configuration of a preoperative retinal detachment and to detect elevated retinal tears and vitreous traction.

It may be impossible to differentiate a rhegmatogenous from an exudative retinal detachment clinically or ultrasonographically. The old rule that shifting subretinal fluid indicates exudative detachment often does not hold in uveitis as inflammation can increase the protein content of the subretinal fluid and result in changes in the shape and extent of detachment with head position. However, stiff, contracted retina or extensive epiretinal membrane formation is likely to occur only in rhegmatogenous detachment.

In eyes with active inflammation, a preoperative trial of oral corticosteroids at high doses for 2 weeks may be helpful in excluding exudative detachment. It is important to remember that rhegmatogenous detachment alone can cause significant intraocular inflammation; patients with retinal detachment and no prior history of intraocular inflammation may have good resolution of the inflammatory changes following repair of the retinal detachment.

Because of associated ocular complications, retinal detachments in uveitis are usually repaired by techniques that include PPV.

17.3.1.2 Epiretinal Membrane

Epiretinal membranes in eyes with past or present cystoid macular oedema can appear worse than their actual effect on vision. Preoperative decision-making regarding epiretinal membrane removal can be assisted by optical coherent tomography to visualize macular thickness, membrane thickness, straightening of the inner retina, cystoid spaces, and vitreous traction. Macular thickening with inner retinal straightening that persists after medical therapy and macular thickening with vitreomacular traction are probably the clearest indications for epiretinal membrane peeling. Removal of epiretinal membranes that cover thinned macular retina in a setting of chronically poor vision (after chronic CME or scar formation) may not be helpful in improving vision.

17.3.1.3 Glaucoma

Uveitic eyes with glaucoma should be evaluated for the feasibility of implantation of a glaucoma drainage device (GDD) at the time of PPV. Anterior segment inflammation can cause early failure of trabeculectomies and also severe complications of fibrosis surrounding anteriorly placed tubes of GDDs [3]. Placement of the GDD in the posterior segment through the pars plana can avoid these severe complications. Excellent cleanup of the peripheral vitreous and posterior cortical vitreous is necessary to prevent aspiration of vitreous with possible retinal complications.

17.3.1.4 Posterior Hyaloid Attachment

Attached posterior hyaloid can create surgical difficulties as it may not be fully peelable, for example in children with predominantly anterior uveitis. If the hyaloid is peeled and elevated, the risk of retinal detachment is increased. Status of the posterior hyaloid and the completeness of its removal should be included in the operative notes.

17.3.2 Concomitant Medical Management

17.3.2.1 Perioperative Immunosuppression

No valid data exist that demonstrate that perioperative control of inflammation reduces the risk of postoperative complications from PPV such as the development of hypotony or CME. Extrapolation from cataract surgery in uveitis patients suggests that surgical results in patients undergoing elective PPV may be better if the inflammation is well controlled. Immunosuppressive treatment seemed to permit safe intraocular lens (IOL) implantation in selected JIA-uveitis children (five patients) [4], a disorder in which IOLs are ordinarily contraindicated. However, profound foreign body reactions around implanted materials are generally not primary concerns in PPV surgery.

A more potent argument for the preoperative use of immunosuppression relates to current understanding of uveitic mechanisms. In autoimmune uveitis, antigen-specific T cells are thought to play a crucial role in orchestrating the infiltration of large numbers of non-antigen-specific leucocytes into the vitreous. The antigen-specific T cells are activated in extraocular lymph nodes and spleen and secondarily invade the eye. Therefore, from an immunological point of view, PPV can remove existing vitreous cells but cannot obliterate the inflammatory response. The value of PPV as an early approach to alter the subsequent course of uveitis is not known. If patients are not adequately immunosuppressed, the inflammatory activity of uveitis will continue after PPV with the risk of postoperative complications such as CME. The definition of adequate immunosuppression is controversial. Moderate doses of oral corticosteroids or treatment with one corticosteroid-sparing drug such as methotrexate cyclosporine or mycophenolate mofetil for 3 months prior to PPV would be predicted to control inflammation in most patients. Despite these theoretical advantages of preoperative immunosuppression, in seven case series that documented medications prior to PPV for uveitis,

