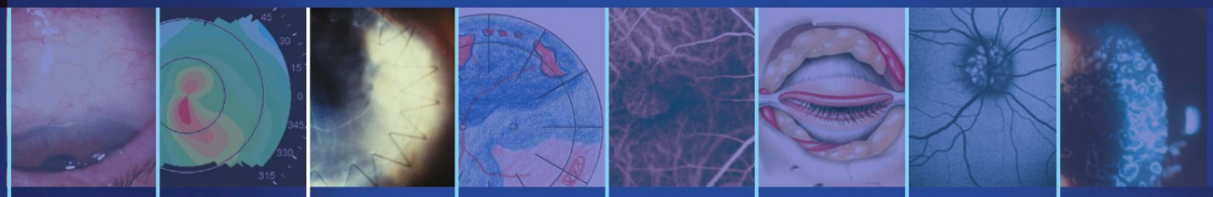
ESSENTIALS IN OPHTHALMOLOGY

G. K. KRIEGLSTEIN · R. N. WEINREB

Series Editors



Glaucoma | Cataract and Refractive Surgery | Uveitis and Immunological Disorders | Vitreo-retinal Surgery | Medical Retina | Oculoplastics and Orbit | Paediatric Ophthalmology, Neuro-ophthalmology, Genetics | Cornea and External Eye Disease

Uveitis and Immunological Disorders

Edited by

U. PLEYER

C. S. FOSTER

Springer

Essentials in Ophthalmology

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Uveitis and Immunological Disorders

With 88 Figures, Mostly in Colour
and 22 Tables

Springer

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Foreword

The series *Essentials in Ophthalmology* was initiated two years ago to expedite the timely transfer of new information in vision science and evidence-based medicine into clinical practice. We thought that this prospicient idea would be moved and guided by a resolute commitment to excellence. It is reasonable to now update our readers with what has been achieved.

The immediate goal was to transfer information through a high quality quarterly publication in which ophthalmology would be represented by eight subspecialties. In this regard, each issue has had a subspecialty theme and has been overseen by two internationally recognized volume editors, who in turn have invited a bevy of experts to discuss clinically relevant and appropriate topics. Summaries of clinically relevant information have been provided throughout each chapter.

Each subspecialty area now has been covered once, and the response to the first eight volumes in the series has been enthusiastically positive. With the start of the second cycle of subspecialty coverage, the dissemination of practical information will be continued as we learn more about the emerging advances in various ophthalmic subspecialties that can be applied to obtain the best possible care of our patients. Moreover, we will continue to highlight clinically relevant information and maintain our commitment to excellence.

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

Preface

This second volume of *Uveitis and Immunological Disorders* in the *Essentials in Ophthalmology* series provides the reader with up-to-date and relevant information. Our knowledge and understanding of immune-mediated diseases has increased exponentially over the past few years, 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 the mechanisms underlying these conditions that incorporate the most recent, up-to-date research material available. The features "Summary for the Clinician" and "Core Messages" enhance the value of the chapters by helping the reader 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, such as contact lens-associated infections, autoimmune keratitis and some forms of uveitis. Several topics, for example handling corneal graft rejection and cataract extraction in uveitis patients are of particular interest for the ocular surgeon. Two chapters focus on recurrent ocular infections, herpes keratitis and ocular toxoplasmosis, which still remain sight-threatening disorders. Our better understanding of the underlying immune pathology has resulted in new treatment approaches, which are highlighted by experts on anti-TNF and gene therapeutic strategies.

This volume contains information of interest to a wide range of ophthalmic subspecialists. For example, the anterior segment subspecialist would have an interest in subjects such as contact lens associated infections, autoimmune Keratitis, ocular allergy, dry eye, corneal transplantation and herpes keratitis. Retina and uveitis specialists have a special interest in the chapters dealing with uveitis and its mechanisms and latest aspects in therapy. Lastly, the chapters on optic neuritis and neoplastic masquerade syndromes are important for interdisciplinary handling of these patients.

We are glad that the previous volume of *Uveitis and Immunological Disorders* had a broad readership and positive acceptance, which is underlined by the fact that it has been translated into Chinese and Italian.

We are sure that this edition will also reach its audience and would like to thank all authors who contributed their valuable time to complete this volume.

U. Pleyer
C. S. Foster
Volume Editors

Contents

Chapter 6
Recent Developments in Herpes Stromal Keratitis

Chapter 7
Genetic Insights into Uveitis

Chapter 8
Cataract Surgery in Patients with Uveitis

Chapter 12
Tumor Necrosis Factor Alpha-Targeted Therapies in Uveitis
Susan Cochrane, Andrew D. Dick

Chapter 13
Immunotherapy of Uveitis: is Gene Therapy in our Future?
Rachel R. Caspi

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Chapter 1

Contact Lens-Related Corneal Infection

1

Barry A. Weissman, Anthony J. Aldave, Bartly J. Mondino

Core Messages

- Complications of contact lens wear are numerous and occur in all external ocular tissues. Only microbial keratitis (MK) and neovascularization, however, are common causes of associated loss of vision.
- MK is an ophthalmic emergency because of the potential for loss of vision.
- Contact lens wear has become a major risk factor for MK, joining trauma, dry eye, and preceding corneal surgery (e.g., cataract extraction, penetrating keratoplasty, refractive surgery).
- Extended wear and poor contact lens care remain the major risk factors for contact lens-associated MK.
- Using modern, highly oxygen permeable contact lenses (Dk values of 100 Fatt units or greater) under open-eye conditions should result in corneal oxygenation similar to that found without any contact lenses. Use of high-Dk rigid and soft lenses for extended wear may moderate the risk of MK, but may not reduce it to the levels found with daily wear of contact lenses.
- Bacteria cultured from contact lens-associated MK are commonly *Pseudomonas* sp. and *Staphylococcus sp.* Bacterial MK is more commonly associated with extended contact lens wear as well as poor contact lens care and hygiene.
- Milder, less threatening, presumed bacterial MK is often initially treated with topical antibiotic monotherapy and close professional supervision, but more severe and/or central infections should first undergo laboratory investigations (cultures, smears, stains) and then be treated aggressively with fortified antibiotics. The clinician should always remain suspicious of *Acanthamoeba* in any contact lens-associated MK.
- *Acanthamoeba* MK is more commonly associated with daily wear, poor contact lens care, and lens exposure to fresh water as opposed to proper contact lens care solutions. *Acanthamoeba* infections can masquerade as herpetic or fungal keratitis in particular, and pain is often out of proportion to the clinical signs.
- Steroid treatment of contact lens-associated MK remains controversial.
- Customized rigid gas permeable contact lenses can often improve vision dramatically after MK has healed, decreasing the need for corneal transplantation.

1

1.1 Introduction

The traditional major risk factors for microbial corneal infection (microbial keratitis or MK) include trauma and preceding corneal compromise such as surgery (e.g., cataract extraction, penetrating keratoplasty, refractive surgery) or herpetic corneal disease. Other participating factors include systemic (e.g., HIV infection, diabetes) and local (topical steroid treatment) immunosuppression, acne rosacea/blepharitis, severe dry eye, and corneal exposure. Contact lens wear has emerged as another risk factor for MK during the past 50 years [14, 43].

Contact lens wear has a long list of potential complications including edema of the various corneal layers, corneal abrasions and neovascularization, and lens soilage and its sequelae (i.e., giant papillary conjunctivitis; Table 1.1), but most patients rarely experience problems that result in permanent vision loss.

Summary for the Clinician

- Complications of contact lens wear can affect all ocular tissues, but are usually benign if patients refrain from sleeping or napping with contact lenses in their eyes, and when patients are compliant with good contact lens care and appropriate hygiene.
- Most complications are self-limiting, reversing even without medical treatment when contact lenses are removed.
- Both MK and neovascularization, however, may result in more serious vision compromise.

Microbial keratitis is unfortunately also a complication of contact lens wear, and, while rare, contact lens-related MK is a sight-threatening disease. For this reason, MK is considered an ophthalmic emergency. Even when "successfully" treated, MK can result in corneal scarring and neovascularization leading to the loss of central corneal clarity, necessitating a corneal transplant in an effort to restore vision. Unsuccessful management may result in the permanent loss of visual function and perhaps even the loss of an eye. Because it can result in a substantial loss of vision, MK is the contact lens wear-associated complication of most concern to both patients and practitioners alike.

Microbial keratitis is identified by the symptoms of sudden-onset ocular pain or foreign body sensation, decreased vision, photophobia, conjunctival vascular injection, discharge and/or lid crusting, blepharospasm, and by the observation of clinical signs of a corneal epithelial/stromal defect with associated inflammatory response (corneal infiltration). MK is often accompanied by an anterior chamber reaction (including a hypopyon in some cases) and lid swelling. Stein et al. [55] found that culture-proven contact lens-associated bacterial corneal infections were more likely:

1. When lesions were single and large rather than multiple, arcuate or small;
2. With epithelial defects, conjunctival discharge, and anterior chamber reactions;
3. When patients were more rather than less symptomatic (pain and photophobia) (Table 1.2).

When suspicious signs and symptoms are found in a contact lens wearer, lesions should be assumed to be infectious in nature and treated accordingly (see below for treatment protocols) until proven otherwise. Whenever any of the signs or symptoms of corneal infection occur, contact lens wear should also be immediately discontinued in both eyes to decrease the potential for bilateral disease.

To add to clinical confusion, however, both corneal infiltrates and epithelial erosions (varying from mild staining to frank abrasion) can occur as nonconcomitant lesions and as such are often noninfectious. Causes include hypoxia, toxic or hypersensitivity reactions, mechanical lens defects and poor fits, lens over-wear, and foreign bodies. Treatment of either of these complications may be similar or differ from that of corneal infection, but is beyond the scope of this chapter.

Other causes of red, painful eyes not specifically associated with contact lens wear that must also be considered in the differential diagnosis include conjunctivitis (allergic as well as infectious), glaucoma (especially acute angle closure), and both iritis and uveitis.

Table 1.1 Physiological complications of contact lens wear. (From [19], with permission.) *GPC* giant papillary conjunctivitis, *SEAL* superior epithelial arcuate lesion, *MK* microbial keratitis

Tissue	Complication type: probable cause(s)
Lids	Toxicity: solution sensitivity
	Allergy: papillary conjunctivitis; GPC due to lens soilage
	Ptosis: GPC; lens insertion and removal
	Blepharitis: bacterial; meibomian gland dysfunction
Bulbar conjunctiva	Injection: mechanical irritation, dry eye; solution sensitivity; hypoxia
	Edema: mechanical irritation; solution sensitivity
	Staining: mechanical irritation; solution sensitivity
Corneal epithelium	3–9 stain: desiccation; contact lens edge chafing
	Pancorneal stain: solution sensitivity; toxicity; blepharitis
	SEAL: mechanical lens problem; lens soilage
	Inferior arcuate stain: desiccation through soft lens
	Foreign body tracks: mechanical foreign body or lens defect
	Cluster stain: contact lens over-wear; hypoxia
	Inferior band (exposure) stain: dry eye (exposure keratopathy); blepharitis
	Abrasion: mechanical foreign body or lens defect; hypoxia; flat contact lens fit, keratoconus, anterior basement membrane dystrophy
	Dimple veil: air bubbles trapped in the tears between the lens and the anterior corneal surface
	Infiltration: infection (viral, bacterial, etc.); solution sensitivity; hypoxia
	Edema (microcysts): hypoxia, endothelial cell dysfunction
Corneal stroma	Edema (central corneal clouding or stromal striae); hypoxia; endothelial cell dysfunction
	Infiltrates: infection (viral, bacterial, etc.); solution sensitivity; hypoxia
Neovascularization	3–9: pseudopterygium: chronic desiccation; chronic lens edge defects, chafing
	Pannus: hypoxia; noncontact lens cause
	Deep stromal vessels: hypoxia; noncontact lens cause (e.g., MK, lues, keratoconus)
Corneal endothelium	Blebs: acute hypoxia, Fuch's dystrophy
	Polymegathism: chronic hypoxia; ageing; anterior segment surgery; Fuch's dystrophy
Microbial corneal infection	Bacterial, protozoal (amoebic), fungal. Viral

Table 1.2 Clinical comparison between bacterial and noninfectious keratitis. (Reprinted from [58], with permission from Elsevier.)

Feature	Bacterial keratitis	Noninfectious keratitis
Onset	Usually acute	Subacute or acute
Predisposing factors	Various: trauma, contact lens wear, prior ocular surface disease, and surgery	Various, including toxic and allergic insults, contact lens wear, blepharoconjunctivitis, herpetic eye disease.
Symptoms	Moderate to severe, increasing pain and light sensitivity	Variable, usually initially mild discomfort or foreign body sensation
Eyelids	Lid edema	Pseudoptosis possible
Conjunctiva	Marked hyperemia with episcleral injection and mucopurulent discharge	Mild hyperemia with mucoid or watery discharge
Corneal epithelium	Usually ulcerated; single larger lesions more common	Usually intact, possibly with punctate staining; can be multiple or arcuate lesions
Corneal stroma	White-yellow suppurative infiltrate with blurred margins and surrounding inflammatory cells and edema, > 1.5 mm, increasing over 24 to 36 hours	White-gray superficial infiltrates usually <1–1.5 mm (tend to remain small)
Corneal endothelium	Pseudoguttata with occasional inflammatory plaque or ring under stromal infiltrate	Minimal changes
Anterior chamber	Variable: cells/flare/hypopyon common	Mild; cells and flare, hypopyon uncommon

Summary for the Clinician

- Microbial keratitis is distinguished from noninfectious kerato-conjunctivitis by its increased severity of symptoms (pain and photophobia) and signs of corneal epithelial defects with associated inflammation (corneal infiltrates, conjunctival injection, and both anterior chamber cell/flare/hypopyon and lid swelling).

1.2 Risk Factors

1.2.1 Extended Wear

Patients can use contact lenses for wear solely during their normal daily activities ("open" eye) or also for use over one or several sleep cycles (extended wear or "closed" eye conditions). "Continuous" wear, alternatively, has been defined as contact lens wear uninterrupted by any intentional occasional lens removal.

The extended and continuous wear of hydrogel contact lenses, in particular, has been shown in several studies to increase the risk of MK [23, 40, 57]. MK has been shown to have an incidence of about 20 per 10,000 people using hydrogel contact lenses for extended wear and about 4 per 10,000 people using hydrogel contact lenses for daily wear per year [9, 48, 52]. Slightly higher rates were recently reported as well [23]. Daily wear of rigid gas permeable (GP) contact lenses is associated with a much reduced risk of MK [9, 36, 44]. The rate of MK with either high Dk silicone hydrogel or GP contact lenses used for extended wear is still in question, but is expected to be less than that found with hydrogel lenses – although it may remain higher than that encountered with daily wear of the same lenses [43, 45].

1.2.2 Contact Lens Care

It seems intuitive that poor contact lens care and hygiene might lead to increased microbial contamination of contact lenses, solutions, and cases. It also seems intuitive that an increased load of micro-organisms in the local environment, available for transfer from the environment to the eye during contact lens "cleaning" and handling, might increase the risks of MK. This particular paradigm of infection may indeed be supported somewhat by data in the case of acanthamoebic infection [16], but – while theoretically attractive – may not be totally supported in the case of bacterial MK [15, 40]. Nonetheless, most clinicians believe that, in general, both extended wear and poor contact lens care increase the risk of MK.

1.2.3 Role of Hypoxia

All rigid contact lenses were made of nonoxygen-permeable polymethyl methacrylate in the mid-1970s, and early hydrogel lenses all had modest oxygen transmissibilities (known as Dk/t). Hypoxia was a very common complication of contact lens use [21, 22, 28, 53].

It is now clear that maintaining oxygen tension in the tear layer (over the metabolizing anterior corneal surface) of about 100 mmHg will preclude physiological hypoxia, although various studies have placed this value between about 20 and 125 mmHg [7, 22, 49].

Most of the modern modest Dk GP and hydrogel contact lenses now available, and particularly those very high Dk silicone hydrogel and GP manufactured from materials with oxygen transmissibility of about 100 Fatt Dk units or greater, generally do not cause clinically observable corneal hypoxia under daily wear conditions [6, 7, 21]. Lenses made from these very high Dk GP and silicone hydrogel materials also appear to provide adequate corneal oxygenation when used on an extended wear basis, even though the precise level of contact lens oxygen permeability necessary to preclude hypoxia under such conditions has yet to be established [7].

When there is clear clinical evidence of hypoxic corneal changes (e.g., epithelial or stromal edema [28], corneal pannus greater than approximately 2 mm unrelated to 3/9 stain [8]), conjunctival and limbal hyperemia (e.g., injection) [47], myopic "creep" [17], or suspected "corneal exhaustion syndrome" [56], the clinician should adjust the contact lens wear schedule or change the contact lens material or design to enhance the availability of oxygen to the anterior corneal surface. Because of all these complications as well as the suspicion that hypoxia increases the risk of MK, increasing contact lens Dk/t is believed to be advantageous.

1.2.4 Role of Immunology

Immunology has been defined as the collection of integrated systems by which an organism defends itself from the assault of micro-organisms. There are both active and passive defenses, including leukocytes, antibodies, skin, and tears. There is a balance at work in that any infection, for example MK, only occurs when the pathogenicity of the microbe overwhelms the immunological defenses of the host.

A major question is whether addressing hypoxia alone is sufficient to reduce the incidence and prevalence of MK during contact lens extended wear to the rate found with daily wear. Several potential paths by which contact lens-driven hypoxia may suppress the immunological defenses of the anterior eye have been proposed. Contact lens wear and hypoxia may cause epithelial defects directly or indirectly (secondary to purely mechanical problems, e.g., abrasions, microtrauma, decreased mitosis and/or adhesion) [4, 20, 35], and any break in the integrity of the ocular surface is known to enhance bacterial infection. Another, more recent, hypothesis is that hypoxia causes changes in the corneal epithelial cell membrane, increasing the potential for bacterial binding [50].

Others believe that there are changes in the closed-eye state, particularly in the constituents of the tears [51], and/or in the ability of the corneal epithelium to resist bacterial invasion [18], beyond hypoxia alone, that makes closed-eye contact lens wear more likely to interrupt the normal immunological defenses of the anterior eye than open-eye contact lens wear. Tears usually

contain multiple antibacterial factors, including lysozyme, lactoferrin, lipocalin, vitronectin, betalysin, phospholipase A2, complement, immunoglobulins, mucins (which may entrap microorganisms for mechanical removal) [24, 51, 59] and occasional leukocytes, all potential targets for disruption. Both local and systemic disease (like Sjögren's syndrome and diabetes) and local or systemic immunosuppression (topical steroid use or HIV infection) are known to disturb one or more aspects of the protective nature of normal tears and/or the ocular surface to increase the risk of corneal infection. Closed-eye contact lens wear, with or without hypoxia, may act similarly. Investigators are actively studying the basic interactions between host and bacteria [11, 18], hoping to unravel the mechanism(s) that allow bacterial invasion of corneal epithelial cells with the goal of discovering ways in which to interrupt these processes.

This is a rapidly evolving research area directed toward enhancing safe contact lens daily and extended wear by assisting the normal immunological defenses of the anterior eye and/or by decreasing the ability of the micro-organisms to attack ocular tissues.

1.2.5 Role of Orthokeratology

Orthokeratology (OK) is the planned use of rigid contact lenses to deliberately modify the anterior corneal surface to neutralize refractive error. OK has been practiced for about half a century, and while efficacy has been questioned by some clinicians, safety has always appeared acceptable.

Recent innovations in rigid GP contact lens manufacture has led to the development of so-called "reverse geometry" contact lenses (with secondary curves steeper rather than flatter than the lens base curve) and the use of these lenses has clearly demonstrated increased efficacy in the OK treatment protocol. At the same time, however, some advocates of this procedure have suggested that OK lenses should be used during sleep (extended wear) as so-called "retainer" lenses and removed during open-eye experience.