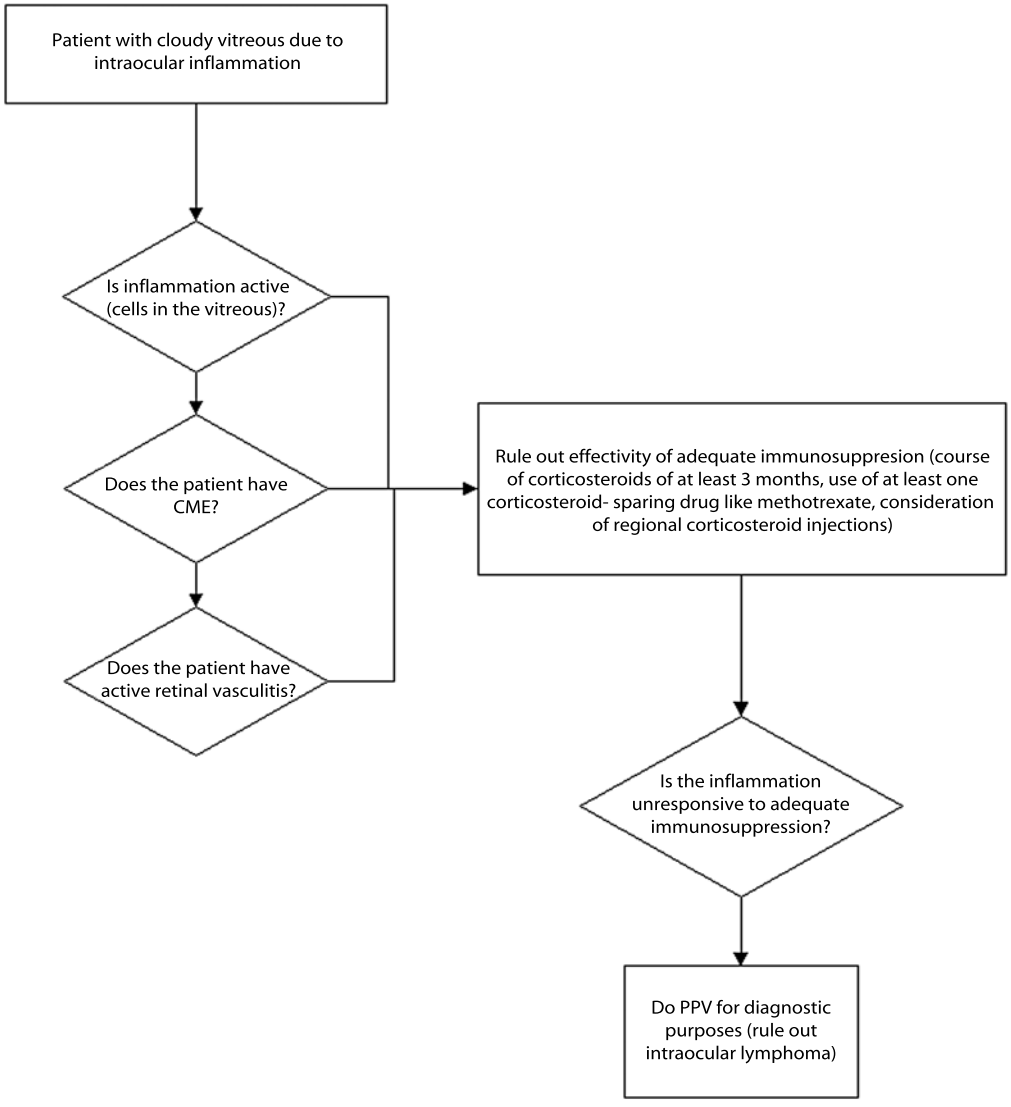

Fig. 17.1. An algorithm approach for decision making for PPV in uveitis

immunosuppressive medications were used in a median of only 26 % of cases (range 10–100 %) [5–12]. Figure 17.1 gives an algorithm approach for the use of immunosuppression during PPV in uveitis.

It is unknown if PPV facilitates postoperative control of inflammation by immunosuppressive drugs, perhaps by removing a depot of activated lymphocytes and cytokines, making it easier for the drugs to work, or by increasing penetration of the drugs into the eye.

17.3.2.2 Intravitreal Corticosteroids

Intravitreal injection of dexamethasone is commonly performed in treatment of severe intraocular inflammation related to bacterial endophthalmitis, although its usefulness has been

debated [13]. Use in PPV in uveitis has been limited. Expanding indications for intravitreal triamcinolone acetonide in the treatment of CME suggest that it may be useful both to reduce postoperative inflammation in uveitis and to treat residual CME after medical therapy. Intravitreal triamcinolone acetonide has been suggested as an adjunct to vitreoretinal surgery in which it is used to define tissue planes for easy removal [14] and may have applicability for PPV for uveitis as well [15].

Although randomized studies are still lacking, clinical experience indicates that macular oedema usually resolves, often with dramatic improvements in vision, within 4–6 weeks of injection. Cataract development and pressure elevations, which usually respond to medical therapy, may occur. Finally, injection of the commercial preparation has been associated with a sterile endophthalmitis, which could markedly confuse the postoperative picture. Although intravitreal triamcinolone appears to be capable of providing rapid improvement in vision, its use should be limited to selected cases until more is known about its effect in uveitis patients undergoing PPV.

17.4 Combined Surgery

17.4.1 Cataract Surgery

17.4.1.1 General Consideration

Cataract formation is one of the most common complications of uveitis, occurring in 50 % of patients.

17.4.1.2 Indications and Contraindications

The major indications for cataract surgery are to remove visually significant opacities or to permit adequate visualization of the fundus for clinical monitoring of disease or during vitreous or macular surgery. The surgery is contraindicated in acute, active inflammation, and in young children with mild cataract and intact accommodation, especially in the amblyogenic age group.

17.4.1.3 Surgical Procedures

Various surgical techniques have been suggested for uveitis patients with cataract. These include lensectomy and extracapsular cataract extraction (or phacoemulsification) with or without the implantation of an IOL. Timing of surgery is critical, as complete quiescence of inflammation must be obtained preoperatively by appropriate anti-inflammatory medication, which is then continued after surgery. The aetiology of uveitis, morphological situation and coexistent complications dictate the perioperative medical regimen and the surgical technique.

Cataract extraction in uveitis patients, especially in children, is commonly followed by secondary cataract formation and by severe inflammation, which often leads to the development of pupillary membranes, vitreous opacities, macular oedema, and ocular hypotony. Excision of the posterior capsule and anterior vitrectomy from an anterior approach may satisfactorily address these concerns in non-uveitic eyes [16], but more severely affected eyes are probably better managed by pars plana lensectomy and vitrectomy. Combined PPV and pars plana lensectomy in chronically inflamed eyes were shown to be effective in improving visual acuity 25 years ago [17]. Lensectomy combined with pars plana vitrectomy is probably still the preferred surgical technique in most patients with juvenile idiopathic arthritis and iridocyclitis [18] although there are recent reports of successful IOL implantation in small numbers of selected patients.

Combined phacoemulsification and PPV may be appropriate in cases in which inflammation can be controlled with medical treatment, or after remission of active uveitis. Combined phacoemulsification and vitrectomy is now practised commonly when both cataract and vitreoretinal diseases are present [19]. This approach is advantageous in improving visualization of the posterior pole, preventing the rapid

progression of cataract after PPV, and avoiding the need for a second operation to remove the cataract, with an increased complication rate and delayed visual rehabilitation [20]. The applicability to patients with uveitis is uncertain. Combined surgery would be predicted to produce higher degrees of anterior chamber inflammation and CME than a two-step approach. Individualized case selection is particularly important when intensive manipulation of the anterior segment is anticipated, and in patients with continued higher grades of activity.

17.4.2 Membrane Peeling

17.4.2.1 General Consideration

Removal of macular epiretinal membranes is a standard indication for PPV in non-uveitis patients, with or without CME. Removal of uveitis-related membranes would be predicted to provide similar benefits as are seen in other patients, provided that membrane formation has been associated with a significant visual acuity drop [21].