This change in lens wear paradigm has unfortunately been accompanied by a number of case reports of subsequent MK in patients treated with unknown OK rigid lenses outside North America and Europe, initially dismissed as "unusual." Recently, reports of MK with OK using known GP lenses of modern designs inside the USA [31, 34] have reached the literature. Is this possible increase in risk more associated just with increased numbers of wearers due to increased popularity, or increased epithelial damage by mechanical pressure on the corneal apex due to OK treatment – or perhaps just closed-eye use as discussed above rather than any specific mechanical or lens design feature of OK? Evolving research will undoubtedly address these questions.

1.3 Microbes

1.3.1 Bacterial Infections

Bacterial corneal infections associated with contact lens (particularly extended) wear are usually attributable to Gram-negative *Pseudomonas aeruginosa*, and less commonly to both Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis* [40, 57]. Other bacteria, both Gram-positive and Gram-negative (such as *Proteus*, *Serratia*, *Bacillus* sp., etc.), are also occasionally cultured from such lesions. For contrast, noncontact lens-associated corneal infections are usually more commonly Gram-positive (*Staphylococcus aureus* or *Streptococcus pneumonia*), Gram-negative *Moraxella* sp., or viral (Herpes). Climate and other environmental factors clearly play a role in the epidemiology of noncontact lens-related corneal infection as well, with more fungal keratitis reported from both the south-eastern United States as well as following direct (e.g., traumatic) exposure to plant matter.

Contact lens-related bacterial corneal infection has been primarily associated with wearing rigid or hydrogel contact lenses of limited oxygen transmissibility through one or more sleep cycles (extended or continuous wear) [9, 12, 29, 40, 48, 52, 57, 61]. Some have suggested that hypoxia alone is necessary and sufficient to account for all or most bacterial corneal infections that occur during contact lens wear, but this has not been proven.

Gram-negative bacterial infections tend to be more aggressive, leading to stromal necrosis with substantial discharge (Fig. 1.1), and Gram-posi-

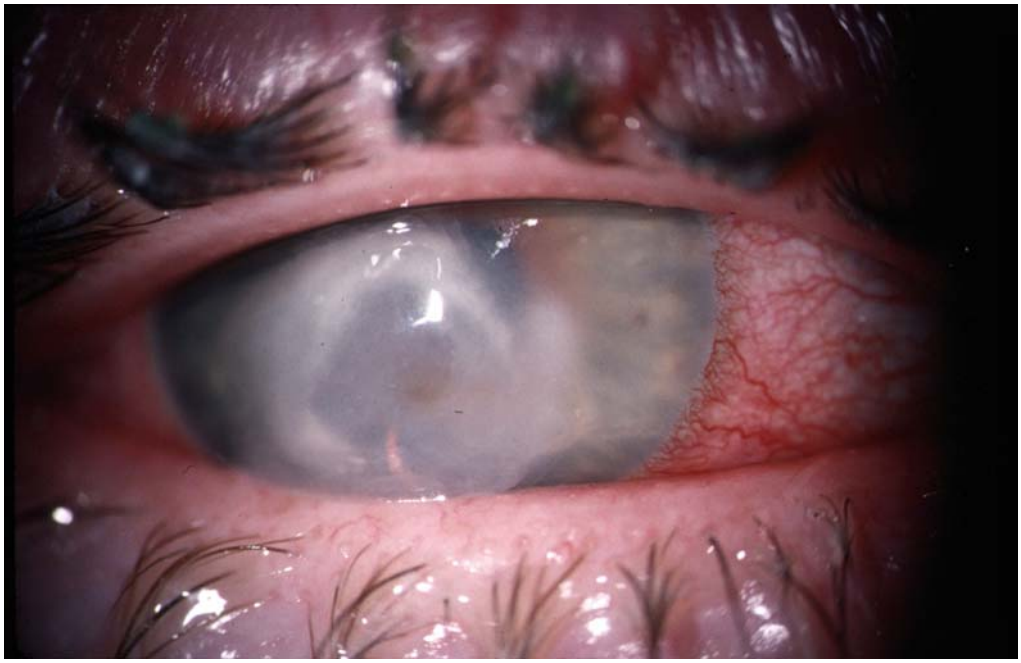

Fig. 1.1 Pseudomonas keratitis following contact lens wear: note both mucopurulent discharge and corneal ring abscess

tive bacterial lesions tend to be less aggressive leading to less discharge and stromal melting, but history and clinical appearance alone may be misleading. Annular corneal infiltrates are seen not only late in the course of acanthamoebic keratitis and early in severe pseudomonas-related corneal infections, but also in the form of an immune ring in herpetic and fungal corneal disease, and sterile anesthetic abuse as well. Results of smears and cultures, and clinical course, are often needed to develop a specific microbiologic diagnosis and hence an appropriate treatment protocol.

Poor compliance with contact lens care procedures leading to enhanced microbiological contamination of lens care solutions, cases, etc., also appears to be a major risk factor for microbial infection, possibly bacterial, but especially due to *Acanthamoeba* [16, 40].

1.3.2 Protozoal Infections

The clinician should always consider the possibility of *Acanthamoeba* species infections in any contact lens-related MK, especially in cases of chronic disease with initially negative culture results that fail to respond to antibiotic therapy. Clinical suspicion should be increased when the patient reports extreme ocular pain and/or a history of exposing his or her contact lenses to nonsterile water, or when an unusual dendritic epitheliopathy (reminiscent of herpetic epithelial disease; Fig. 1.2) or peripheral corneal radial neuropathy (Fig. 1.3) is observed [27, 41, 42, 54].

Acanthamoeba infections can be particularly challenging to confirm by laboratory investigations. Special culture techniques are available, such as culturing on nonnutrient agar coated with an *E-coli* overlay, but corneal biopsy is often necessary. Amoeba cell walls stained with calcofluor white will be seen when examined with fluorescent microscopy. Confocal microscopy can be useful for the diagnosis of corneal infections with *Acanthamoeba*; unfortunately, the limited availability of such instruments in the USA makes cultures and biopsies the more commonly employed diagnostic tests.

Misdiagnosis and medical failures in the treatment of *Acanthamoeba* infections are common.

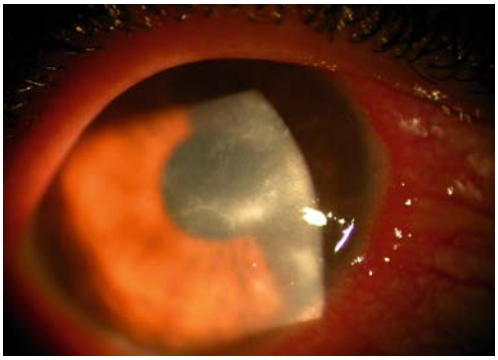

Fig. 1.2 Acanthamoeba keratitis: dendritiform lesion that often leads to misdiagnosis

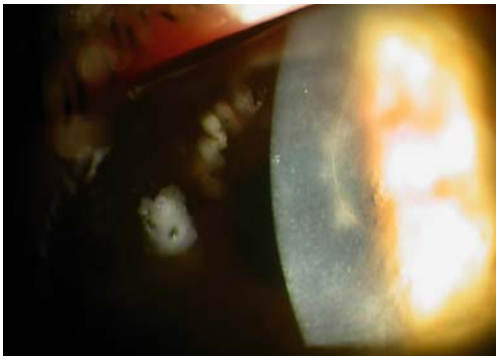

Fig. 1.3 Acanthamoeba keratitis: radial perineuritis

1.3.3 Fungal Infections

Fungal corneal infections (keratomycosis) have been extremely rare among cosmetic contact lens wearers, with the exception of an unusual worldwide collection of Fusarium keratitis possibly related to one brand of soft lens solution in 2006 (under investigation at the time of writing). Most previous cases reported in the literature have involved the use of contact lenses for treatment of aphakia, bandage use of contact lenses, or concomitant chronic treatment with topical steroids in patients suffering from concurrent ocular disease (e.g., neurotrophic epithelial defects, diabetes, trauma) [26, 60]. Fungal corneal infections are often distinguished as "fluffy"-appearing infiltrates with feathered borders, associated with separate satellite lesions. It is important to note that atypical mycobacterium and *Acanthamoeba* infections often mimic fungal corneal ulcers and vice versa.

1.3.4 Viral Infections

Adenoviral and herpetic viral corneal infections can occur during contact lens wear. No causative association has been uncovered for such viral infections. Round subepithelial corneal infiltrates and follicular conjunctivitis can occur with both infections, and discharge tends to be more watery than mucopurulent as in bacterial infections. Both epithelial dendrites and decreased corneal sensitivity are common signs of herpetic infection in particular. Contact lens wear should be discontinued during viral infections unless the contact lens is being used in a treatment protocol. Adenovirus infection is usually successfully managed by supportive therapy such as tear supplements and topical decongestants. Effective topical (Viroptic) and oral antiviral agents are available for the treatment of herpetic eye disease. The clinician who observes apparent herpetic keratitis in association with the use of contact lenses, however, should always consider the possibility of an *Acanthamoeba* infection masquerading as herpes.

It is prudent to consider discarding contact lenses, especially inexpensive disposable soft lenses of any type, that have been worn during an active viral infection and then dispense new contact lenses once the infection has resolved. More expensive customized (primarily rigid GP but also occasionally soft) lenses should be disinfected using the appropriate techniques prior to advising the patient that contact lens wear can be resumed.

Although both the human immunodeficiency virus (HIV) and the prions that cause Creutzfeldt-Jakob disease have been isolated from human ocular tissues (e.g., cornea, conjunctiva, and tears), no reports of disease transmission have been reported from ocular contact. Nonetheless, it is prudent to minimize risks to both patients and clinicians by appropriate disinfection of diagnostic instrumentation, and particularly disinfection (or discarding) of diagnostic contact lenses (whether disease is known, suspected, or unsuspected).

Summary for the Clinician

- Most clinicians believe that both extended wear and poor contact lens care increase the risk of MK.
- When there is clear clinical evidence of hypoxic corneal changes, the clinician should adjust the contact lens wear schedule or change the contact lens material or design to enhance the availability of oxygen to the anterior corneal surface.
- Both local and systemic disease and local or systemic immunosuppression are known to disturb one or more aspects of the protective nature of normal tears and/or the ocular surface and increase the risk of corneal infection. Closed-eye contact lens wear, with or without hypoxia, may act similarly.
- Bacterial corneal infections associated with contact lens wear are usually attributable to Gram-negative *Pseudomonas aeruginosa*, and less commonly to both Gram-positive *Staphylococcus aureus* and *Staphylococcus epidermidis*.
- The clinician should always consider the possibility of *Acanthamoeba* species infections in any contact lens-related MK, especially in cases of chronic disease with initially negative culture results that fail to respond to antibiotic therapy.
- Fungal corneal infections (keratomycosis) have been extremely rare among cosmetic contact lens wearers.
- Adenoviral and herpetic viral corneal infections can occur during contact lens wear. No causative association has been uncovered for such viral infections
- The clinician who observes apparent herpetic keratitis in association with the use of contact lenses, however, should always consider the possibility of an *Acanthamoeba* infection masquerading as herpes.

1.4 Treatment

1.4.1. Bacterial Infections

Traditional management of MK begins with the acquisition of cultures on blood and chocolate agar, on Sabaroud's (for fungi), or in thioglycolate broth (for anaerobes), and with Gram and Giemsa-staining of smears for more immediate microscopic evaluation. A sterilized Kimura or similar spatula is used to acquire material for these laboratory investigations by scraping the base and leading edge of the corneal ulcer [1, 33]. Cultures of lids, conjunctiva, and both contact lens cases and solutions may prove helpful upon occasion.

It should be recognized that the diagnosis and management of corneal infection continues to evolve. In general, the trend among community doctors is toward treating peripheral and small corneal infiltrates without laboratory investigation, while central and large corneal lesions are almost universally cultured prior to treatment [37–39]. Laboratory investigations prior to treatment of all suspected MK remain the standard of care at university medical centers, however, both because such centers direct the teaching of young clinicians and usually the more resistant and aggressive corneal infections present at these tertiary care centers.

Topical fluoroquinolone antibiotics were introduced into ophthalmic care in the early 1990s [32], all but replacing several earlier antibiotics. Several studies discussed the clinically successful use of 0.3% commercial-strength topical fluoroquinolone antibiotics (e.g., Ciloxan) as monotherapy for suspected bacterial corneal infections without cultures, especially when the lesions were relatively small (<2 mm), and neither central nor deep [25, 46]. Many clinicians found fluoroquinolone monotherapy to be as effective as previous dual therapy with "fortified" aminoglycosides (e.g., gentamicin, tobramycin, amikacin) and cephalosporins (see below), and initial cultures were believed unnecessary in many cases. As emerging resistance to the fluoroquinolone antibiotics followed [30], some clinicians discussed a new form of dual therapy, utilizing both fluoroquinolone and cephalosporin agents [5]. The recent availability of fourth generation fluoroquinolones (in off-label use as these agents

are only FDA-approved for the treatment of conjunctivitis) has reversed the trend of treatment back toward monotherapy without cultures, particularly for small and peripheral suspected bacterial infections.

At the time of writing, it appears that small, peripheral, possibly bacterial corneal infections are often initially treated by a combination antibiotic such as Polytrim or a third or fourth generation topical fluoroquinolone antibiotic agent as monotherapy without cultures. With the fluoroquinolones, an initial "loading" dose is established using one drop every 15 min for the first hour of treatment, followed by one additional drop every 1–2 h while the patient is awake. A loading dose may not be needed with fourth generation fluoroquinolones (as their penetration characteristics are excellent), but it is still often used. Professional supervision should be frequent, often at 24-h intervals.

Central and/or larger, more severe, corneal ulcers are treated more aggressively. After scraping, performing a Gram or Giemsa stain, and obtaining cultures, aggressive topical treatment should begin with dual therapy: specially prepared fortified (15 mg/ml) topical aminoglycosides (e.g., gentamicin, tobramycin, amikacin), which have greatest activity against Gram-negative bacteria, and fortified (50 mg/ml) cephalosporins (e.g., cefazolin) or vancomycin (25–50 mg/ml) which are most effective against Gram-positive bacteria, used hourly. Cycloplegic agents may be used to decrease the pain of secondary ciliary spasm and preclude development of posterior synechiae. Treatment may be modified by observation of the patient's clinical course and the laboratory identification of likely microorganisms and their antibiotic sensitivities [1]. Adjunctive patching should be avoided [10].

1.4.1.1 Role of Steroids

The early use of topical steroids is usually contraindicated because these drugs suppress the host immune response, but some doctors will intervene with steroids early with the intention of limiting scar formation from stromal infiltration and neovascularization. This treatment runs the risk, however, of allowing inadequately controlled infections of such microbes as *Pseudomonas* sp., herpes, and *Acanthamoeba*, to escape therapy.

1.4.1.2 Additional Diagnostic and Therapeutic Steps

Occasionally, topical antibiotic management fails and the clinician must consider the accuracy of the initial diagnosis. Further laboratory investigation, corneal biopsy, and more aggressive medical treatment including subconjunctival injections, hospitalization, and perhaps corneal transplantation, may be necessary. Systemic antibiotic treatment may be necessary if infectious scleritis develops.

Proper treatment of bacterial corneal infection, therefore, remains an area of much debate and concern. Clinicians are advised to keep abreast of the latest clinical recommendations and research regarding this evolving subject.

1.4.2 Acanthamoeba Infections

Combinations of the following four types of pharmacological agents have been used successfully for the medical treatment of *Acanthamoeba* keratitis [3, 13]. Most of these drugs are not commercially available in topical formulations for ophthalmic use so they must be specially prepared by the pharmacist:

1. Antibiotic/aminoglycoside: paromomycin, neomycin;
2. Antifungal: clotrimazole, ketoconazole, itraconazole, miconazole, fluconazole;
3. Antiparasitic/aromatic diamidine: propamidine isethionate (Brolene), hydroxystibamidine, hexamidine di-isethionate;
4. Biocide/cationic antiseptic: polyhexamethylene biguanide, chlorhexidine gluconate, povidone-iodine.

Some recommend initial topical treatment with both a diamide and cationic antiseptic immediately after corneal debridement every hour for 48 h, followed by continued treatment during waking hours for the following 3 days, and reduced thereafter to every 3 h. Treatment may continue for several weeks before improvement

is observed. Concomitant steroid treatment is very controversial.

Medical treatment failure may occur in the treatment of *Acanthamoeba* corneal infections, leading to tectonic corneal transplantation.

1.4.3 Fungal Infections

Antifungal pharmaceutical agents (both commercial – topical 5% Natamycin used every 1–2 h – and custom-made by the pharmacist from systemic drugs: amphotericin B, fluconazole, miconazole, and ketoconazole, are commonly used) are available, but medical treatment is often quite difficult, prolonged, and may fail, again leading to tectonic corneal transplantation.

Summary for the Clinician

- Treatment of bacterial infections includes a combination antibiotic or a third or fourth generation topical fluoroquinolone antibiotic agent as monotherapy without cultures.
- Central and/or larger, more severe corneal ulcers are treated more aggressively with specially prepared fortified topical aminoglycosides and fortified cephalosporins or vancomycin
- Treatment of *Acanthamoeba* keratitis includes antibiotic/aminoglycoside, antifungal, antiparasitic/aromatic diamidine, biocide/cationic antiseptic
- Antifungal pharmaceutical agents are available for the treatment of fungal infections, but may fail.

1.5 Successful Treatment

Hallmarks of successful treatment/healing include improved patient comfort (decreasing pain), reduced inflammatory signs (such as corneal infiltrates, lid, and anterior chamber reactions), and closing of epithelial defects. Antibiotic treatment is tapered or discontinued according to clinical response, but because treatment can be prolonged for weeks, toxicity from these drugs is always a potential additional complication. When corneas have been completely healed for a reasonable length of time, rigid GP contact lenses may be helpful in restoring good vision compromised by subsequent central corneal scars and irregular astigmatism as an alternative to corneal transplantation.

1.6 Concluding Remarks

In summary, MK associated with CL wear, while rare, remains an issue for concern [23, 36] and management is complex. Regardless of culture results, aggressive medical treatment and perhaps corneal transplantation may be necessary, especially in cases of indolent, refractory, or nonbacterial corneal infections. The referral of patients with severe or refractory inflammatory or infectious ocular disease to a corneal and external eye disease specialist is often prudent.

Avoidance of this disease is probably the best protocol. Minimizing the risk of contact lens-related MK begins with proper patient selection and continues with appropriate mechanical fitting and use of modern contact lenses made from plastics of reasonable oxygen transmissibilities. Clinicians should advise appropriate wear schedules and counsel their patients about the importance of routine professional supervision. Maintaining hygienic contact lens cleaning techniques, cases, and solutions are other important factors in decreasing the risk of this devastating complication of contact lens use. As stated above, however, MK is fortunately infrequent when patients refrain from extended wear and are compliant with both good contact lens care and hygiene.

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Chapter 2

New Insights into the Diagnosis and Treatment of Dry Eye Syndrome

2

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Core Messages

- Aqueous deficient dry eye is not commonly associated with Sjögren's syndrome (SS), but aqueous-deficient dry eye is often most severe in patients with SS.
- Ocular and oral symptoms, ocular signs, histopathology of salivary and/or lacrimal glands, salivary gland involvement and investigation of serum autoantibodies are key points for establishing the diagnosis of primary or secondary SS.
- The use of dry eye questionnaires, measurement of the tear quantity (Schirmer test), tear stability (tear film break-up time [TBUT]) and ocular surface vital staining are important in the ophthalmological diagnosis of SS.
- Artificial tear solutions, autologous serum eye drops, and lacrimal punctal occlusion are still the mainstays of treatment of SS. Newer approaches such as anti-inflammatory agents, immunosuppressants, and lacrimal gland stimulation may be promising therapeutic options in the near future.