17.4.2.2 Indications and Contraindications

Formation of epiretinal membranes is common in uveitis but does not always lead to a significant decrease in visual acuity. Some membranes do not directly affect the foveal zone and central involvement should be confirmed by OCT or biomicroscopy. If surgery is indicated, with metamorphosia being the primary indicator, the inflammation should be well controlled.

17.4.2.3 Surgical Procedure

In the perioperative period, systemic immunosuppression is increased, often in the form of increased systemic steroids. Periocular steroids are often given at the time of surgery. Systemic corticosteroids are rapidly diminished after surgery, based on the ocular response. The surgical procedure is identical to pucker surgery in other settings.

17.4.3 Cryotherapy

17.4.3.1 General Considerations

Cryocoagulation can have either an adjunctive role in retinal reattachment surgery or a therapeutic role in treating uveitis. It causes a fairly intense inflammatory response, which is easily recognized by an increase in anterior chamber flare and which often lasts 3–4 days, and may lead to significant uveal effusion and epi- and subretinal membrane formation. For this reason, laser should be substituted for cryotherapy whenever possible, either by a transpupillary delivery system or a transscleral diode infrared laser.

17.4.3.2 Indications and Surgical Technique

When cryocoagulation is used to provide chorioretinal adhesion around retinal tears in uveitis patients, a light retinal freeze, possibly with several adjacent applications to cover the edges of the tear, is superior to a large central iceball.

Cryocoagulation is also used therapeutically to treat pars plana exudation associated with active, visually significant intermediate uveitis that is unresponsive to medical treatment. It is also indicated for neovascularization of pars plana exudates that is complicated by vitreous haemorrhage. Here also, minimal treatment is best. Laser can also be applied to the snow banks, if they are not too large, often in repeated sessions to reduce the risk of traction on the peripheral retina.

17.4.4 Diagnostic PPV

17.4.4.1 General consideration

Recent developments in molecular biology allow novel diagnostic approaches in intraocular

inflammation. Genetic markers as well as species-specific sequences are used for the diagnosis of infection or masquerade syndromes.

An established collaboration with a microbiology department to test for infectious agents in vitreous fluid as well as an immediate transfer of vitreous cells to a pathology department to prevent rapid autolysis of the cells are prerequisites for diagnostic vitrectomy and should only be done in specialized centres.

Besides classical culture techniques, detection of antibody titres and DNA by polymerase chain reaction (PCR) has revolutionized the possibilities of diagnostic PPV. Additionally, cytological determination of cell surface markers and cytokine levels adds valuable information to the diagnostics of intraocular lymphoma.

With modern diagnostic tests like enzyme-linked-immunosorbent (ELISA) or radial immunodiffusion assays (RIA), antibodies against *Toxoplasma gondii, Toxocara canis,* herpes simplex virus, varizella zoster virus, cytomegalovirus and Epstein-Barr virus can be determined quantitatively. The Goldmann-Wittmer coefficient compares the antibody level from the serum with the vitreous and can be used to detect intraocular antibody production.

PCR is a highly sensitive method for the detection of DNA from small samples sizes. However, the limits and possibilities of this technique have to be carefully considered. Positive results have to be weighed against the risk of false-positive results. Therefore, the results have to be seen in the clinical context of the sensitivity and specificity of a particular test in a particular laboratory. Also, the results have to be seen as a simple "on/off" result; this means that the clinician has to decide if the DNA is "old/new", "live/dead" or "active/inactive".

17.4.4.2 Indications and Contraindications

Diagnostic PPV is usually performed in uveitis with an atypical course, for example, if standard immunosuppressive therapy does not produce the expected outcome. The intent is to exclude infectious aetiology or masquerade syndromes such as intraocular lymphoma.

Because of cost, diagnostic PPV is contraindicated if there is no possibility of reaching a diagnosis with available testing. However, research involving assays of vitreous specimens from uveitis patients would be of great scientific interest.

17.5 Postoperative Complications

17.5.1 Hypotony

Hypotony following surgery in uveitic eyes is felt to relate to shutdown of the aqueous humour production from acute, severe inflammation. Following PPV there are additional concerns about the possibility of wound leakage or retinal detachment, both of which should be excluded.

Hypotony usually responds to oral corticosteroids, very frequent applications of topical corticosteroids and corticosteroid injections i.v. or even i.o. Prolonged hypotony can occur in certain eyes with a poor preoperative prognosis. High-resolution scanning of the ciliary body region may be useful to detect membranes bridging the ciliary processes that might be amenable to removal. In practical terms, only non-contact ultrasound probes can be used, as contact with a hypotonous globe collapses it and distorts the anatomy.

Assessment of atrophic ciliary processes and the presence of any cyclitic membranes at the time of the initial PPV, using indirect ophthalmoscopy and deep scleral depression, is very useful in assessing postoperative hypotony. Due to the limited visualization of cyclitic membranes under clinical circumstances, UBM may be the preferred technique. However, the morphological abnormalities underlying hypotony may not be clearly distinguished in all patients.

17.5.2 Retinal Detachment

Retinal detachment is a severe complication in uveitic eyes since the intraocular inflammation

would be predicted to produce more epiretinal membrane formation and a worse prognosis. Causes of retinal detachment that are specific to uveitis patients include instrument passage through pars plana exudates, contracture of pars plana exudates and tractional detachment, and retinal breaks at the edge of chorioretinitis.

Replacement of vitreous fluid with air, gas or silicone oil may be required because of prior PPV, but may worsen the situation because of concentration of inflammatory mediators and cells in the residual fluid phase. This may lead to epiretinal membrane formation that jeopardizes the retinal reattachment.

17.5.3 Vitreous Haemorrhage

Vitreous haemorrhage is relatively common following PPV of all types, presumably from oozing from the sclerotomies. Blood generally clears from a vitrectomized eye within 1 month. Neovascularization or retinal tear formation should be kept in mind as possible complications leading to vitreous haemorrhage that require treatment.

17.5.4 Uveitis Recurrence

Recurrence of uveitis following PPV can occur. Transient increases in inflammation immediately after surgery are generally easily treated with oral corticosteroids. Because the vitreous gel is absent, vitreous cells are sparser and affect vision less. In the absence of vitreous cellular reaction, there may be a false sense that the inflammation is entirely under control. Another marker for disease activity should be used for monitoring, which could include anterior segment inflammation, visible posterior segment lesions, visual field or ERG as a measure of retinal function, or macular oedema.

17.6 PPV in Specific Diseases

17.6.1 Fuchs Uveitis Syndrome

Two articles report PPV for Fuchs uveitis [22, 23]. All 25 operated eyes (100 %) had an improvement in vision with complications limited to one postoperative hyphaema. The risk:benefit ratio for PPV with patients with Fuchs uveitis seems similar to that for cataract surgery, which is generally well tolerated [24–26].

17.6.2 Behçet's Disease

Behçet's disease accounts for about one-fourth of patients in case series of PPV for uveitis in which systemic associations are documented [27–29]. The number is skewed by a large series of uveitis patients published by a group in France with a special interest in Behçet's disease [28]. No detailed results of surgery were reported by that group. A report of three patients with Behçet's disease undergoing PPV [29] concluded that PPV is of no benefit for this subgroup of uveitis patients due to a high rate of complications.

17.6.3 Intermediate Uveitis

Most patients (46 %) in the published literature are classified as intermediate uveitis. There are five reports specifically focused on PPV for intermediate uveitis [8, 11, 28, 30, 31, 32]. Most authors support PPV in patients with intermediate uveitis, especially if systemic corticosteroids have failed to control inflammation preoperatively. PPV may reduce CME in these patients [8, 11].

17.6.4 Childhood Uveitis

Three case series report the outcome of PPV in young uveitis patients [31, 33, 34]. Other case series include small groups of children with idiopathic anterior, intermediate, posterior, or panuveitis. A minority of reported cases have associated systemic diseases, such as sarcoid, juvenile idiopathic arthritis [18], or Behçet's disease.

The principal indication for vitrectomy in children is intermediate uveitis. The value of PPV as compared to immunosuppression in children with intermediate uveitis is not well defined. PPV may be favoured to avoid the untoward side effects of systemic corticosteroids or immunosuppression. However, PPV may engender surgical complications, including subsequent cataract formation.