2.1 Introduction

One of the problems in dealing with dry eye conditions is the lack of a precise definition. Until several years ago, the term dry eye implied only tear volume deficiency, which was associated mainly with Sjögren's syndrome (SS). With heightened awareness of dry eye, the term now includes a variety of tear film abnormalities arising from multiple causes, such as aqueous deficiency, mucin or lipid layer abnormalities or impaired lid function. Dry eye syndrome (DES) represents a heterogeneous group of conditions that share inadequate lubrication of the ocular surface as their common denominator. DES is characterized by symptoms of ocular surface dryness and discomfort due to insufficient tear quantity or quality caused by low tear production and/or excessive tear evaporation [23].

The term keratoconjunctivitis sicca (KCS) has been used loosely to describe dry eye disorders. In the UK, KCS tends to be used to describe the autoimmune destruction of the lacrimal gland, whereas in the United States the term is more loosely used to describe other dry eye disorders. Because of the previous confusion associated with terminology in KCS, "dry eye syndrome" may be the more appropriate term to use for classifying these disorders. The National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes in 1993/1994 sought to provide consensus definitions to assist in clinical trial development and communication and reported a global definition of dry eye as follows [11]:

"Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporation that causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort."

In 2004, the Dry Eye Workshop was held and suggested a new definition of dry eye emphasizing the visual disturbance as follows:

"Dye eye is a multifactorial disorder of tears, the ocular surface associated with symptoms of discomfort and/or visual disturbance."

In the decade since the 1994/1995 workshop, numerous advances have been made in relation to dry eye diagnostic markers, technologies and treatment options. Whether these advances can be translated into a greater understanding of and capability for "widely accepted" standards for the diagnosis and treatment of dry eye disorders still remains to be resolved. Dry eye is referred to as Sjögren's syndrome in the presence of hypergammaglobulinemia, rheumatoid arthritis, and antinuclear antibody. Involvement of the salivary glands causes dry mouth. Other mucous membranes, such as bronchial epithelium and the vagina, may also be affected. When these features occur in isolation, the disorder is termed primary SS. When they are associated with a generalized connective tissue disorder, the condition is classified as secondary SS [23]. Although aqueous-deficient dry eye is not commonly associated with SS, aqueous-deficient dry eye is often most severe in patients with SS; thus, this article will mainly focus on SS-associated dry eye.

Summary for the Clinician

- Definition of dry eye is a multifactorial disorder of tears and the ocular surface, associated with symptoms of discomfort and/or visual disturbance.
- Sjögren's syndrome (SS) is a multifactorial autoimmune disorder, mainly affecting the salivary and lacrimal glands, which is influenced by genetic as well as environmental factors.
- In the absence of an associated connective tissue disease, dry mouth and dry eye are referred to as the sicca complex, or primary SS.
- Secondary SS refers to the full triad of xerophthalmia, xerostomia, and a connective tissue or collagen disease such as rheumatoid arthritis (RA), scleroderma or systemic lupus erythematosus (SLE).

2.2 Sjögren's Syndrome

Large population-based studies of dry eye are indeed few. In the Melbourne study, dry eye had been previously diagnosed in less than 1% of subjects and SS in 0.2%. The incidence of primary SS reported in the literature varies from less than 1:1,000 to more than 1:100 [20]. Fox et al. estimated the prevalence of primary SS at approximately 1:1,250 [7]. In studies of secondary SS, the prevalence of sicca symptoms among Greek patients with rheumatoid arthritis (RA) has been estimated to be 31%, with systemic sclerosis at 20% and systemic lupus erythematosus (SLE) at 8% [33]. Approximately 17,000 new cases of SS are being diagnosed in Japan each year. The incidence of SS in Japan employing the Japanese diagnostic criteria is far lower than that of other nations and is reported to be 0.026%, which may owe to racial differences and varying diagnostic criteria [5]. A cautious but realistic estimate from the studies presented until recently is that primary SS is a disease with a prevalence not exceeding 0.6% of the general population [7].

Sjögren's syndrome (SS) is a multifactorial autoimmune disorder, mainly affecting the salivary and lacrimal glands, that is influenced by genetic as well as environmental factors that are not completely understood as yet. Henrik Sjögren described the syndrome in 1933 in the context we understand today. The systemic manifestations of SS are generally the result of a lymphocyte-mediated toxicity. Although dry eyes and dry mouth characterize the disease, the expression of the clinical spectrum is diverse, extending from a solitary organ-specific autoimmune exocrinopathy to a systemic disorder affecting several organs. In the absence of an associated connective tissue disease, dry mouth and dry eye are referred to as the "sicca complex, or primary SS." Secondary SS refers to the full triad of xerophthalmia, xerostomia, and a connective tissue or a collagen disease such as RA, scleroderma or SLE.

2.3 Diagnosis of Dry Eye Disease

A questionnaire is useful in assessing severity and duration of dry eye-associated symptoms, while vital staining identifies the ocular surface disease. Measurement of the tear quantity (Schirmer

test) and tear stability (tear film break-up time [TBUT]) provides useful information on the assessment of tear functions in dry eye syndrome.

2.3.1 Symptoms

The ocular surface does not contain specific receptors for dryness and patients therefore complain of a wide variety of symptoms such as grittiness, irritation, burning and/or stinging, redness, pain, ocular fatigue or visual disturbance. It is thought that a diurnal variation of symptoms and exacerbation by certain activities are indicative of dry eye disease. Symptoms usually become worse during the day, but may be present on waking, which may reflect reduced production during sleep. Long periods of reduced blinking (VDT use or driving), as well as air conditioning and the low humidity in aircraft cabins, all exacerbate the condition. Iatrogenic causes of dry eye include systemic and topical drugs, and certain autoimmune diseases are associated with SS.

2.3.2 Assessment of Ocular Surface Epithelial Health Status

2.3.2.1 Ocular Surface Vital Staining: Fluorescein staining

Fluorescein staining indicates increased epithelial permeability of the cornea or conjunctiva. A minimal amount of dye (1 µl of 1% fluorescein) should be applied with a sterile disposable applicator using a micropipette, or by using a fluorescein-impregnated strip. The strip is wetted with a drop of sterile preservation-free solution, the excess is shaken off and the strip is touched to the lower fornix. An improved view can be gained by using a yellow barrier filter with the cobalt blue light of the slit lamp.

2.3.2.2 Ocular Surface Vital Staining: Rose Bengal/ Lissamine Green Staining

Rose Bengal stains dead and degenerating conjunctival cells and corneal epithelial filaments in a dark pink color. It is toxic to the epithelium and causes irritation on instillation so only a small amount (1 µl of 1% Rose Bengal) should be applied with a sterile disposable applicator. Lissamine green is similar to Rose Bengal in that it is seen best over the sclera and least over the dark iris, but it is less toxic and better tolerated.

2.3.3 Assessment of Tear Film Stability: Invasive TBUT Using Fluorescein

Invasive TBUT using fluorescein (FTBUT) is carried out using a small amount of fluorescein, which is applied into the lower fornix using an applicator. The patient is asked to blink and then keep the eyes open. The time taken for the first black spot in the stained tear film is recorded as the FTBUT. The test is ideally repeated three times and the mean is recorded as the TBUT of that eye. Currently, a TBUT value greater than 10 s is regarded as normal.

2.3.4 Assessment of Aqueous Tear Quantity

Aqueous tear quantity is measured using the Schirmer test, which may be performed with or without the use of a topical anesthetic. For the Schirmer test a strip of filter is placed with its tip in the lower lateral conjunctival fornix, and the length that becomes wet within 5 min is measured.

The Schirmer test I measures the reflex of tear secretion, but the result depends on temperature, humidity, and evaporation, so these values need to be reasonably consistent for all tests. Avoid touching all but the end of the strip to prevent skin secretion affecting the result. The patients should blink normally during the test. A dry eye is present if less than 5 mm of the strip is wetted in 5 min. The Schirmer test I with anesthetic gives a lower value than without anesthetic. It is believed to measure the basal secretion rate in the absence of a reflex tear component.

2.3.5 Newer Diagnostic Tools

Relatively new diagnostic techniques with improved sensitivity and specificity include tear film osmolarity (usually in excess of 323 mOsm/kg in dry eye disease of SS), tear fluid immunoassays measuring tear lactoferrin levels (decreased in SS), and tear fluorescein clearance rate (TCR) (<8×: suspected KCS; <4×: definite KCS). We have shown that TCR strongly correlates with tear secretion and have proposed a useful new measurement of tear dynamics, the tear function index (TFI), calculated as follows: Schirmer II value/TCR. A TFI lower than 34 was 78.9% sensitive and 91.8% specific for dry eyes in SS [28, 35]. Another simple and useful test is tear ferning, which is widely used in Europe. Tears are collected with a glass capillary, placed on a glass slide, and left to dry at room temperature. The samples are then observed in white light or by polarized microscopy and classified into four grades according to the appearance following crystallization. The tears of SS and other severe dry eye patients exhibit less ferning than those of normal patients [9]. A skillfully performed impression or brush cytology is an indispensable tool in the evaluation of the severity of ocular surface changes, associated inflammatory responses, assessment of the response to treatment, and definitely for research purposes. Impression cytology samples from SS patients reveal snake-like chromatin cells, decreased cellular cohesion, increased grades of squamous metaplasia, decreased goblet cell density and mucin pick-up, and inflammatory cells [4, 18, 19]. The value and safety of lacrimal gland biopsy has always been a

Table 2.1 Revised International Classification Criteria for Sjögren's Syndrome

I. Ocular symptoms: (+) response to at least one of the following questions
1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
2. Do you have a recurrent foreign body sensation in the eyes?
3. Do you use tear substitutes more than three times a day?
II. Oral symptoms: (+) response to at least one of the following questions
1. Have you had a daily feeling of dry mouth for more than 3 months?
2. Have you had recurrently/persistently swollen salivary glands as an adult?
3. Do you frequently drink fluids to aid in swallowing dry food?
III. Ocular signs: (+) result for at least one of the following two tests
1. Schirmer's I test without anesthesia (<5 mm in 5 min)
2. Rose Bengal score (>4 points – Van Bijsterveld scoring)
IV. Histopathology: Focal lymphocytic sialadenitis in minor salivary glands
Evaluated by an expert pathologist, with a focus score >1 point, defined as a number of lymphocytic foci adjacent to normal-appearing mucous acini and containing more than 50 lymphocytes per 4 mm2 of glandular tissue.
V. Salivary gland involvement: (+) result for at least one of the following tests
1. Unstimulated whole salivary flow (<1.5 ml in 15 min)
2. Diffuse sialectasis in parotid sialography without obstruction in the ducts
3. Salivary scintigraphy showing delayed uptake, decreased concentration and/or delayed excretion of the tracer
VI. Autoantibodies: presence in the serum of the following antibodies
Autoantibodies to Ro (SSA) or La (SSB) antigens or both

concern, but has been performed in Japan since the 1950s with proven efficacy and safety, and contributes significantly to the potential diagnosis of SS. Biopsy can be considered in patients with dry eyes with poor reflex tearing [31], usually showing extensive lymphocytic infiltration and acinar cell destruction.

2.3.6 Diagnosis of Sjögren's Syndrome

The criteria for the diagnosis of SS remain controversial, and several sets of diagnostic criteria have been proposed. Recently, a consensus has been reached between the US and European study groups resulting in revised international classification criteria and revised rules for SS that have found wide acceptance (Tables 2.1, 2.2) [34].

The proper diagnosis of SS depends on the recognition of the characteristic clinical findings, such as dryness of the skin and pruritus, arthralgia (approximately 20% of patients with RA have secondary SS), coughing, xerostomia, problems of deglutition, mild hepatitis or pancreatitis diagnosed by routine laboratory testing and KCS [2]. Neurological disease is perhaps the most common significant extraglandular manifestation of SS and can involve the cranial nerves and peripheral nerves, but rarely the central nervous system. A laboratory work-up for SS should contain antinuclear antibody (ANA) and rheumatoid factor (RF), which are prevalent in SS. The Sjögren antibodies SSA and SSB are specific, but are seen in 30% and 15% respectively of SLE patients as well [2]. Longitudinal monitoring of laboratory parameters is appropriate and essential for SS patients especially for monitoring the therapeutic response. The recommended work-up for SS is shown in Table 2.2.

Table 2.2 Revised Rules for Classification of Sjögren's Syndrome

For primary SS (in those without any potential disease associations)
1. Presence of any 4 of the 6 items, as long as either item IV or VI is (+)
2. Presence of any 3 of the 4 objective criteria (items III–VI)
For secondary SS
In patients with a potential disease association such as a well-defined connective tissue disease, the presence of item I or item II, plus any 2 from items III, IV, and V may be considered as indicative of secondary SS
Exclusion criteria
Past neck or head radiation treatment
Hepatitis C infection
AIDS
Pre-existing lymphoma
Sarcoidosis
Graft versus host disease
Use of anticholinergic drugs

2.3.7 Ocular Features of Sjögren's Syndrome

The evaluation of a presumed SS-related dry eye patient should include a medical history on systemic diseases including information on medications in use, since antihistamines, decongestants, antihypertensives, diuretics, muscle relaxants, and psychotropic drugs may decrease tear production, and in patients with marginal tear production they can trigger clinical dry eye [6].

Sjögren's syndrome may cause symptoms such as increased awareness of eye pain, heaviness of the eyelids, blurred vision, increased mucus secretions, burning, foreign body sensation, photophobia, and tearing [27]. Infrequent blinking and prolonged staring at monitors result in the worsening of the symptomatology [2]. The frequency of each symptom and the degree of interference with daily activities should be assessed. It is our belief that it is helpful to use a currently available questionnaire such as McMonnies's Dry Eye Questionnaire, The National Eye Institute Visual Function Questionnaire, The Dry Eye Questionnaire or the Ocular Surface Disease Index for the diagnosis of dry eyes in understanding the burden the dry eye state imposes on a patient's daily life and tailoring a treatment approach suitable for the needs of the patients [1, 24]. Further study

is obviously required to assess the usefulness of the available questionnaires in clinical trials of dry eye.

Clinical ocular signs of SS include decreased or fluctuating visual acuity, conjunctival hyperemia, low tear meniscus, the presence of excess debris in the tear with occasional mucus strands, foam, and debris. Blepharitis and meibomitis are commonly associated with SS-related dry eye. Corneal findings consist of superficial punctate keratopathy (usually interpalpebral, generalized or variable), band keratopathy, corneal epithelial erosions, sterile or infectious corneal ulcers, peripheral corneal infiltrates or ulceration, stromal melting, and perforation [14].

Summary for the Clinician

- A questionnaire is useful in assessing the severity and duration of dry eye-associated symptoms, while vital staining identifies the ocular surface disease.
- Measurement of the tear quantity (Schirmer test) and tear stability (tear film break-up time [TBUT]) provides useful information on the assessment of tear functions in dry eye syndrome.

2.4 Treatment of Dry Eyes

2.4.1 General Health Care in Sjögren's Syndrome

The role of the health care personnel should focus not only on the ophthalmological aspects of the disease, but should also ensure that the quality of life of the subject involved is increased. Initial efforts for the SS dry eye patient must include communication with rheumatology and dentistry subspecialties. The health care should involve counseling the SS patient with regard to simple environmental measures designed to enhance moisture, such as the sufficient daily intake of fluids, avoidance of excessive alcohol, use of humidifiers, avoidance of excessive air conditioning and forced hot air heating systems, daily baths (not allowing the skin to dry completely afterward), and use of skin moisturizers [2]. More frequent instillations of tear substitutes, the use of moisture chambers or dry eye spectacles, or warming the eyelids using warming sheets are of help, especially during times of prolonged computer work or reading.

Traditional therapies for dry eye are palliative, in that their purpose is to replace or conserve the patient's tears without correcting the underlying disease process.

Tear replacement by topical artificial tears and lubricants is currently the most widely used therapy for dry eye, and a variety of components are used to formulate a considerable number of commercially available preparations [3, 15, 16, 21, 25, 30]. The goal of using tear substitutes is to increase humidity at the ocular surface and to improve lubrication while decreasing evaporation.

2.4.2 Artificial Tear Solutions

Most artificial tear preparations contain cellulose ethers, carbomers, polyvinyl alcohol, polyvinyl pyrrolidones or sodium hyaluronate as their main components [26].

Among them, cellulose ethers (e.g., hypromellose, hydroxyethyl cellulose, methylcellulose, carboxymethyl cellulose) are viscoelastic polysaccharides that increase the viscosity of tears, are not influenced by blinking, have a good retention time on the ocular surface, and mix well with other ophthalmic products. They are sometimes co-formulated with electrolytes as hypotonic solutions and are also available as sustained release tear inserts. Yet, hypromellose can cause crusting of lids and mimic blepharitis [26].

Carbomers are synthetic polymers with high viscosity and a good retention time on the ocular surface, but tend to blur vision and may be uncomfortable to patients [26].

Polyvinyl alcohols are also synthetic polymers with low viscosity and optimal wetting properties at a concentration of 1.4%. They are water soluble and do not cause visual blurring. They are beneficial in aqueous, lipid and mucin layer deficiencies. However, they have a short ocular surface retention time and do not mix well with other ophthalmic products [26].

Povidones (Polyvinyl pyrrolidones) are synthetic polymers that have superior wetting properties when co-formulated with polyvinyl alcohols. They can also be co-formulated with electrolytes. These formulations are beneficial in mucin layer deficiencies [26].

Sodium hyaluronate is a mucopolysaccharide with a viscous formulation and a good ocular surface retention time with beneficial effects in corneal wound healing [26].

The instillation of eye drops on the ocular surface results in temporary thickening of the precorneal tear film [17], followed by the subsequent increase in tear evaporation [13] and clearance through the canaliculi. The distribution of the tear film itself has been reported to be thicker at the superior portions of the cornea after blinking [12], which may account for the characteristic fluorescein staining patterns of the inferior portions of the cornea in certain types of dry eyes. Although artificial tears are the first choice in the treatment of such dry eye patients, there is no information about the possible effects that the distribution of eye drops on the cornea may have on the tear film or on the epithelial lesions in these patients.

2.4.3 Autologous Serum Eye Drops

Natural tears have a complex composition of water, salts, hydrocarbons, proteins, and lipids, which artificial tears cannot completely substitute. In 1984, Fox et al. reported the beneficial effects of autologous serum application to dry eye in SS [32]. The rationale for their observation was based upon the fact that vitamins or growth factors present in tears are also present in serum. The application of autologous serum offered an advantage over artificial tears, which lack such essential components [32]. We also demonstrated a clear benefit of using autologous serum for the treatment of dry eye associated with SS. We measured EGF, vitamin A, and TGF-β concentrations in serum and found that these components can be supplied to the ocular surface by this method [10]. Furthermore, it was confirmed that the autologous serum samples can be preserved for more than 1 month in the refrigerator and more than 3 months in the freezer. A 40-ml sample of venous blood from an SS patient is enough to last for at least 3 months. Twenty milliliters of serum can be obtained from 40 ml of whole blood, while diluting it 1:5 with saline provides 100 ml of serum solution (Fig. 2.1). If each eye drop is 50 μl, 2,000 drops can be obtained from 100 ml. SS dry eye patients use a maximum of 20 drops a day (10 times for each eye); thus, 2,000 drops are enough for more than 100 days. Patients are supplied with twenty 5-ml bottles of 20% autologous serum and are advised to store bottles in the freezer until use. They are advised to keep bottles in current use in the refrigerator. Objective observations of Rose Bengal and fluorescein scores with autologous serum treatment dramatically improved in our patients.