Combined pars plana lensectomy and vitrectomy has been proposed for the treatment of complicated cataract in children with uveitis [18].

17.6.5 Infectious Uveitis

17.6.5.1 Toxoplasmosis

PPV for toxoplasmic chorioretinitis is used in three settings. First, diagnostic PPV is useful in severe, atypical cases without classic manifestations of a chorioretinal inflammatory focus adjacent to a pigmented scar. PCR to amplify toxoplasmosis genome and local antibody production are useful in making the diagnosis. Culture of toxoplasmosis organisms from vitreous has also been reported [35]. Second, PPV can relieve vitreous traction at the edge of chorioretinal lesions. This is particularly helpful following intense inflammatory reactions, when vitreous condensation leads to transvitreous tractional membranes. Third, PPV will often be required in order to properly manage retinal detachments related to toxoplasmosis. Selection of the exact surgical procedure depends on the degree of residual inflammation, if any. If the eye is inflamed, an aggressive approach with long-term tamponade such as silicone oil will often be needed. Avoidance of cryotherapy may help reduce postoperative inflammation.

Prophylactic antibiotics active against toxoplasmosis are probably advisable in the perioperative period of any ocular surgery to reduce the risk of recurrent disease.

17.6.5.2 Toxocariasis

Pars plana vitrectomy may help in establishing a diagnosis through intraocular antibody determinations. More commonly, PPV is used to relieve vitreous traction, either from peripheral lesions, or from paramacular lesions that are distorting the retina. Rarely, an active toxocaral organism may be removed from the subretinal space, thereby avoiding the inflammatory response which occurs following the organism's death, and is the cause of much of the ocular pathology observed in this condition [36].

17.6.5.3 Cytomegalovirus Retinitis

PPV is mainly indicated in the management of retinal detachments [37]. These can occur within the area affected by the CMV virus, or in an area of intact retina. If the area of detachment is located in, or adjacent to, the CMV retinitis, a pars plana vitrectomy removing all traction to the area with CMV as well as other holes is recommended. Silicone oil is usually selected as the intraocular tamponade, especially when CMV progression is to be expected because of the lack of response to highly active antiretroviral agents. An encircling band may help in the repair of inferior detachments or when the vitreous base cannot be adequately dissected. Cases with limited disease may respond well to short-term gas tamponade without the side effects associated with the use of oil [38].

17.6.5.4 Necrotizing Herpetic Retinitis

Pars plana vitrectomy can be helpful in removing the massive vitreous condensations that often accompany this infection. It is also required to repair retinal detachment, which often appears as the infection subsides, particularly in those cases that were not prophylactically lasered along the leading edge of the necrotic retina. The surgical approach will depend on the extent of necrosis and the area of detachment. A laser barrier should be placed in healthy retina. While a buckle is usually not required, silicone oil is often used initially. Postoperative reproliferation of epiretinal membranes is common [39].

17.6.5.5 CME

CME refractory to medical therapy may be a relative indication for PPV, especially when another vitreoretinal complication exists. It is controversial whether PPV improves CME. PPV could theoretically reduce CME by eliminating macular contact with the inflamed vitreous gel or by allowing better tissue penetration of medications. Since PPV may also be complicated by postoperative CME, adequate immunosuppressive treatment prior to PPV may help reduce the risk of late CME.

Chronic CME probably causes secondary changes to the photoreceptors. An increased size of the foveal avascular zone in fluorescein angiography should alert the clinician that the chance of an increase in visual acuity after PPV is probably minimal. However, an accepted indication for PPV in CME is the presence of vitreous traction to the macula, which can be demonstrated by optical coherence tomography (OCT).