2.4.4 Albumin as a Tear Supplement in the Treatment of Severe Dry Eyes

The use of serum as a tear replacement is not without problems, especially when handling serum from patients with transmissible disease such as HIV, hepatitis B, hepatitis C, and prion disease. The logical solution to this would be to develop artificial tear solutions that contain some of the key components of tears, including tear proteins. Although tears contain proteins with specific functions, such as lactoferrin and immunoglobulins, a substantial quantity of other nondefined proteins in the pre-albumin and albumin fractions are also found [29, 32]. Human albumin was chosen as a protein source since it is widely used in infusion therapy. The results of our clinical pilot study showed that staining scores significantly improved over the 4-week study period. Of special interest was Rose Bengal, which was said to stain areas with poor protection by the preocular tear film. Topical albumin may therefore compensate for the lack of soluble mucin in patients with SS. Our data also showed that fluorescein staining improved with topical albumin. In addition, we revealed accelerated wound healing in experimental animal model eyes receiving albumin drops. Although no adverse effects were observed during the clinical study, a minute risk of transmissible viral and prion disease still cannot be ruled out.

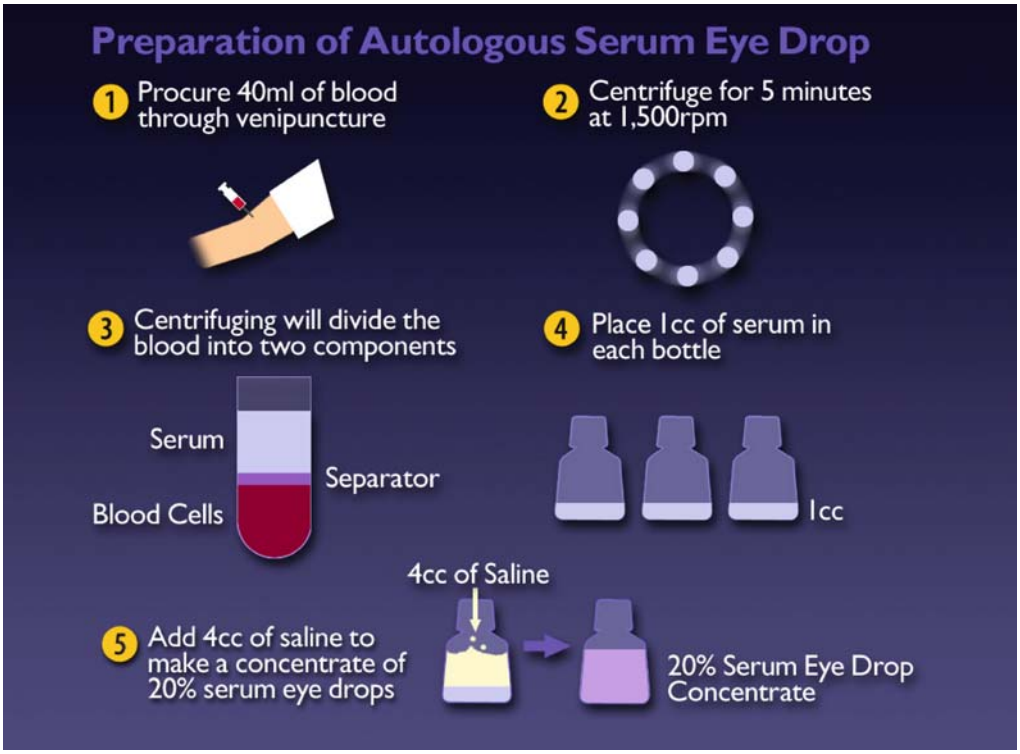

Fig. 2.1 The protocol for the preparation of autologous serum eye drops

2.4.5 Lacrimal Punctal Occlusion

Punctal plug insertion is a simple, safe, effective, and reversible method of treating aqueous tear deficiency and ocular surface epitheliopathies not controlled with preservative-free lubricants. Lacrimal punctal occlusion may help by maximizing the time that essential tear components are in contact with the ocular surface epithelium (Fig. 2.2). Punctal occlusion performed with collagen-rod, silicone, or plastic plugs, seems to be very effective in patients with reflex tearing of 1–9 mm [8]. Patients with no reflex tearing can receive both upper and lower punctate occlusion. It is important to recognize the possibility of the longer retention of deleterious components on the ocular surface with punctate occlusion. Thus, punctate occlusion should be performed only after or in conjunction with adequate management of local inflammatory conditions by topical steroids or nonsteroidal anti-inflammatory agents, or cyclosporine eye drops. Patients with KCS must also be instructed to wash their eyes with preservative-free drops three to four times a day, especially before going to sleep [8].

Summary for the Clinician

- The main treatment for dry eye and Sjögren's syndrome consists of preservative-free artificial tear solutions and punctal occlusion.
- Severe cases can be treated with autologous serum and albumin eye drops.

2.4.6 Future Therapeutic Expectations

Future research hopes to define the pathogenesis of dry eye disease in more detail and thus provide better diagnostic tools. Further controlled trials

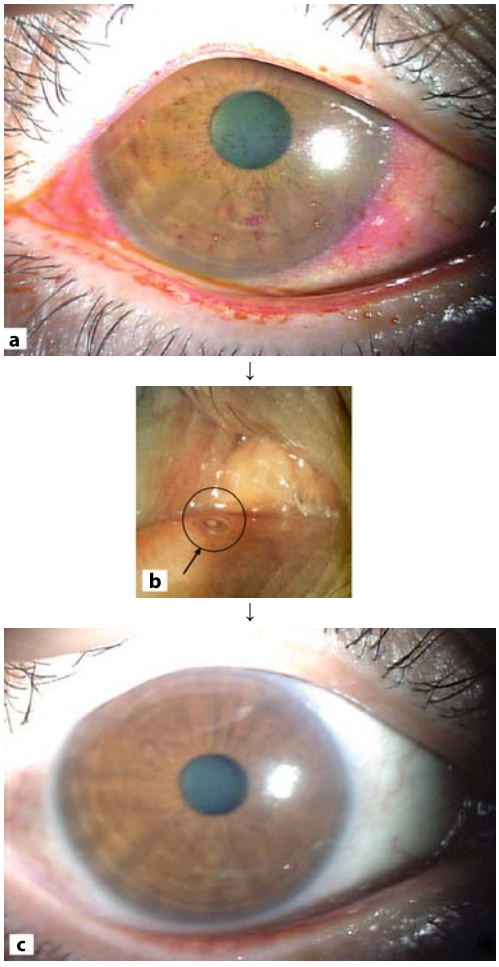

Fig. 2.2 Ocular surface Rose Bengal staining before and after the punctum plug insertion in a patient with SS. **a** Note the intense Rose Bengal staining in an SS patient before the punctum plug insertion. **b** Silicone punctum plug inserted in a lower punctum (*arrow*: punctum plug). **c** Note the improvement in the ocular surface Rose Bengal staining after punctum plug insertion

are definitely needed on the topical application of newer medications, such as anti-inflammatory agents, immunosuppressants, such as cyclosporin A and FK-506, growth hormones, androgens, topical mucins, and ocular surface-stimulating drugs like INS365 with potential therapeutic benefits for the ocular surface disease of patients with KCS and these trials will pave the way for newer treatment protocols in SS dry eyes. Indeed, the first topical cyclosporine prescription product was launched in early April 2003. INS365 is expected to be launched in 2004, 15-S-HETE in 2005, and androgen tears in 2006 [1].

It is our belief that the efforts of numerous talented dry eye researchers with an interest in regenerative medicine coupled with efforts to overcome the autoimmune disease process will lead to the development of artificial lacrimal glands or at least the development of lacrimal gland stem cell cultures that can then be transplanted to regenerate and/or replace the diseased gland in SS patients.

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Chapter 3

Allergic Conjunctivitis: Clinical Consequences and an Update on Understanding Its Pathophysiology

3

Andrea Leonardi

Core Messages

- Allergic conjunctivitis is not a single disease.
- Seasonal and perennial allergic conjunctivitis are the most common entities.
- An accurate clinical history and evaluation of signs and symptoms allow the diagnosis of ocular allergy and the definition of possible sensitizing antigens.
- Recurrent and chronic allergic conjunctivitis require laboratory tests for their etiologic diagnosis and for a definition of their pharmacological and nonpharmacological management.
- The cornea may be involved in vernal keratoconjunctivitis , atopic keratoconjunctivitis or contact blepharoconjunctivitis, but never in seasonal or perennial allergic conjunctivitis.
- IgE-mediated hypersensitivity and mast cell degranulation are the initial pathophysiological mechanisms.
- The Th2-type of cytokines and chemokines are over-expressed in ocular allergy.
- Nonpharmacological measures and avoidance are extremely important for disease management.
- Therapy should not include vasoconstrictors and, if possible, corticosteroids.
- Mast cell stabilization and histamine antagonism are the main pharmacological interventions.
- Dual action drugs are the first choice in the treatment of ocular allergy.
- Severe cases need intense treatment.

3.1 Introduction

Environmental allergens such as those derived from pollens, house dust mites, molds, and domestic animals are ubiquitous in modern life. While both atopic and non-atopic subjects develop an immunological response, only in atopic subjects does it lead to an IgE-mediated hypersensitivity. The factors that determine these dissimilar immunological outcomes are complex and are determined by genetics, environmental factors, and the efficiency of normal regulatory mechanisms [26]. Approximately one-third of the world population is affected by some form of allergic disease [42], and ocular/conjunctival symptomatology is estimated to be present in 40–60% of this population. Allergic conjunctivitis is a localized allergic condition frequently as-

sociated with rhinitis, but often observed as the only or prevalent allergic manifestation. This disease ranges in severity from mild forms, which can still interfere significantly with quality of life, to severe cases characterized by potential impairment of visual function.

The term allergic conjunctivitis refers to a collection of hypersensitivity disorders that affect the lid, conjunctiva, and/or cornea. Various clinical forms are included in the classification of ocular allergy: seasonal (SAC) and perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC), giant papillary conjunctivitis (GPC), and contact or drug-induced dermato-conjunctivitis (Table 3.1) [1, 37, 42].

This chapter will review the clinical outcome of the various forms of ocular allergy as well as their diagnostic procedures, highlighting new knowledge of their pathophysiology that may lead to advances in therapeutical strategies.

3.2 Clinical Forms

3.2.1 Seasonal and Perennial Allergic Conjunctivitis

Seasonal allergic conjunctivitis (SAC) is without a doubt the most common form of allergy and is associated with sensitization and exposure to environmental allergens, particularly pollen. The perennial form, perennial allergic conjunctivitis (PAC), usually involves sensitization to mites or to multiple antigens. Both forms are characterized by onset in childhood or early adulthood. They are typical IgE-mediated diseases, featuring spikes of histamine and other mediators released from conjunctival, activated mast cells. Patients present with ocular itching, conjunctival hyperemia, and at times lid and conjunctival edema of varying severity, mild serous or sero-mucous secretions, and/or slight papillary or follicular hypertrophy of the conjunctiva [1]. This symptomatology may be occasional, seasonal, or chronic in PAC. The clinical picture is quite nonspecific since none of the signs or symptoms related to SAC or PAC is specific or pathognomic. The disease is bilateral, but not always symmetrical, and frequently associated with rhinitis. The only diagnostic factor is the presence of itching; in fact, if the patient does not complain of conjunctival or periocular itching, it is almost certainly not allergic conjunctivitis. The most important diagnostic tool for SAC and PAC are a thorough medical history. This facilitates determining offending allergens, as symptoms arise and subside with the patient's exposure. While these conditions are not serious, they are very disturbing to patients and can significantly affect their quality of life.

Acute or hyperacute episodes of ocular allergy, also called anaphylactoid reactions, are characterized by acute itching and eyelid swelling, as urticaria (hives and wheals) in the superficial layers of the skin, or angioedema (in the deeper subcutaneous tissues), or both. These reactions can be unilateral or bilateral and the conjunctiva may or may not be affected. Insect bites, food allergy or contact hypersensitivity can be involved in the etiology of these reactions.

3.2.2 Vernal Keratoconjunctivitis

Vernal keratoconjunctivitis (VKC) is a severe ocular allergic disease that occurs predominantly in children [27]. Most of the VKC patients in the Mediterranean area complain of symptoms between early spring and fall, with differences among climate zones. In warm or subtropical countries, symptoms may persist all year. It is typical that, in addition to chronic inflammation, exacerbations of the diseases and acute episodes arise, triggered by allergen exposure or, more frequently, by nonspecific stimuli such as wind, light, and dust. VKC is an IgE- and Th2-mediated disease [1, 27, 37, 42]; however, only 50% of patients present a clearly defined allergic sensitization.

Intense itching, tearing, and photophobia are the classic symptoms of these patients. The presence of pain associated with photophobia is indicative of corneal involvement. Foreign body sensation may be caused by mucous hypersecretion, papillae hypertrophy, and superficial keratopathy. Various grades of conjunctival hyperemia and chemosis are always present in both forms of the disease. The tarsal form is characterized by irregularly sized hypertrophic

Table 3.1 Ocular allergic diseases. SAC, PAC, VKC, AKC, GPC, M, F

Condition	Prevalence	Severity	Causes	Signs/symptoms
SAC/PAC	Most frequent ocular allergic disease. 10–15% of population	Mild/ moderate	• Genetic predisposition • Associated with rhinitis • Seasonal allergens (pollens, molds, chemicals) • Perennial allergens (dust, animal dander, foods, chemicals	• Itching • Redness • Tearing • Watery discharge • Chemosis • Lid swelling
VKC	Rare Ages 3-20 Under 14 M>F In adults M=F	Severe	• Genetic predisposition? • Associated with atopic disorders (50%) • Th2 upregulation • Nonspecific eosinophil activation	• Extreme itching • Ropy mucous discharge • Cobblestone papillae • Trantas' dots • Keratitis/ulcer • Conjunctival eosinophilia
AKC	Rare 2nd to 5th decade of life M>F	Severe/ sight threatening	• Genetic predisposition • Associated with atopic dermatitis • Environmental allergens: food, dust, pollens, animal dander, chemicals	• Itching • Burning • Tearing • Photophobia • Chronic redness • Blepharitis • Periocular eczema • Mucous discharge • Keratitis/ulcer • Conjunctival and corneal scarring • Cataract
GPC	Iatrogenic 2nd to 5th decade	Mild	• Trauma induced by contact lens edge, ocular prosthesis, exposed sutures, aggravated by concomitant allergy	• Lens intolerance • Blurred vision • Foreign body sensation • Abnormal thickening of conjunctiva • Giant papillae
Contact dermatitis	Not known	Moderate	• Contact delayed type hypersensitivity • Exogenous haptens (cosmetics, metals, chemicals) • Topical preparation (drugs, preservatives)	• Eyelid eczema • Eyelid itching • Conjunctival redness • Punctate keratitis

papillae, leading to a cobblestone appearance on the upper tarsal plate (Fig. 3.1). They may be distributed over the entire tarsal area or limited to one isolated zone. Giant papillae may also form in the lower tarsal conjunctiva. Abundant mucus may adhere to the papillae or may be incarcer-

ated between them and in the fornix. A variation of the tarsal form of VKC may appear as diffuse upper tarsal conjunctival thickening with fine and diffuse subepithelial fibrosis without papillae formation. Asymmetrical tarsal involvement is not unusual. The limbal form of the disease is characterized by multiple gelatinous, yellow-gray limbal infiltrates and papillae, whose size and location may change over time. The limbus may appear thickened and opacified for 360°, accompanied by peripheral, superficial neovascularization. The apices of infiltrates may appear as punctiform, calcified concretions called Trantas' dots. In the mixed form of the disease, both tarsal and limbal signs are observed to varying degrees. Blepharospasm, tearing, and mucus hypersecretion may be present in all VKC forms, while pseudoptosis is usually secondary to the presence of heavy tarsal giant papillae.

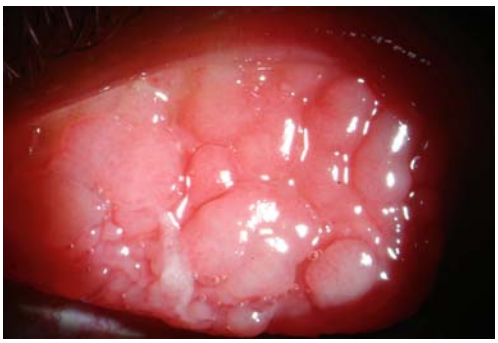

Fig. 3.1 Giant papillae on upper tarsal conjunctiva in a vernal keratoconjunctivitis patient

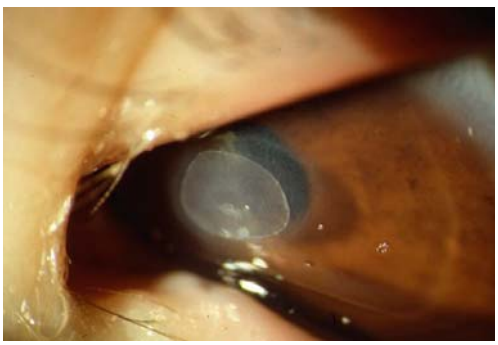

Fig. 3.2 Corneal ulcer in vernal keratoconjunctivitis

Corneal involvement is common in VKC, and is more frequent in tarsal than limbal patients, taking the form of superficial punctate keratitis, epithelial macroerosion or ulcers, plaque (Fig. 3.2), subepithelial scarring, or pseudogerontoxon. Ulcer formation is preceded by a progressive deterioration of the corneal epithelium, which appears irregularly stained and covered with fine filaments. Diagnosis is based on typical signs and symptoms.

3.2.3 Atopic Keratoconjunctivitis

Atopic keratoconjunctivitis (AKC) is a rare disease that constitutes less than 1% of all ocular allergies. Generally, it emerges in young adults and continues through the fifth decade of life, reaching its peak incidence between the ages of 30 and 50 [7, 21]. Young children with active atopic dermatitis may also develop early signs of AKC. A family history of allergic conditions is common, while 95% of patients have a history of eczema and 87% have a history of asthma. AKC presents as chronic bilateral conjunctivitis with seasonal exacerbation corresponding to the offending allergen/s. Some patients experience exacerbation after dust or food exposure. The common presenting symptoms are bilateral ocular itching, burning, tearing, and mucous discharge. These are generally present all year round. The hallmark sign of AKC is erythematous, exudative lesions of the lids [7]. Eyelids tend to be thickened, indurated, erythematous, and fissurated, due to eczema, which is often associated with chronic blepharitis, meibomian gland dysfunction, and staphylococcal infection. About 90% of atopic patients are colonized with *Staphylococcus aureus* rather than the usual staphylococcal flora, including the lids; however, this does not correlate with the incidence or severity of the keratopathy [44]. The conjunctival hyperemia and chemosis affect predominantly the inferior forniceal and palpebral conjunctiva. The limbus may also be involved, presenting gelatinous papillary hyperplasia and Trantas' dots. Hypertrophic papillae, leading to a cobblestone appearance of the upper tarsal plate, may be similar to those observed in VKC patients. Cicatrizing conjunctivitis, subepithelial fibrosis, and symblepharon have also

been reported, with the lower fornix possibly shrinking subsequent to scarring. Reduced tear function and tear volume may also be observed. Punctate keratitis, persistent epithelial defects and ulcer with plaque formation are possible complications. Keratoconus is also often associated with AKC. Herpes keratitis and microbial infections may complicate the disease, particularly if chronic topical steroid therapy is required. Severe keratopathy with corneal neovascularization and pannus formation may develop as a consequence of repeated corneal inflammation. This can result in marked astigmatic changes and permanent visual impairment. Anterior "atopic" or posterior subcapsular cataract contributes to the visual deterioration associated with AKC.

3.2.4 Giant Papillary Conjunctivitis

Giant papillary conjunctivitis (GPC) is a non-IgE-mediated inflammation induced most frequently by the use of contact lenses [8]. All types of contact lenses can trigger GPC, as can the use of ocular prostheses, the presence of corneo-conjunctival sutures or protruding scleral buckling [9]. The upper tarsal conjunctiva is subjected to repetitive or constant micro-trauma generated by a conjunctival "foreign body"; this phenomenon is then complicated by an immune reaction against a protein or residue deposited on the lens. Suspension of contact lens wear initiates the immediate regression of the disease. Previously considered an allergic condition, GPC is similar to VKC with regard to the morphology of giant papillae, and some immunopathological findings such as increased mast cell number, eosinophil and T cell infiltration, expression of Th2-type cytokines, such as IL-4, and chemokines [25]. Contact lens GPC is mostly seen in young patients, and gender is not related to this condition. A history of atopy may be a predisposing factor. In fact, although not IgE-mediated, GPC is commonly observed in allergic patients and exacerbated by seasonal allergic sensitivities. The early stages of GPC may be asymptomatic. In contact lens GPC, mild lens intolerance progresses to foreign body sensation, itching, blurred vision and increased mucus production. Intolerance progresses until patients are no longer able to wear their lenses. In other forms of GPC, mild to severe irritation, discomfort, itching, and burning continue until removal of the external device or suture. The defining characteristic of GPC is the presence of giant papillae greater than 0.3 mm in diameter. The papillae are smaller and more uniform in size than those of VKC. There can be a single papilla or the entire tarsal plate may be covered. Conjunctival hyperemia, limbal infiltrates, Trantas' dots, and conjunctival thickening are common findings. Mucous discharge and lens deposits are typical.

3.2.5 Contact Blepharoconjunctivitis

Contact blepharitis or dermato-conjunctivitis involves the skin of the eyelid and/or conjunctiva [37, 57]. It is related to contact T cell-mediated delayed hypersensitivity reaction to haptens (incomplete antigens), which become immunogenic only after they bind to tissue protein. The antigen is captured by Langerhans cells of the eyelid skin or the conjunctiva and presented to T-helper lymphocytes in the regional lymph nodes. The sensitized T cells react by secreting cytokines and several chemotactic factors, resulting in recruitment and activation of inflammatory cells and resident cells. Various haptens and antigens that might come in contact with the eyelid and/or the conjunctiva have been implicated, including drugs, topical eye drops, preservatives, metals, nail polish, and cosmetics. An "allergic" reaction may occur following instillation of topical anti-glaucoma agents, such as beta-blockers, prostaglandins and prostanoids, or mydriatics used for diagnostic purposes, usually phenylephrine. Other alpha-agonists are commonly used as decongestants in over the counter anti-allergy eye drops. Topical antibiotics such as neomycin, as well as ocular solutions based on herbal extracts can provoke contact allergic reactions. Among preservatives, benzalkonium chloride, thimerosal, parabens, and ethylenediaminetetraacetici acid (EDTA) may cause either a toxic reaction or a cell-mediated (delayed) hypersensitivity (DH) response. The most prominent symptoms are itching and burning of the eyelid and eczematoid dermatitis. Other signs and symptoms

are redness, eyelid swelling, tearing, and mucous discharge. The ocular surface may also be involved as conjunctival hyperemia, punctate staining of the cornea and conjunctiva, especially on the inferonasal bulbar conjunctiva, and a follicular reaction. Similar signs and symptoms and conjunctival staining patterns occur with drug or preservative toxicity. Marginal corneal infiltrates may rarely occur in reactions to neomycin, phenylephrine, dorzolamide, gentamicin and atropine; however, the exact nature of these hypersensitivity reactions is not clear. Eczema on the eyelid skin in the absence of conjunctival hyperemia indicates that the cause of the reaction is due to something that has become in contact only with the eyelid. Diagnosis is based on accurate clinical history of agents/drug exposure and the results of patch tests on the dorsal skin. Customized patch tests may be prepared using eye drops or other suspected agents [50].

Summary for the Clinician

- Accurate history is essential.
- If it doesn't itch, it is not an allergy.
- Diagnosis of SAC is usually clinical.
- VKC and AKC have unique clinical features and typical signs.
- Chronic forms of allergy require laboratory tests.
- If lids are involved, suspect contact allergy.

3.3 Diagnosis

Each of these clinical entities requires a differential diagnosis that is usually clinical, yet can be substantiated by objective laboratory parameters. While their clinical characteristics allow a relatively convincing diagnosis of SAC, PAC, VKC, AKC, GPC, and contact blepharoconjunctivitis in the milder or initial stages of these diseases, there can be some confusion as to what form of allergy is present. At times, pseudoallergic forms, with clinical manifestations similar to allergy, but with a non-allergic equivocal pathogenesis, are difficult to distinguish from allergic forms, with their precisely defined pathogenic mechanisms.

Table 3.2 Differential diagnoses of chronic allergic disease

Dry eye
Blepharitis
Uncorrected visual defects
Chlamydia
"Medicamentosa" (drug-induced conjunctivitis)
Viral conjunctivitis
Contact lenses intolerance
Nonspecific hyperreactivity
Hyperuricemia
Toxic conjunctivitis
Mechanical conjunctivitis

Several clinical forms may mimic the clinical pictures of ocular allergy, including tear film dysfunction, subacute and chronic infections, and toxic and mechanical conjunctivitis (Table 3.2).

3.3.1 Diagnostic Assays in Ocular Allergy

The first step is to determine definitively that the inflammation is not nonspecific, but is allergic in origin, caused by an IgE-mediated sensitization to an antigen. The second phase of diagnosis consists in identifying which of the various forms of ocular allergy are present based on the clinical characteristic observed.

3.3.1.1 Skin-Prick Test

The skin-prick test is a simple, rapid, and inexpensive procedure that is commonly included in the work-up of systemic allergy. Allergen extracts (grass and tree pollens, molds, mites, animal dander) are directly applied to the flexor side of the forearm and pricked with a sterile lancet. It provides evidence of specific sensitivity to environmental and food allergens, and reinforces the concept of allergy to the patient. The test is highly sensitive for systemic allergies, but unfortunately does not correlate well with the ocular allergic status. Because the conjunctiva can be a uniquely

sensitized organ in allergy, the skin test remains a confirmatory test that requires the support of additional local tests.

3.3.1.2 Patch Test

If there is question of a contact sensibility, particularly in the presence of eczematous blepharitis or blepharoconjunctivitis, a patch test is necessary. This involves applying to the skin of the back a series of hapten-containing cellulose disks that are removed after 48 h. Benzalkonium chloride and thimerosal, preservatives present in the majority of drops and contact lens solutions, should always be tested. If topical drugs are suspected, the patch tests can be performed using the exact solution in question. It must be remembered that eyelid skin is quite different from that of the back, not only with regard to the depth of the epithelial and dermal layers, but also to the limited number of mast cells present and to its limited exposure to the external environment compared with lid skin. It is possible, for example, that sun exposure exacerbates specific and nonspecific hyperreactivity reactions only on the lid skin.

3.3.2 In Vitro Assays

The assaying of specific IgE in serum is particularly indicated and preferred to skin tests in certain cases. In children affected by VKC or AKC, the direct determination of serum-specific IgE is recommended for various reasons: the age of the patient and his/her usually insufficient collaboration during skin testing; the frequent presence of cutaneous hyperreactivity and/or eczema; the prolonged use of topical and systemic drugs; the ability to quantify sensitizations to diverse antigens simultaneously, and lastly, the ability to compare over time the numerical IgE values obtained. It must be remembered that VKC and AKC are not always associated with specific allergic sensitization. Particularly with VKC, IgE sensitization occurs in only half the population, although the disease is indistinguishable between IgE-positive and -negative patients [15, 27].

Total serum IgE is no longer considered indispensable for diagnosis, since normal values do not exclude an allergic diagnosis, and conversely, high levels of IgE can be observed in numerous pathologies other than atopy.

Eosinophilia, while indicative, is not pathognomic for allergic disease. Generally, SAC and PAC, contact allergy, and GPC are not accompanied by an abnormal number of blood eosinophils, while in AKC and VKC their numbers are often increased. Conversely, levels of eosinophil cationic protein (ECP) do increase significantly in active phases of ocular allergy [32]. If ECP levels are high, even in the exclusively local presence of an ophthalmic sensitization, systemic therapy is recommended.

Summary for the Clinician

If allergy is suspected:

- An accurate clinical history and ocular examination are required;
- Perform skin tests;
- Identify specific and total serum IgE levels;
- Analyze hemochrome with the eosinophil count;
- If all of these systemic tests are negative, perform local tests.

3.3.3 Local Tests

3.3.3.1 Conjunctival Provocation Test or Conjunctival Allergen Challenge

Defining the conjunctival allergic response to sensitizing allergens can be extremely helpful in understanding a patient's disease. The conjunctival provocation test (CPT) may confirm conjunctival reactivity to allergens with a positive result in the prick test [3, 39]. It is also particularly useful in patients with a negative prick test or serum IgE and a positive clinical history of allergic ocular disease to evaluate the specific, local conjunctival response [28]. Furthermore, CPT can be used to define the most important allergen/s in patients with several positive skin tests; to follow the local success of specific systemic immunotherapy; to evaluate the effects of anti-allergic drugs; and

to study the pathophysiology of the allergic conjunctival reaction.

Standardized allergens are available for the provocation test. At the moment of the test, patients must be asymptomatic and without pharmacological treatment for at least 1 week. The allergen dose is instilled every 15 min until a moderate clinical reaction is obtained. The immediate positive response is characterized by the same signs (redness, chemosis, and lid swelling) and symptoms (itching and tearing) that the patients experience after natural exposure to the antigen. The positive reaction usually subsides gradually within 20 min. A late phase inflammatory reaction may also occur, depending on allergen dose and patient sensitivity [14]. Systemic side effects (generalized itching, bronchospasm, anaphylaxis) are rare, but possible in particularly sensitized patients.

The use of the test is only recommended in specialized centers and rapid escape treatment for systemic reactions should always be available. In asthmatic patients, the test should be performed only when necessary.

Elimination tests, which involve the patient's suspension of exposure to the suspected allergen, can be very useful. While for an allergist the classic elimination test is the exclusion diet, the ophthalmologist must exclude above all local eye drop therapy, subsequently evaluating any change in symptoms. Similarly, it is useful to exclude the presence of animals, suspend time at work, or change climactic or home conditions and evaluate what effect this has on ocular symptoms.

3.3.3.2 Measurement of Specific IgE in Tears

When an IgE-mediated conjunctival reaction is suspected in the presence of negative systemic tests, tear levels of specific IgE can provide important information [11, 28, 54]. Levels of specific IgE are undetectable in tears of non-allergic subjects. For each assay, at least 50 µl of tears are required; thus, it is not always possible to obtain a sufficient quantity of sample. Considering the greater sensitivity of new in vitro diagnostic assay techniques not yet applicable to the tear sample, serum-specific IgE testing and reserve tear assays are recommended for selected cases. The assay is identical to that used for serum IgE measurement, even if there are no standardized reference parameters for the eye. The difficulty of tear collection, the potential to induce reflex, and therefore abnormally dilute tears, and above all the quantitative limits of sample have led to a preferential use of specific provocation tests in that they better define the level of clinical response of the conjunctiva.

3.3.3.3 Measurement of Total IgE in Tears

Normal values of IgE in tears are normally very low, less than 2.5 kUI/l (3 ng/ml), due to the blood–tear barrier. Detectable tear IgE levels indicate local production of antibodies and a diagnosis of allergic conjunctivitis. This parameter has come back into fashion recently after the commercialization of a simple and rapid diagnostic test for the semi-quantitative determination of total IgE in tears, the Lacrytest (Adiatec). The test utilizes paper strips that are applied directly to the lower fornix of the conjunctiva in a manner similar to Schirmer's test. Inside the strip are anti-IgE antibodies and, with a colorimetric system, a semi-quantitative total IgE result is obtained in a matter of minutes by reading color intensity. In our experience, the test is well correlated with CPT results, but poorly correlated with skin test results, confirming that local sensitization may occur.

3.3.3.4 Conjunctival Cytodiagnosis

Evaluation of the number and percentage of leukocytes on the ocular surface in the active phase of conjunctival inflammation can be essential to the decision of how to proceed with further diagnostic tests. The presence of even one eosinophil is highly indicative of an allergic pathology, while their absence does not exclude an allergic diagnosis.

Tear cytology is rapid and easy to perform: a few microliters of tears collected from the external canthus with a glass capillary are immediately placed on a slide. We prefer precolored slides,

such as those treated with May Grunwald-Giemsa.

Conjunctival scrapings, performed with a spatula, allow for the collection of more cells than with tear cytology. Samples are placed onto a glass slide, fixed and stained with rapid dyes. This method is also indicated for the differentiation of intracytoplasmic inclusion bodies when Chlamydia is suspected, or in combination with immunofluorescence when looking for viruses.

Brush cytology allows the recovery of a higher number of cells with little trauma to the conjunctival epithelium [36]. This technique can be associated with flow cytometry to phenotype tear cells.

Impression cytology using nitrocellulose membranes is most frequently indicated for tear film pathology in that it allows by nontraumatic means evaluation of the morphology of the superficial conjunctival epithelium by either light or electron microscopy. It can be performed without topical anesthetic.

Conjunctival biopsy is required for histological and immunohistochemical analyses if a neoplastic pathology is suspected, or for the diagnosis of autoimmune diseases such as pemphigoid.

3.3.3.5 Tear Chemical Mediator Measurement

Quantitative analyses of inflammatory mediators or inhibitors in tear fluid have been extensively used in ocular allergy to find either a "disease marker," to better understand the immune mechanisms involved in the ocular surface inflammation, or to identify potential targets for therapeutic interventions.

The measurement of tear histamine, carried out with radioimmunoassay or ELISA, is a relatively simple assay to perform given the commercially available kits. High levels are observed in tears immediately after massive mast cell degranulation, such as after a provocation test, or in VKC [4].

Tear tryptase is a marker for conjunctival mast cell activation and, as such, its measurement might be useful in the diagnosis of allergic disease [16]. Tear tryptase can be assayed used the automatized UNICAP method (Pharmacia). The problem remains that of sample quantity. Both tear histamine and tryptase levels are valuable more for evaluating the effects of mast cell stabilizers than for diagnostic purposes.

Eosinophil Cationic Protein (ECP) is released from activated eosinophils and thus can be considered a marker of eosinophil activation. In fact, ECP is significantly increased in tears in all forms of allergic conjunctivitis, correlating with the clinical status of the patient and with the severity of corneal involvement [29, 40, 47].

Determination of tear mediator, cytokine, chemokine levels or expression of adhesion molecules is not yet used for diagnosis, but only for the study of allergic physiopathology or for the evaluation of efficacy of anti-allergic agents. Tear cytokine analysis is currently carried out using ELISA techniques [30, 43, 53]. The disadvantage of these assays is again related to the limited quantity of samples. Recent advances in "multiplexed bead-based flow cytometry" may open up new opportunities for the study and diagnosis of ocular allergy [19, 35].

Summary for the Clinician

- Cytological tests are useful in the active phase of the disease.
- Conjunctival allergen provocation proves local hypersensitivity.
- Low tear volume limits its potential usefulness in analytical diagnosis.
- The measurement of tear-specific IgE is not practical.
- The measurement of total tear IgE by paper strips is easy, but not highly specific.

3.4 Immunopathogenesis

The etiology of allergic disease involves interactions between multiple genes and environmental factors, such as protein allergens, air pollutants, and viral or bacterial proteins. Various molecules participate during the different phases of the allergic reaction, such as cytokines, chemokines, their receptors, MHC molecules, and transcrip-

tion factors. All these molecules are potential candidates for being allergy-predisposing genes [51]. The nature of causative genes and the degree to which they contribute to the disease is probably variable from patient to patient. It is hoped that further advances in research on genetic factors for ocular allergy will lead to more effective prophylaxis and therapeutic strategies being established for these diseases.

3.4.1 Sensitization

Like the respiratory mucosa, the conjunctiva is normally exposed to picogram quantities of environmental allergens such as pollens, dust mite fecal particles, animal dander, and other proteins [26, 37, 42, 50]. When deposited on the mucosa, these antigens are processed by Langerhans cells or other antigen-presenting cells (APC) in the mucosal epithelium, bind to the antigen recognition site of major histocompatibility complex (MHC) class II molecules, and presented to naive CD4+ lymphocytes at some unknown location that could be the local draining lymph nodes. Complex and multiple simultaneous contacts and cytokine exchanges between APC and T cells expressing antigen-specific T cell receptors are necessary to trigger the antigen specific T cells to differentiate into Th2 lymphocytes [26].

Non-atopic subjects usually develop a low-grade immunological response to aeroallergens with the production of allergen-specific IgG1 antibodies and a modest T cell proliferative response in vitro to allergens with the production of interferon gamma (IFN-γ) typical of Th1 cells. Non-atopics also appear to have a normal T-regulatory cell response [26]. In contrast, allergic subjects mount an exaggerated allergen-specific IgE response with elevated serum levels of IgE antibodies and positive skin tests to extracts of common aeroallergens [51]. In fact, T cells derived from allergic subjects and grown in vitro proliferate in the presence of specific allergens, responding with the production of typical Th2-type cytokines, IL-4, IL-5, and IL-13 [26, 42]. This may be the result of an inappropriate balance between allergen activation of regulatory T cells and effector Th2 cells.

The major driving force that polarizes CD4+ T cells to the Th2 phenotype is IL-4, whereas IL-12 favors a Th1 response. However, many other cytokines, chemokines, and mediators with potential relevance to allergy and allergic conjunctivitis have been described since this initial definition of the Th1/Th2 paradigm. This may explain the disappointing results of single cytokine-directed therapy that have been recently proposed with regard to allergy.

The control of Th1 and Th2 development depends on several transcription factors. For example, GATA3 is implicated in Th2 development, nuclear factor kappa B (NFκB) may control GATA3, and signal transducer and activator of transcription (STAT)-6 transduces signals for IL-4 and IL-13 [26]. These mechanisms have not been well defined in allergic conjunctivitis, but they may represent future targets for therapeutic intervention.

Th2 cytokines influence a wide range of events associated with chronic allergic inflammation. These include IgE production (IL-4, IL-13), maturation of eosinophils (IL-5, IL-9), upregulation of eosinophil-selective adhesion molecules VCAM-1 (IL-4, IL-13), mast cell development (IL-3, IL-9), mucus overproduction (IL-4, IL-9, IL-13), and collagen production (IL-4, IL-13). All these effects are present in chronic allergic conjunctivitis.