Summary for the Clinician

Indications for PPV in Uveitis

Accepted Indications

- Urgent indications
  - Rhegmatogenous retinal detachment
  - Phacolytic uveitis
- Elective indications
  - Visually significant vitreous opacities despite adequate treatment with corticosteroids or immunosuppressive therapy
  - Vitreomacular traction associated with macular oedema
  - Non-clearing vitreous haemorrhage
  - Visually significant epiretinal membranes
  - In combination with other ocular procedures: pars plana lensectomy, placement of GDD
  - For diagnosis of infections or intraocular lymphoma

Relative or Controversial Indications

- Alternative to immunosuppressive or corticosteroid therapy
- CME unresponsive to medical treatment
- Hypotony due to cyclitic membrane
- Combined with extracapsular cataract surgery and intraocular lens implantation
- Removal of chronic vitreous opacities with mild to moderate decrease in vision

Contraindications to PPV

- Epiretinal membrane not causing decreased visual acuity
- Chronic CME with damaged photoreceptors
- Stable traction detachments
- Inflammatory choroidal effusion
- Exudative retinal detachments
- Anterior uveitis or posterior uveitis without significant vitreous inflammation
- Acute active uveitis, as PPV may be followed by severe postoperative inflammation
- Suspected retinoblastoma masquerading as uveitis

References

1. Becker MD, Harsch N, Zierhut M, et al. (2003) Therapeutic vitrectomy in uveitis. Ophthalmologe 10:787–795
2. Fujii GY, De Juan E Jr, Humayun MS, et al. (2002) A new 25-gauge instrument system for transconjunctival sutureless vitrectomy surgery. Ophthalmology 10:1807–1812
3. Sheppard JD, Shrum KR (1995) Pars plana Molteno implantation in complicated inflammatory glaucoma. Ophthalmic Surg 3:218–222
4. Lam LA, Lowder CY, Baerveldt G, et al. (2003) Surgical management of cataracts in children with juvenile rheumatoid arthritis-associated uveitis. Am J Ophthalmol 6:772–778
5. Heiligenhaus A, Bornfeld N, Foerster M, et al. (1994) Long-term results of pars plana vitrectomy in the management of complicated uveitis. Br J Ophthalmol 7:549–554
6. Leuenberger P (1984) L'interet de la vitrectomie dans le traitement des uveites posterieures. Klin Monatsbl Augenheilkd 5:445–448
7. Sikic J, Suic S (2001) Surgical treatment of uveitis. Coll Antropol 71–76
8. Stavrou P, Baltatzis S, Letko E, et al. (2001) Pars plana vitrectomy in patients with intermediate uveitis. Ocul Immunol Inflamm 3:141–151
9. Thumann G, Bartz-Schmidt K, Esser P, et al. (1997) Vitrektomie in der Behandlung von Augen mit komplizierter Uveitis. Klin Monatsbl Augenheilkd 4:241–244
10. Verbraeken H (1996) Pars plana lensectomy in cases of cataract with juvenile chronic uveitis. Graefes Arch Clin Exp Ophthalmol 10:618–622
11. Wiechens B, Nolle B, Reichelt J (2001) Pars-plana vitrectomy in cystoid macular edema associated with intermediate uveitis. Graefes Arch Clin Exp Ophthalmol 7:474–481
12. Wiechens B, Reichelt J, Urbat C, et al. (2003) Pars-plana-Vitrektomie bei zystoidem Makulaödem bei verschiedenen Formen der chronischen Uveitis. Ophthalmologe 1:33–43
13. Shah GK, Stein JD, Sharma S, et al. (2000) Visual outcomes following the use of intravitreal steroids in the treatment of postoperative endophthalmitis. Ophthalmology 3:486–489
14. Enaida H, Hata Y, Ueno A, et al. (2003) Possible benefits of triamcinolone-assisted pars plana vitrectomy for retinal diseases. Retina 6:764–770
15. Sonoda K, Enaida H, Ueno A, et al. (2003) Pars plana vitrectomy assisted by triamcinolone acetonide for refractory uveitis: a case series study [In Process Citation]. Br J Ophthalmol 8:1010–1014
16. Ram J, Brar GS, Kaushik S, et al. (2003) Role of posterior capsulotomy with vitrectomy and intraocular lens design and material in reducing posterior capsule opacification after pediatric cataract surgery. J Cataract Refract Surg 8:1579–1584
17. Diamond J, Kaplan H (1978) Lensectomy and vitrectomy for complicated cataract secondary to uveitis. Arch Ophthalmol 10:1798–1804
18. Flynn H, Davis J, Culbertson W (1988) Pars plana lensectomy and vitrectomy for complicated cataracts in juvenile rheumatoid arthritis. Ophthalmology 8:1114–1119
19. Lahey JM, Francis RR, Kearney JJ (2003) Combining phacoemulsification with pars plana vitrectomy in patients with proliferative diabetic retinopathy: a series of 223 cases. Ophthalmology 7:1335–1339
20. Koenig SB, Han DP, Mieler WF, et al. (1990) Combined phacoemulsification and pars plana vitrectomy. Arch Ophthalmol 3:362–364
21. Dev S, Mieler W, Pulido J, et al. (1999) Visual outcomes after pars plana vitrectomy for epiretinal membranes associated with pars planitis. Ophthalmology 6:1086–1090
22. Scott R, Sullivan P, Aylward G, et al. (2001) The effect of pars plana vitrectomy in the management of Fuchs heterochromic cyclitis. Retina 4:312–316
23. Waters F, Goodall K, Jones N, et al. (2000) Vitrectomy for vitreous opacification in Fuchs' heterochromic uveitis. Eye 2:216–218
24. Avramides S, Sakkias G, Traianidis P (1997) Cataract surgery in Fuchs' heterochromic iridocyclitis. Eur J Ophthalmol 2:149–151
25. Daus W, Schmidbauer J, Buschendorff P, et al. (1992) Results of extracapsular cataract extraction with intraocular lens implantation in eyes with uveitis and Fuchs' heterochromic iridocyclitis. Ger J Ophthalmol 6:399–402
26. Jones NP (1996) Cataract surgery in Fuchs' heterochromic uveitis: past, present, and future. J Cataract Refract Surg 2:261–268
27. Limon S, Boscher C, Abenhaim A, et al. (1985) Interet de la vitrectomie precoce au cours de la maladie de behcet compliquee de hyalite, a propos de 20 cas. Bull Soc Ophtalmol Fr 3:363–365
28. Limon S, Renard J, Waligora G, et al. (1989) Interet de la vitrectomie dans les uveites intermediaires et la maladie de Behcet avec hyalite. Ophtalmologie 3:206–208
29. Ozdemir O, Erkam N, Bakkaloglu A (1988) Results of pars plana vitrectomy in Behcet's disease. Ann Ophthalmol 1:35–38
30. Eckardt C, Bacskulin A (1992) Vitrectomy in intermediate uveitis. Dev Ophthalmol 23:232–238