3.4.2 Allergic Response

In sensitized subjects, mast cells residing in the conjunctival mucosa have specific IgE antibodies bound to the cell surface by high affinity receptors. After exposure to natural environmental allergens, antigens bind to adjacent specific IgE on mast cells, causing a cross-linking of the molecules and signaling the subsequent degranulation of the mast cell, with the release of inflammatory mediators, such as histamine, tryptase, prostaglandins, and leukotrienes. These trigger the clinical manifestations of the acute phase of the disease (early phase; Fig. 3.3) [38]. Mast cell degranulation also induces activation of vascular endothelial cells and thus the expression of chemokines and adhesion molecules such as RANTES [regulated on activation normal T cell expressed and secreted], MCP, IL-8, eotaxin, MIP-1a, ICAM, VCAM, and p-selectin. These factors initiate the recruitment phase of inflammatory

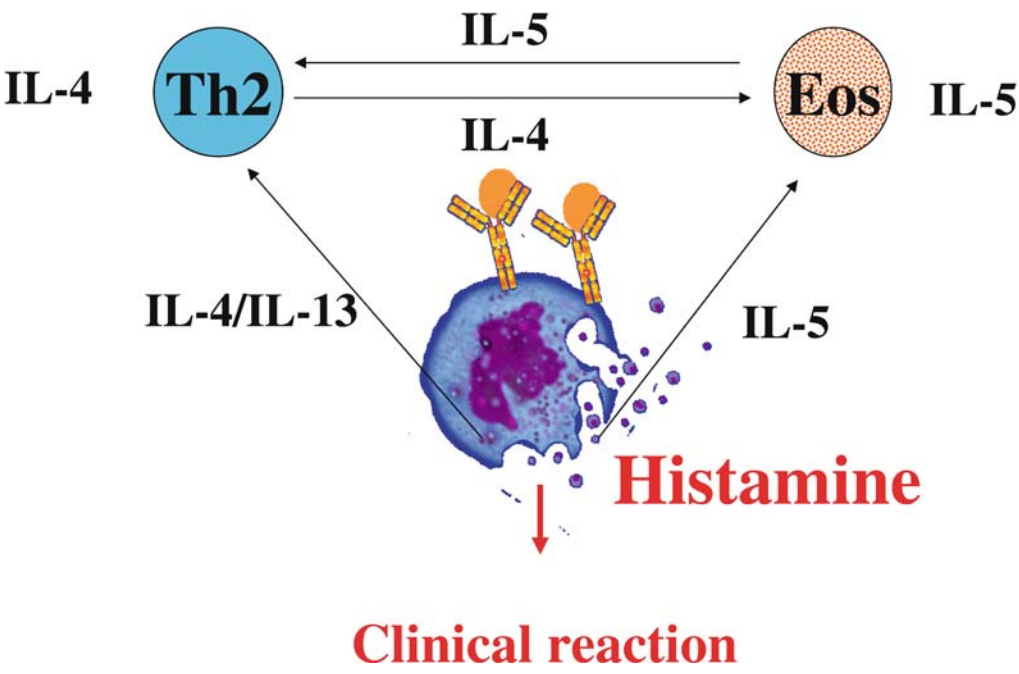

Fig. 3.3 Schematic diagram of immediate IgE- and mast cell-dependent allergic reaction. *Th2* T lymphocyte T helper type 2, *Eos* eosinophil, *IL* interleukin

cells in the conjunctival mucosa. Fibroblast and epithelial cells contribute to the conjunctival inflammation producing, when stimulated, cytokines and chemokines, and thus playing a key role in both the induction and chronicity of ocular diseases (Fig. 3.4).

The late-phase reaction to allergen stimulation occurs hours after allergen exposure and is typified by the recurrence or prolongation of symptoms, characterized by mucosal infiltration of eosinophils, neutrophils, basophils, and T lymphocytes. This late phase reaction mirrors the findings in persistent/chronic allergic disorders.

Late-phase reactions can be induced in atopic asthma without a preceding immediate, IgE/mast cell-dependent response [45]. A direct activation of allergen-specific T cells by T cell peptides may be an alternative system for initiating an allergic reaction in subjects with no evidence of specific IgE sensitization, such as many VKC patients.

In normal individuals, mast cells are particularly abundant in the conjunctival stroma, especially at the limbus. Two types of mast cells have been demonstrated in the conjunctiva, depending on their content of tryptase (mucosal mast cell or MCT) or both tryptase and chymase (connective type mast cell or MCTC). The vast majority of mast cells in the conjunctiva belong to the MCTC subtype [24]. Activated mast cells, in addition to the well known gamut of inflammatory mediators, can also release several cytokines such as IL-4, IL-6, IL-8, IL-13, tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β), all of which have profound effects on the mucosa, including the induction of chemokines and adhesion molecules that contribute to the recruitment of inflammatory cells [10].

Eosinophils are considered an important effector cell in producing the macroscopic appearance of the late phase [52]. Eosinophils are activated by interactions with other inflammatory cells, mediators such as platelet-activating factor (PAF), C5a, allergen-antibody complexes, and possibly IgE through high and low affinity receptors (Fcε-I and Fcε-II). Activated eosinophils are an important source of leukotrienes, prostaglandins, cytokines, and chemokines and release very basic, highly charged polypeptides, including

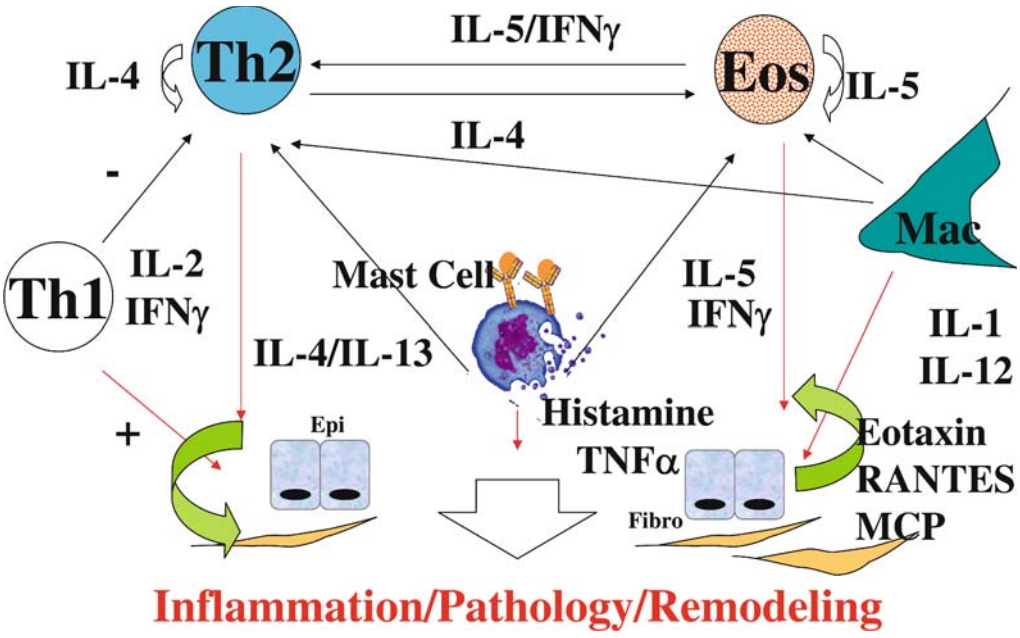

Fig. 3.4 Schematic diagram of chronic allergic reaction. *Th1* lymphocyte T helper type 1, *Th2* lymphocyte T helper type 2, *Eos* eosinophil, *Mac* macrophages, *Epi* conjunctival epithelial cells, *Fibro* conjunctival fibroblasts, *IL* interleukin, *IFN* interferon, *TNF* tumor necrosis factor, *MCP* monocyte chemotactic protein, *RANTES* regulated on activation normal T cell expressed and secreted

major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EPX), and eosinophil peroxidase (EPO). These proteins may bind to basement membrane proteoglycans and hyaluronic acid to cause cellular disaggregation and epithelial desquamation. ECP and MBP are also epitheliotoxic and are involved in the corneal damage (epitheliopathy and ulcers) that occurs in severe chronic allergic conditions. Several chemotactic factors for eosinophils have been described in allergic conjunctivitis. RANTES, MCP, eotaxin-1 and -2 are produced in large amounts in ocular allergic patients. Conjunctival fibroblasts and epithelial cells, when stimulated by IL-4 and TNF, are potential sources of these chemokines [33].

Helper lymphocytes (CD4+ T cells) are the predominant population in inflamed conjunctival tissues [17, 31]. Increased expression of Th2-type cytokines has been demonstrated in SAC, VKC, and AKC [19, 30, 53]. However, in addition to typical Th2-derived cytokines, increases in pro-inflammatory cytokines and INF-γ have been recently shown both in VKC and AKC, suggesting that in the most severe atopic conditions, cell-mediated hypersensitivity may also occur [17, 31, 41]. The recruitment of Th2 cells appears to be under the control of several CC chemokines that bond with receptors such as CCR3 (i.e., eotaxin 1–3, RANTES, MCP-3, MCP-4), CCR4 (i.e., MDC and TARC), and CCR8 [6].

Summary for the Clinician

- Genetic and environmental influences determine IgE-mediated hypersensitivity.
- IgE/mast cell-dependent reactions are the basic mechanism in ocular allergy.
- There is a central role for CD4+ Th2 cells in the allergic response.
- Th2 cells and Th2-type cytokines are involved in the pathological changes associated with chronic allergic disease.

3.5 Treatment of Ocular Allergy

Ocular allergic patients need fast relief from signs and symptoms, long-lasting therapeutic effects, comfortable and safe topical drugs, and a convenient treatment regimen (Table 3.3). The most common diseases, SAC and PAC, are classic IgE-mediated disorders, in which the therapeutic focus is mostly confined to the suppression of mast cells, their degranulation, and the effects of histamine and other mast cell-derived mediators [34]. Conversely, severe chronic disorders such as VKC and AKC are both IgE- and T cell-mediated, leading to chronic inflammation where eosinophil, lymphocyte, and structural cell activation characterizes the conjunctival allergic reaction. In these cases, stabilization of mast cells and histamine or other mediator receptor antagonists are frequently insufficient for the control of conjunctival inflammation. Severe disease necessitates the use of potent drugs and a more complex therapeutic regimen, often associated with more frequent side effects. None of the available drugs covers all therapeutic requirements for the suppression of conjunctival allergies.

Table 3.3 Topical ocular allergy medications

Class	Drug	Indication	Comments
Vasoconstrictor/antihistamine combinations	Naphazoline/pheniramine	• Rapid onset of action	• Short duration of action • Tachyphylaxis • Mydriasis • Ocular irritation • Hypersensitivity • Hypertension • Potential for inappropriate patient use
Antihistamines	Levocabastine Emedastine	• Rapid onset of action • Relief of itching • Relief of signs and symptoms of SAC	• Short duration of action
Mast cell stabilizers	Cromolyn Nedocromil Lodoxamide NAAGA Pemirolast	• Relief of signs and symptoms	• Long-term usage • Slow onset of action • Prophylactic dosing
Antihistamine/mast cell stabilizers (dual-acting)	Azelastine Epinastine Ketotifen Olopatadine	• Treatment of signs and symptoms of SAC • Rapid onset of action • Long duration of action • Excellent comfort	• Bitter taste (azelastine) • No reported serious side effects
Corticosteroids	Loteprednol Fluorometholone Desonide Rimexolone	• Treatment of allergic inflammation • Use in severe forms of allergies	• Risk for long-term side effects • No mast cell stabilization • Potential for inappropriate patient use • Requires close monitoring

3.5.1 Anti-Allergic Medication

Currently available topical drugs for allergic conjunctivitis belong to different pharmacological classes (Table 3.3): vasoconstrictors, antihistamines, mast cell stabilizers, mast cell stabilizers and antihistamines called "dual-acting" agents, nonsteroidal anti-inflammatory agents, and corticosteroids.

3.5.1.1 Vasoconstrictors

Vasoconstrictors are alpha-adrenergic agonists approved topically for the relief of conjunctival redness. The number and commercial names of these products are countless all over the world. Although they are well known and widely used, their short duration of action (2 h or less) limit their value compared with the newer anti-allergic drugs available. Topical vasoconstrictors are not usually recommended because they are nonspecific, and not pharmacologically active in the cascade of events that leads to the allergic reaction.

3.5.1.2 Antihistamines

Antihistamines are H_1 receptor competitive antagonists of varying specificity, potency, and duration of action. The first generation antihistamines, pheniramine and antazoline, have a long safety record, but are known for their burning upon instillation, their rapid onset and disappearance of effects, and limited potency [20]. These are still available in over the counter products, particularly in association with vasoconstrictors. The newer antihistamines are still H1 antagonists, but have a longer duration of action (4 to 6 hs), and are better tolerated than their predecessors. These include levocabastine hydrochloride (Livostin, 0.5%) and emedastine difumarate (Emadine, 0.05%). Both drugs are effective and well tolerated, even in pediatric subjects with allergic conjunctivitis.

3.5.1.3 Systemic Antihistamines

Allergic rhinoconjunctivitis is an equally frequent condition generally treated with systemic antihistamines, which have been proven effective at relieving nasal and conjunctival signs and symptoms [13]. First generation H1 receptor antagonists may provide some relief of ocular itching, but are sedating and have anti-cholinergic effects such as dry mouth, dry eye, blurred vision, and urinary retention. Second generation antihistamines offer the same efficacy as their predecessors, but with a low-sedating profile and lack of anti-cholinergic activity. These drugs include acrivastine, cetirizine, ebastine, fexofenadine, loratadine, and mizolastine. Desloratadine and levocetirizine are considered the subsequent evolution of these second generation agents.

3.5.1.4 Mast Cell Stabilizers

Mast cell stabilizers inhibit degranulation by interrupting the normal chain of intracellular signals resulting from the cross-linking and activation of the high affinity IgE receptor (FcεRI) by allergen [20]. These drugs inhibit the release of histamine and other preformed mediators and the arachidonic acid cascade of mediator synthesis. Several mast cell stabilizers are available for use in the eye: cromolyn sodium 4%, nedocromil sodium 2%, lodoxamide tromethamine ophthalmic solution 0.1%, spaglumic acid 4%, and pemirolast potassium ophthalmic solution 0.1%.

Cromolyn sodium was the first of these molecules to be developed. The recommended dosing schedule is four to six times daily, with a loading period of at least 7 days and an onset of activity after as much as 2 weeks. However, in human conjunctival-derived mast cells in culture, cromolyn only partially inhibited cell degranulation and histamine release, confirming the limited efficacy of this drug in the clinical treatment of ocular allergy.

Nedocromil appears to be more potent than cromolyn, and is approved for a dosage of two times daily. Nedocromil appears to inhibit mast cells, eosinophils, epithelial cells, and sensory nerves by a common pathway.

Lodoxamide has been shown to be effective in the treatment of seasonal allergic conjunctivitis and vernal keratoconjunctivitis. In addition to mast cell stabilization, lodoxamide reduces eosinophil activation and degranulation, thus improving corneal signs such as keratitis and shield ulcers in severe allergic disease.

Pemirolast is another mast cell stabilizer that has been shown to alleviate the signs of allergic conjunctivitis. In vitro and in vivo studies have demonstrated the efficacy of pemirolast in inhibiting the antigen-induced release of inflammatory mediators (e.g., histamine, leukotriene C4, D4, E4) from human mast cells and subsequently in preventing signs and symptoms associated with allergic conjunctivitis. Pemirolast is currently approved for a dosing regimen of four times daily (QID).

Dipeptide N acetyl-aspartyl glutamic acid (NAAGA; 6%) is approved in Europe for the treatment of allergic conjunctivitis, VKC, and GPC. NAAGA inhibits leukotriene synthesis, histamine release by mast cells, complement-derived anaphylatoxin production, and leukocyte adhesion to endothelial cells. These pharmacological properties confer a potential anti-inflammatory activity to NAAGA.

3.5.1.5 Dual Action Anti-Allergic Molecules

This class includes azelastine (Optivar/Allergodil), epinastine (Elestat/Relestat), ketotifen (Zaditor/Zaditen), and olopatadine (Patanol/Opatanol). The advantage offered by these molecules is the rapidity of symptomatic relief given by immediate histamine receptor antagonism, which alleviates itching and redness, coupled with the long-term disease-modifying benefit of mast cell stabilization [20].

Azelastine was shown to reduce ICAM-1 expression on conjunctival epithelium, and inflammatory cell infiltration during both early and late phase allergic reactions [18]. It was demonstrated to be significantly effective in adults and children of at least 4 years of age, and to be at least as effective as levocabastine. The most significant side effect with azelastine is an unpleasant taste following instillation.

Epinastine is a histamine H_1-receptor antagonist with mast cell stabilizing activity and no effect on muscarinic receptors. Its safety and efficacy have been investigated in the clinical conjunctival allergen challenge model, and in patients with active seasonal allergy, where it was shown to rapidly and significantly inhibit hyperemia, chemosis, and lid swelling for at least 8 h [56].

Ketotifen has been shown to inhibit the release of inflammatory mediators from mast cells, and, clinically, to be effective in the treatment of ocular allergy, and a safe option for children with allergic conjunctivitis [5].

Olopatadine has been shown to effectively and potently inhibit conjunctival mast cells in allergic patients with seasonal and perennial allergic conjunctivitis [2], to reduce levels of histamine, the cellular infiltrate, and ICAM expression compared with placebo after conjunctival allergen challenge.

3.5.1.6 Nonsteroidal Anti-Inflammatory Drugs

Ketorolac 0.5% (Acular) is the only ophthalmic nonsteroidal anti-inflammatory drug (NSAIDs) currently approved by the FDA for use in acute seasonal allergic conjunctivitis, for the relief of ocular itching. Blocking the synthesis of prostaglandins, particularly, PGD_2, NSAIDs partially inhibit the cascade of events following mast cell activation. Diclofenac sodium 0.1% was also shown to have some effect on the control of signs and symptoms of seasonal allergic and vernal conjunctivitis.

3.5.1.7 Corticosteroids

Corticosteroids should be the last choice when treating allergic disease. They should be avoided in seasonal and perennial allergic conjunctivitis, while their use is at times inevitable in VKC and AKC. Corticosteroids do not directly stabilize immune cell membranes and do not inhibit histamine release; however, they may modulate the mast cell response by inhibiting cytokine production and inflammatory cell recruitment and

activation. Thus, they are not the ideal therapy choice for allergic disease, although clinically they are the most effective anti-inflammatory agents in active ocular allergy.

Fluorometholone, medrysone, loteprednol, rimexolone, and desonide, called "soft" steroids, are considered to be the medications of choice when a mild, weakly penetrating drug is needed.

For severe ocular allergy, stronger prednisolone and dexamethasone should be used, opting for the lowest dose for the shortest duration of time. The therapeutic effects of the drug, as well as the potential side effects, must be monitored, and topical therapy tapered slowly over several days.

3.5.1.8 Immunomodulators

Several reports proved the clinical benefit of topical cyclosporine (1–2% in the treatment of severe VKC and AKC patients, ameliorating the signs and symptoms without significant side effects [7, 12, 23, 46, 49].

Tacrolimus is a potent drug similar to cyclosporine in its mode of action, but chemically distinct. A skin ointment of tacrolimus has recently been licensed for the treatment of moderate to severe atopic eyelid diseases [48]. However, patients may be at increased risk of folliculitis, acne, and herpes simplex. A recent study reported great efficacy of tacrolimus (0.1%) ointment in the treatment of severe VKC patients [55].

Although worldwide there has been an overall positive clinical experience with the use of topical cyclosporin A, this drug has not been approved for the treatment of allergic keratoconjunctivitis, and as such, its use should be reserved for selected patients followed in Referral Centers.

Summary for the Clinician

- Dual acting components are the first line treatment for ocular allergy.
- Avoid vasoconstrictors.
- Avoid abuse of steroids.
- Severe diseases must be treated with two or more drugs.

3.5.2 Nonpharmacological Management

The first treatment for ocular allergy should be avoidance of the offending allergens. This can usually be achieved for indoor, professional or food allergens. Thus, the identification of allergens by skin or blood testing is necessary to allow for avoidance of precipitating factors.

Nonpharmacologic treatments include tear substitutes and lid hygiene for the washing out of allergens and mediators from the ocular surface, and cold compresses for decongestion. Patients should be informed of the duration of the disease based on allergen diffusion and exposure.

In the management of VKC and AKC, patients and parents should be informed of the duration of the disease and its possible complications. Avoidance of nonspecific triggering factors such as sun, wind, and salt water with the use of sunglasses, hats with visors, and swimming goggles must be recommended. Frequent hand and face washing should be also suggested.

Particularly in AKC and in contact blepharoconjunctivitis, tear substitutes are useful for the removal and dilution of antigens and mediators, as well as for improving corneal involvement. Lid hygiene is required to prevent infectious blepharitis, and to improve meibomian gland function and tear film quality.

Disease prevention and nonpharmacological management are particularly important in contact lens-related diseases such as GPC [22]. This approach involves prescribing the appropriate lens type and edge design, and encouraging strict lens hygiene. Enzymatic cleaning of the lens is essential to minimize the accumulation of lens coatings and to remove protein build-up. The most essential treatment for early stage GPC is removal of the device that is causing the condition. In fact, patients are asymptomatic several days after discontinuation or removal of the contact lens, device or suture. Re-initiation of lens wear with a clean lens, or lens of a different type or design may occur within days of symptom resolution.

Specific immunotherapy is indicated only when a clearly defined systemic hypersensitivity to identified allergens exists. In these cases, oral-

specific immunotherapy, which is better tolerated in children, can be considered since it is equally effective as traditional subcutaneous injections. However, successful treatment requires at least 2 years of therapy and adjustment of tolerated doses during the pollen season.

3.5.3 Surgical Treatment

Surgical treatment is at times indispensable. Physical removal of corneal plaques is recommended to resolve severe symptoms such as photophobia, pain, and irritation, and to allow for corneal re-epithelialization. Giant papillae excision may be indicated in cases of mechanical pseudoptosis or gross papillary formations. More invasive procedures such as oral mucosal grafting and saphenous vein transplantation, or, more recently, amniotic membrane application, have been used for the treatment of severe tarsal VKC patients, but should be avoided in young children since these procedures do not have proven immunosuppressive effects. Cryotherapy of the tarsal mucosa is not indicated since it may favor conjunctival scarring, does not resolve signs and symptoms and has no immunosuppressant effects on the conjunctiva. As such, surgical procedures that permanently change the anatomy and physiology of the lid are not recommended.

Summary for the Clinician

- Avoidance of antigen exposure is the first therapy.
- Cold compresses and tear substitutes may be helpful.
- Specific immunotherapy is especially indicated if other allergic manifestations are present.
- Removal of corneal plaques is the only surgical procedure recommended in cases of corneal complications.

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Chapter 4

Current Aspects of the Immunobiology and Prevention of Corneal Graft Rejection: What Have We Learned from 100 Years of Keratoplasty?

4

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Core Messages

- Keratoplasty is considered the most successful transplant procedure; however, several studies indicate that the long-term survival of corneal grafts is below that of, for example, kidney and heart transplantation.
- Even when considered an immune privileged organ, the cornea is subject to the same principles of transplantation immunology; this applies in particular to high-risk recipients.
- Beside the well-known risks of *acute* corneal graft rejection there is growing evidence that postoperative endothelial cell loss is probably a *chronic* immune mediated rejection process.
- Current strategies to improve corneal graft survival have been adopted from other fields in transplant medicine and include histocompatibility matching as well as immunomodulatory drug regimens with the (limited) option of topical treatment.
- Experimental keratoplasty models have contributed with many details to our current understanding of the underlying immune mechanisms in corneal grafting.
- Our more detailed understanding of the underlying immune mechanisms in graft rejection may allow more specifically directed preventive and therapeutic measures in the future that may include "biologicals" and gene therapy.

4.1 Introduction

4.1.1 History and Prevalence of Allograft Rejection

Corneal transplantation definitely has its paradoxes. With a century of experience, it is the oldest and most frequent kind of transplantation [140]. At the same time it is considered as the most successful form of transplantation in man, but several long-term studies indicate that the opposite might be the case. Whereas for conditions such as keratoconus and corneal dystrophies the results of keratoplasty are excellent; the prognosis of acquired corneal opacities is worse than for kidney- and heart transplantation. In contrast to an overall survival rate of renal grafts of greater than 90% [74] the 5-year prognosis for penetrating keratoplasty in the presence of risk factors is estimated to be approximately 50% [134]. From a clinical point of view the initially favorable prognosis of penetrating keratoplasty can be narrowed by immunological considerations.

Table 4.1 Corneal graft rejection rate in clinical studies. *n.m.* not mentioned

Reference	Number	Incidence (%)	Irreversible (%)
[27]	600	44	n.m.
[118]	88	49	25
[127]	290	28	n.m.
[121]	100	46	23
[1]	156	29	5
[4]	869	n.m.	5
[105]	226	43	13
[80]	740	4–37[a]	3–28[a]
[133]	7741	n.m.	32–33
[93]	646	18	n.m.
[89]	49	53	16
[76]	394	23	7
[10]	417	n.m.	28–40
[126]	126	37	30

[a]Dependent on risk factors

The effects of immune mediated graft rejection on keratoplasty have been clearly shown in numerous studies and interestingly it seems the rejection rate has not changed in the last 50 years (Table 4.1). Therefore, more efficient preventive and therapeutic efforts are required to improve the prognosis after keratoplasty. In recent years the use of experimental keratoplasty models in rodents has broadened not only our understanding of the immunobiology of corneal grafts, but has also led to new preventive and therapeutic options. We would therefore like to focus our attention first to the underlying immune mechanisms before we approach current and new treatment possibilities.

4.1.2 Local Immunological Features of the Cornea

The eye has unique features that make it quite different from other organs with regard to its immunobiology. Accordingly, some principles involved in general transplantation immunology usually cannot be applied directly in corneal transplantation.

4.1.2.1 Corneal Immunogenicity

The cornea has been thought to lack immunogenicity because of its low antigen expression: major histocompatibility complex (HLA) class I antigens are expressed particularly in the corneal epithelium and, much less densely, in the stroma and endothelium. HLA class II antigens have been found to be only scattered in the corneal epithelium, particularly in the limbus region and in the corneal stroma [78]. However, the expression can change under certain conditions. An enhanced expression of HLA class I and II antigens could be detected during allograft rejection and even induced by the surgical process itself [78]. In contrast, extending storage [2] or low-dose UVB radiation [14] decreased HLA class II antigen (DR) expression in human corneas. Moreover, studies using rodent models of keratoplasty have shown that, in some strain combinations, allografts carrying minor histocompatibility (H) alloantigens are more likely to be rejected than

those bearing major histocompatibility complex (MHC) alloantigens [102, 113]. Interestingly, by using a murine model, which differed only in defined minor-H antigens, Haskova [34] revealed that some minor-H antigens are even more immunogenic in corneal transplantation than skin allografts. In addition, more recent studies demonstrated that minor-H antigens are devoid of immunogenicity in the absence of IFN-γ [33]. Depletion of IFN-γ by either in vivo neutralization or disruption of the IFN-γ gene resulted in an amazing acceptance in MHC-matched, minor H-mismatched corneal allografts, but not in a MHC-mismatched setting. In addition, MHC class II antigens were found to play a more important role as targets of T cell-mediated alloimmunity than MHC class I antigens [75].

As a consequence of antigen distribution in all cellular layers, corneal allograft rejection can occur in each of the three main layers independently or simultaneously in any combination. Epithelial rejection is thought to be unimportant since the donor epithelium is gradually replaced by the host epithelium derived from the limbus without further consequences (Fig. 4.1a). Stroma rejection is relatively rare, and might be reversed by intensive application of topical corticosteroids (Fig. 4.1b). The endothelium is the most critical cell layer because of its essential physiological function and at the same time the most important target of allograft rejection (Fig. 4.1c). Surgical trauma, early postoperative inflammation as well as endothelial rejection can result in a rapid and irreversible decrease in corneal endothelial cell density (Fig. 4.1d). In fact, endothelial cell density decreased at an accelerated rate of 4.2% per year [15] after transplantation even without apparent rejection. The widely embraced explanation for this phenomenon is chronic subclinical immune response. Interestingly, more recent

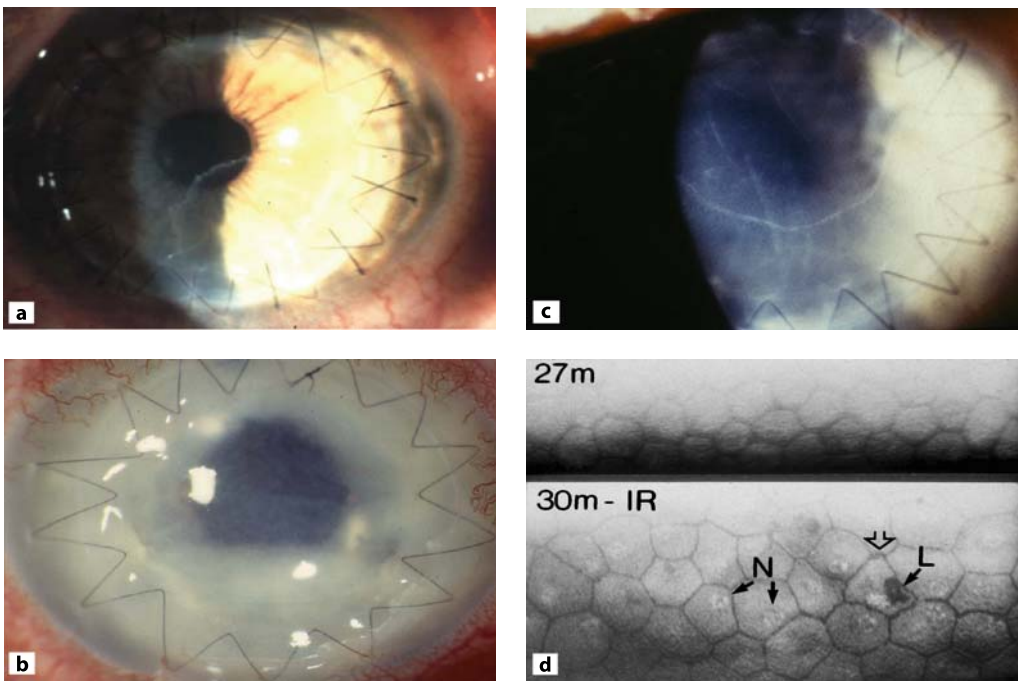


Fig. 4.1 Corneal graft rejection. In principle, since all layers of the tissue are able to express MHC antigens, rejection may occur at the **a** epithelial, **b** stromal, and **c** endothelial levels. **d** Direct adherence of leukocytes can be seen by specular microscopy (*clear arrow*). The same (keratoconus) patient was seen 27 and 30 months following transplantation. Reduced corneal endothelial density with increased cell size is easily seen. *N* neutrophil, *L* lymphocyte

studies have shown that epithelium and stroma bear the immunogenic ability while endothelium has the immune privileged property, which can subsequently abrogate the immunogenicity of the stroma [43].

4.1.2.2 Immune Privilege

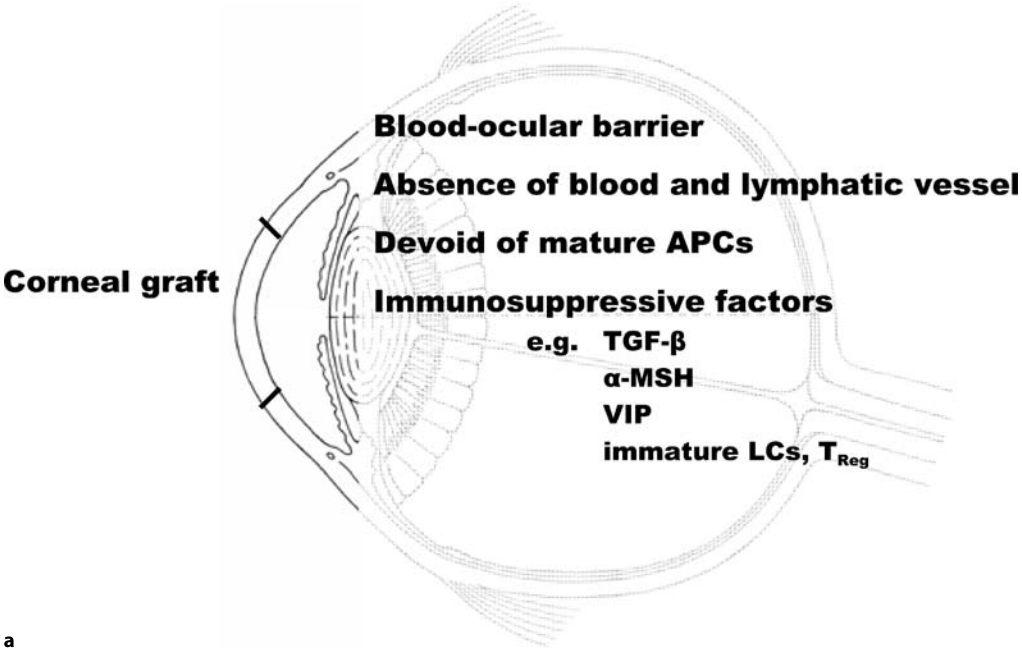
Compared with other forms of organ transplantation, keratoplasties performed in patients, with for example keratoconus, have a relatively favorable prognosis even without tissue typing or systemic immunosuppressive therapy. The most widely used explanation for this is that the transplantation is performed at an immunologically privileged site. Medawar (1948) was the first to realize that the anterior chamber of the eye has some unique immunological features. He substantiated the "sequestration" of the corneal graft from the systemic immune apparatus by the avascularity of the cornea and the graft bed, the absence of sensitizing histocompatibility antigens in the graft and the rapid replacement of donor tissue by the recipient.

It later became clear that of these criteria only avascularity remained a significant factor and that immune privilege is an active immunoregulatory process (Fig. 4.2). The underlying mechanisms include:

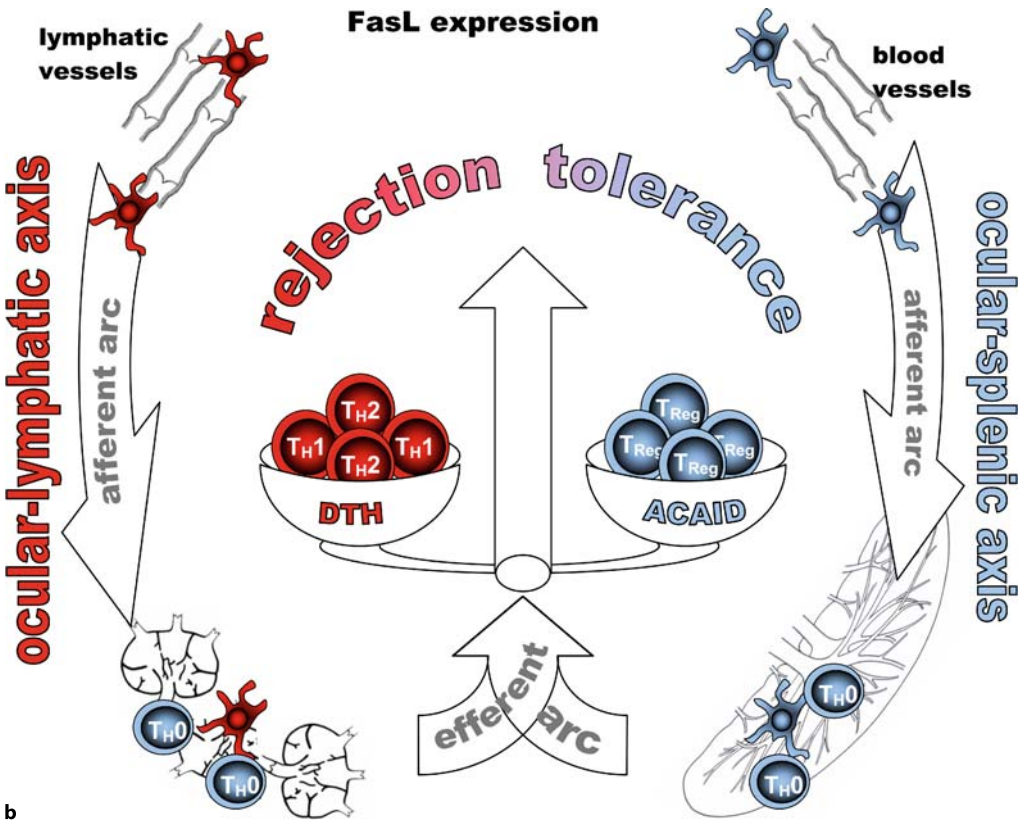
1. An *afferent blockade* of the immune response. The transmission of immunogenic stimuli to the peripheral lymphoid tissues is actively interrupted. As mentioned above, the normal cornea and the graft bed are devoid of lymphatic and blood vessels; there is a very low expression of MHC class I antigen on corneal cells and in the central cornea only MHC class II antigen negative LCs appear.
2. An *efferent blockade*. Immune effector cells are neutralized by Fas ligand (FasL) expression at the host–graft interface. FasL induces programmed cell death (apoptosis) in cells bearing its receptor (neutrophils, activated T cells, CTL) [30].
3. Immunoregulatory mechanisms deviate the immune response into a nondestructive pathway and thus inducing an antigen-specific tolerance (*immune deviation at a central stage*). The presence of immunosuppressive cytokines, e.g., tumor growth factor-β (TGF-β) and interleukin-10 (IL-10), neuropeptides and hormones in the anterior segment of the eye, accounts for the immaturity of LCs. This tolerogenic milieu and immature dendritic cells provide an anergic status of Th1/2 cells and immune modulation/suppression by regulatory T cells (T_{REG}).
4. Ocular innervation stimulates the secretion of immunomodulatory neuropeptides such as substance P (SP), VIP (vasoactive intestinal peptide), α-MSH (α-melanocyte-stimulating hormone) and CGRP (calcitonin gene-related peptide) into the aqueous humor (neural control). They are typically released from nerve termini and inhibit T-cell activation and effector functions of macrophages [115]. Recent investigations revealed, furthermore, that besides ocular innervation an intact sympathetic nervous system is required for the induction of splenic regulatory T-cells [60].

Fig. 4.2 Immune tolerance vs. corneal graft rejection. **a** Local factors contributing to immune privilege. **b** Simplified scheme on the immunoregulation of corneal grafts. Antigens introduced into the anterior chamber are captured by antigen-presenting cells (*APCs*) and transported to the lymph organs. In experimental models antigen presentation to naïve T cells (T_H0) via the ocular–lymphatic axis may lead to delayed type hypersensitivity (DTH), whereas the presentation via the ocular–splenic axis may induce T_{REG}-triggered downregulation of T_H1 and T_H2 immune responses and therefore promotes an anterior chamber-associated immune deviation (*ACAID*). *α-MSH* α-melanocyte stimulating hormone, *FasL* Fas ligand, *LCs* Langerhans cells, *TGF-β* tumor growth factor-β, T_{REG} regulatory T cells, *VIP* vasoactive intestinal peptide

Disintegration of any of these components leads to the breakage of immune privilege. "Danger signals" (viral or bacterial antigens, inflammation) as well as traumatic stimuli cause the activation of DCs and thus increase their allostimulatory capacity. In the absence of danger signals the introduction of antigens into the anterior chamber, i.e., the orthotopic corneal allograft, leads to an antigen-specific downregulation of Th1 immune responses. This phenomenon has been termed anterior chamber-associated immune deviation (ACAID) [50].


Blood-ocular barrier
Absence of blood and lymphatic vessel
Devoid of mature APCs
Corneal graft
Immunosuppressive factors
e.g. TGF-β
α-MSH
VIP
immature LCs, T_{Reg}

a


lymphatic vessels
FasL expression
blood vessels
rejection
tolerance
ocular-lymphatic axis
afferent arc
ocular-splenic axis
afferent arc
T_H2
T_H1
T_H2
T_H1
DTH
T_{Reg}
T_{Reg}
T_{Reg}
T_{Reg}
ACAID
efferent arc
T_H0
T_H0
T_H0

b

4.1.2.3 Anterior Chamber-Associated Immune Deviation

The induction of an antigen-specific down-regulation of delayed-type hypersensitivity (DTH) has been demonstrated with a wide array of antigens, including the MHC and minor histocompatibility antigens [73]. It was postulated that antigens injected into the anterior chamber of the eye are internalized by intraocular tolerogenic APCs, which then subsequently migrate to the spleen and to a lesser extent to regional lymph nodes where they deviate a systemic immune response from a cytotoxic, inflammatory response to a noncytotoxic response, thus promoting graft success. Indirect support for the involvement of regulatory T cells was found in studies with splenectomized mice, which showed a significant increase in corneal graft rejection compared with controls [114]. Further experiments using depleting monoclonal antibodies (mAb) or knockout mice proved the requirement of regulatory αβ NK T cells [112] and γδ T cells (cells bearing a γδ chain TCR) [135] for the induction of ACAID. These γδ T cells play a crucial role in the well-known phenomenon of oral tolerance. In fact, feeding recipient mice with donor cells enhanced corneal graft survival and prevented graft rejection in high-risk settings [63]. Although removal of the thymus did not affect experimental induction of oral tolerance, an intact thymus is essential for the development of ACAID in adult mice [129]. Ocular antigen laden F4/80$^+$ monocytic APCs were proposed to be present in the thymus where they activate thymic NK T cells, which in turn subsequently home to the spleen and activate regulatory T cells [28].