31. Kroll P, Romstock F, Grenzebach U, et al. (1995) Frühvitrektomie bei endogener juveniler Uveitis intermedia – eine Langzeitstudie. Klin Monatsbl Augenheilkd 4:246–249
32. Schönfeld C, Weissschadel S, Heidenkummer H, et al. (1995) Vitreoretinal surgery in intermediate uveitis. Ger J Ophthalmol 1:37–42
33. Bacskulin A, Eckardt C (1993) Ergebnisse der Pars plana-Vitrektomie bei chronischer Uveitis im Kindesalter. Ophthalmologe 5:434–439
34. Ulbig M, Kampik A (1989) Pars plana-Vitrektomie bei chronischer Uveitis des Kindes. Klin Monatsbl Augenheilkd 1:10–12
35. Miller D, Davis J, Rosa R, et al. (2000) Utility of tissue culture for detection of *Toxoplasma gondii* in vitreous humor of patients diagnosed with toxoplasmic retinochoroiditis. J Clin Microbiol 10:3840–3842
36. Belmont J, Irvine A, Benson W, et al. (1982) Vitrectomy in ocular toxocariasis. Arch Ophthalmol 12:1912–1915
37. Davis JL (1999) Management of CMV retinal detachments in the new era of antiretroviral therapy. Ocul Immunol Inflamm 3–4:205–213
38. Canzano JC, Morse LS, Wendel RT (1999) Surgical repair of cytomegalovirus-related retinal detachment without silicone oil in patients with AIDS. Retina 4:274–280
39. Ahmadieh H, Soheilian M, Azarmina M, et al. (2003) Surgical management of retinal detachment secondary to acute retinal necrosis: clinical features, surgical techniques, and long-term results. Jpn J Ophthalmol 5:484–491

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