By targeting fluorescence-labeled, intracamerally injected antigens Camelo et al. showed that most of the injected antigen readily drains to local lymph nodes, hemolymph nodes, and spleen via the conventional ocular outflow pathways in a soluble form. In contrast, ocular APCs appear unable to migrate to secondary lymphoid organs [19]. Despite the initial paradigm of antigen-presentation by ocular APCs, these recent findings strongly suggest that the ACAID-inducing signal is more likely associated with networks of resident macrophages and DCs in the secondary lymphoid organs.

Summary for the Clinician

- Features that support corneal graft survival include unique immunological characteristics such as a low MHC antigen expression, absence of MHC class II$^+$ dendritic cells as well as the lack of an afferent drainage system.
- Long-term corneal storage advances down regulation of MHC antigens, which resulted in improved graft prognosis. However, the role of other antigens that revealed a great impact in experimental keratoplasty, e.g., minor H antigens, is still unknown.
- Several factors such as surgical trauma, neovascularization and "danger signals" impair the protective mechanisms and significantly increase the risk of graft rejection.
- Immune privilege is a highly regulated active immune process involving local and systemic mechanisms rather than a simply passive antigen sequestration.

4.2 Mechanisms of Corneal Allograft Rejection

The precise cellular and molecular mechanisms still remains poorly understood. Experimental keratoplasty models have contributed in great detail to our understanding and indicate that both local and systemic immune mechanisms contribute to transplant rejection. To simplify the underlying complex mechanisms of corneal graft rejection, it was suggested that a three-step process might occur:

1. The donor antigen has to be released, recognized, and transported to the draining lymph tissue (afferent arm)
2. Alloantigens have to be processed to induce a specific cellular immune response (central stage)
3. Effector cells gain access to and attack the graft, eventually destroying it (efferent arm)

4.2.1 Antigen Presentation

4.2.1.1 Antigen-Presenting Cells

As the initiator and the modulator of the immune response, APCs are not only critical for the induction of antigen-specific allograft immune responses, but also for the induction of immunological tolerance.

4.2.1.1.1 Langerhans Cells

Langerhans cells (LCs), derived from bone marrow cells, are professional antigen-presenting cells. They display large amounts of MHC-peptide complexes on their surface, express much higher costimulatory molecules, e.g., CD80 and CD86, than other APCs and are thought to be the only cell type that can activate naive T cells. Only few LCs are necessary to provoke a strong T cell response. The presence of donor-derived LCs dramatically increases the immunogenicity and doubles the incidence of corneal allograft rejection [71]. Interestingly, while donor tissue is kept in culture, LCs can gradually migrate out of the transplant and MHC class II^+ LCs are not found in cultured cornea 1 week after storage [2]. A recent retrospective clinical study demonstrated that prolonged storage of donor cornea (⊠7 days) enhances the acceptance of the allograft, especially in high-risk cases [109].

Usually, LCs reside in tissue in an immature state with limited APC function. However, they are extremely well equipped to capture and process antigen and subsequently form MHC-peptide complexes. Once they entrap antigens, they migrate to the lymphoid organ and complete their maturation. Mature LCs can easily prime T lymphocytes and initiate the immune response. Previous studies have shown that only low numbers of LCs are present in the normal cornea, mainly in the periphery of the corneal epithelium. However, more recent studies demonstrated that large amounts of LCs reside in the epithelium and anterior stroma of the normal cornea. Most of them, locating in the central and paracentral area, are $CD45^+$, $CD11c^+$, $CD11b^-$, MHC class II^-, $CD80^-$, $CD86^-$, $CD3^-$, indicating their immature status [31]. However, cytokines such as IL-1 and TNF-α released, e.g., after surgical trauma can increase the expression of MHC antigens and CD80, CD86 costimulatory molecules on the surface of LCs, and promote their migration [139]. On the one hand, the host-derived LCs could migrate centripetally to the grafts [72], capture and process the antigen, then present the donor-derived peptide to host T cells (indirect pathway). On the other hand, donor-derived LCs migrate centrifugally out, and gain access to the host bed and the lymphoid tissue [62], subsequently priming the T cells directly (direct pathway, see below).

4.2.1.1.2 Macrophages

In contrast to LCs, which have been extensively studied, the role of macrophages is not clear. Macrophages have phagocytic capability, secrete a variety of inflammatory cytokines, and express low levels of MHC class II antigens and costimulatory molecules. They participate in a wide range of critical immunologic functions: acting as APCs or as effector cells. Interestingly, the normal murine corneal stroma contains a significant number of resident macrophages [18], and macrophages constitute the predominant cellular infiltrate in rejected grafts [57]. Consequently, experimental depletion of macrophages, e.g., by subconjunctival administration of clodronate liposomes remarkably prolonged the survival of allografts [110]. However, depletion of macrophages before adoptive transfer of antigen-specific activated $CD4^+$ T cells to CD4 KO mice did not influence the incidence of allograft rejection, indicating that macrophages mainly function as APCs rather than as effector cells [37].

4.2.1.2 Direct/Indirect Pathway of Antigen Presentation

Presentation of alloantigens to recipient T lymphocytes is the first step to initiating allograft rejection. Two pathways have been proposed, either the direct or the indirect (Fig. 4.3). During direct allosensitization, the donor-derived APCs migrate from the graft to the host's lymphoid tissue and present allogeneic MHC molecules to host T cells. In contrast, in the indirect pathway, recipient-derived APCs pick up and process alloantigens and present them to T cells in the pres-

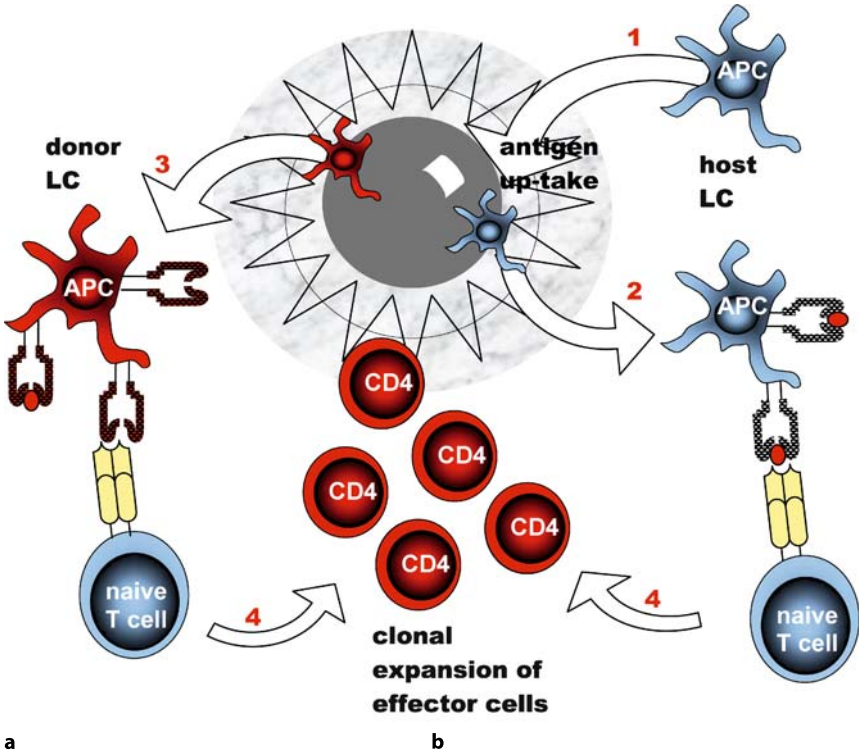


Fig. 4.3 Direct and indirect antigen presentation. *1* Recipient LCs migrate from the limbus centripetally into the corneal graft. There they internalize and process alloantigens. *2* The presentation of foreign peptides to host T cells leads indirectly to a donor-specific immune response. *3* Donor LCs migrate centrifugally out of the allograft and directly prime T cells in the draining lymph nodes. Alloantigen-specific T cells recognize either corneal antigens or allogenic epitopes presented on the major histocompatibility complex (MHC) molecules. *4* Activated T cells proliferate and infiltrate the graft tissue

ence of its own MHC molecules. It is now clear that both pathways are involved in the corneal alloresponse; however, the exact contribution still remains elusive.

The indirect pathway is considered to be the driving force of corneal allograft rejection [45]. This differs from other tissue transplantation, in which direct antigen presentation plays a critical role in initiating the rejection process. Several reasons may contribute and explain this difference:

1. The normal cornea is devoid of MHC class II$^+$ APCs, that is, lack of so-called passenger leukocytes.
2. The expression of MHC antigens is relatively low.
3. Minor H is the primary antigen to be presented to T cells. This was supported by the evidence that minor H antigen-specific, but not MHC antigen-specific DTH response was detected in the murine model of keratoplasty, bearing minor H and MHC mismatched graft [102].
4. In the mice model of normal-risk keratoplasty, T cells could be activated through both pathways. Interestingly, CD4$^+$ T cells were activated exclusively via the indirect pathway, while CD8$^+$ T cells were activated through the direct pathway. Moreover, only the CD4$^+$ T cells, which are activated via the indirect pathway, had the ability to reject the allograft [13].

All these observations suggest that the direct pathway is not likely to occur. However, more recent investigations have challenged this concept.

1. The normal cornea possesses resident MHC class II^- dendritic cells, which are capable of expressing MHC class II antigens after stimulation [31, 62].
2. Donor-derived APCs can migrate centrifugally out of the grafts and gain access to the host bed and to the ipsilateral draining lymph nodes [62].
3. The presence of donor-derived LCs dramatically increases the rejection rate [71]. In contrast, depletion of donor APCs, e.g., by pretreatment with ultraviolet radiation or hyperbaric oxygen, leads to fewer rejections [35].
4. In the mice model of high-risk keratoplasty, $CD4^+$ Th1 cells, which were activated via the direct pathway, could be detected as early as 72 h after transplantation. In addition, abrogation of the direct T cell response by using MHC class II KO donor grafts significantly decreased the frequency and tempo of rejection [44].

Taken together, both the indirect and direct pathways are involved in cornea allograft rejection. In experimental models, the indirect pathway is the driving force in both normal-risk and high-risk keratoplasty, while the direct pathway may play a role in the high-risk setting.

4.2.2 T Cell Activation: Principle of Costimulation

It is well accepted that a variety of signal transductions involved in cell-to-cell interactions are required for an effective immune response. Antigen-specific activation of T lymphocytes is dependent on a series of signal transductions between T cells and APCs. For full activation and proliferation of T cells, at least two signals are required (Fig. 4.4):

1. Signal 1, the triggering signal transferred from the peptide-MHC complex expressed on the APCs to T cell receptor-CD3 complex, which provides antigen specificity to immune response.
2. Signal 2, costimulatory signals transferred through cell surface receptors with their ligands between APCs and T cells (e.g., CD28-B7, CD40L-CD40, LFA-1-ICAM-1, etc.), which amplify the triggering signal. If T cells are not triggered efficiently via these two pathways they will become unresponsive (anergic).

4.2.2.1 CD28-CD80/CD86 Costimulatory Signal Pathway

The interaction of the T cell surface molecule CD28 with its ligands, CD80/CD86, expressed on the surface of activated APCs, is the best understood costimulatory signal. The ligation of the CD28 molecule leads to full activation of T cells, triggers T cells without CD28 interaction, and may subsequently lead to anergy or apoptosis. In order to prevent an excessive immune response, activated T cells express the CTLA-4 (CD152) molecule, which can interact with the same counter-receptors on APCs. In contrast to the stimulatory interaction of CD28 with CD80/CD86, the interaction of CTLA-4 with CD80/CD86 is thought to inhibit a subsequent T cell response by sending negative signals to T cells. Theoretically, blockade of this signal pathway either by anti-CD28 monoclonal antibody or soluble CTLA-4 constructs would have similar beneficial effects on allograft survival. Interestingly, in experimental models, anti-CD28 monoclonal antibody seems to be more effective in enhancing graft survival than CTLA-4Ig [123]. In addition, although CD28 KO mice show relatively longer allograft survival, rejection can still develop [3]. This indicates that other costimulatory pathways are operational in such conditions.

4.2.2.2 CD40-CD154 (CD40L) Costimulatory Signal Pathway

CD40-CD154 is another important costimulatory pathway that has been intensively studied. CD40, a member of the TNF receptor family, is expressed on a variety of cells including APCs, and interacts with CD154 (CD40L), a member of the TNF family, which is expressed on activated T cells. CD40 can upregulate the expression of CD80, CD86 as well as IL-12 and promote T cell differentiation in a Th1-type direction

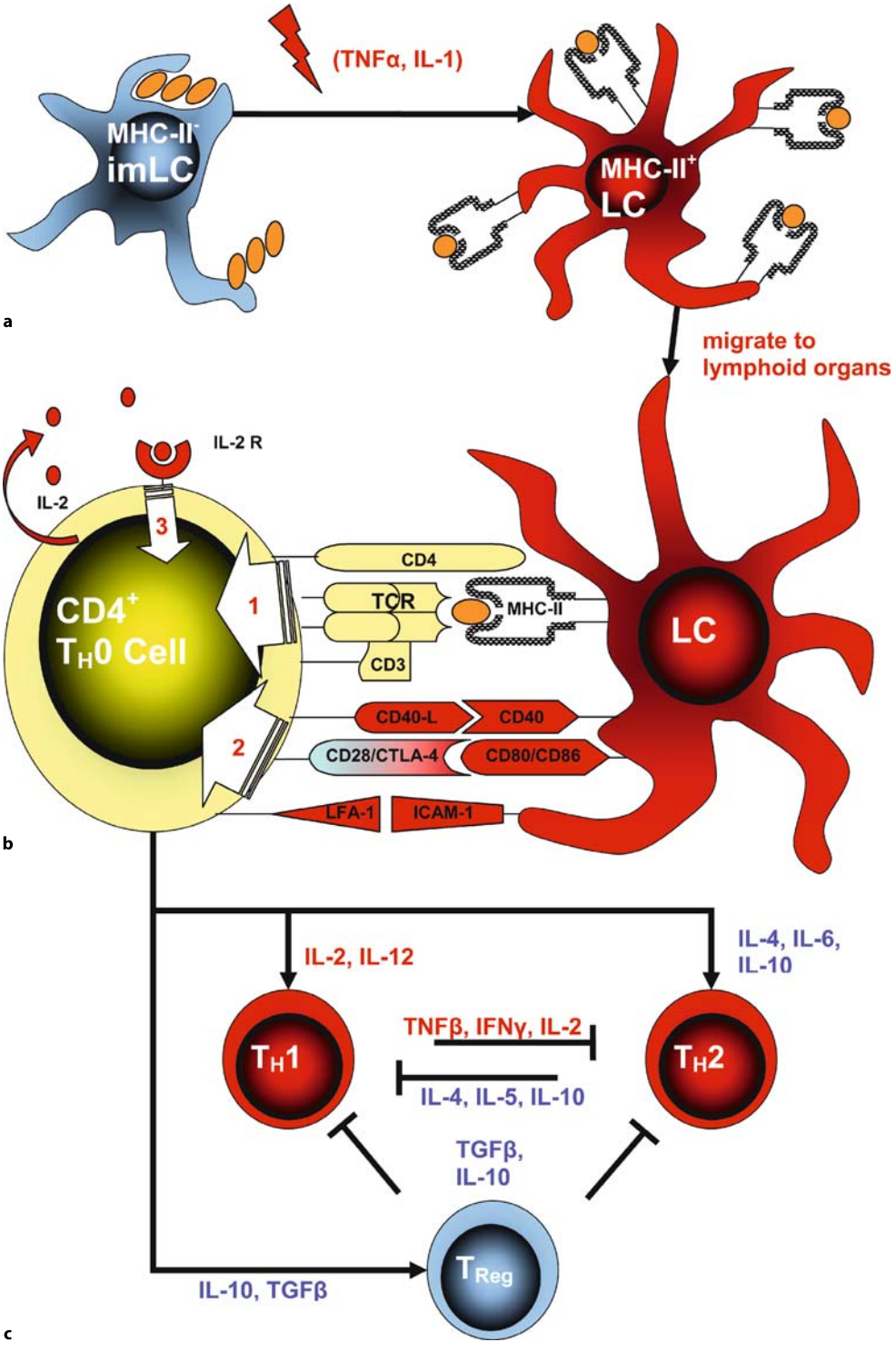

(TNFα, IL-1)
MHC-II-
imLC
MHC-II+
LC
a
migrate to
lymphoid organs
IL-2 R
IL-2
3
CD4+
TH0 Cell
1
2
CD4
TCR
MHC-II
CD3
LC
CD40-L
CD40
CD28/CTLA-4
CD80/CD86
LFA-1
ICAM-1
b
IL-2, IL-12
IL-4, IL-6,
IL-10
TH1
TNFβ, IFNγ, IL-2
IL-4, IL-5, IL-10
TH2
TGFβ,
IL-10
TReg
IL-10, TGFβ
c

Fig. 4.4. Mechanisms leading to T cell activation in corneal grafts. **a** Uptake and processing of antigens and induction of maturation by inflammatory stimuli: immature, MHC class II-negative LCs constitutively internalize antigens. Pro-inflammatory stimuli, e.g., bacterial lipopolysaccharides, viral dsRNA, tumor necrosis factor-α (*TNF-α*), and interleukin-1 (*IL-1*) induce the maturation of LCs. This process is characterized by a strong upregulation of MHC class II and costimulatory (CD80, CD86, and CD40) molecules. The expression of chemokine receptors provides for the homing of the mature LCs to the regional lymph nodes. There peptide fragments of processed antigen can effectively be presented to naïve T cells. **b** Schematic figure of interactions between T cells and LCs. A naïve T cell can only be fully activated if it receives at least two different signals: the recognition of the peptide–MHC complex by the T cell receptor (TCR) delivers the antigen-specific signal 1. The interaction of costimulatory (CD80/CD86–CD28, CD40–CD40L) and accessory (ICAM-1–LFA-1) molecules (signal 2) leads to an amplification and completion of the signal triggering. The driving force for T cell proliferation is the auto-/paracrine ligation of IL-2 and its high-affine ($CD25^+$) receptor (IL-2 R). This is often referred to as signal 3. **c** Activated effector populations and their cross-regulation. In principle, the development of the T helper cell populations (Th1 and Th2) and regulatory CD4+ T cells (T_{REG}) is dependent on the circumjacent cytokine milieu. The patterns of cytokines expressed by each T cell population stimulate their own proliferation and inversely act as inhibitors of that of the others

[77]. Blockade of the CD40-CD154 costimulatory pathway, e.g., by systemic administration of anti-CD154mAb profoundly reduced corneal allograft rejection in both normal-risk and high-risk keratoplasty. This effect was associated with a downregulation of chemokine gene expression, a decrease in leukocyte recruitment and suppression of Th1-type response [90]. Interestingly, this blockade cannot bias the T cell differentiation to a Th2-type response, as found in other organ transplantation.

However, selective blockade of each of the costimulatory signal pathways can prolong allograft survival. Surprisingly, the combined blockade seems to have no additional effect. In addition, CD154 blockade had no effect on CD28 KO mice indicating that CD40-CD154 depends on a functioning CD28 costimulatory pathway [3].

4.2.3 Role of Immune Cells and Molecular Mediators in Graft Rejection

4.2.3.1 Cellular Infiltration

Clinical studies have demonstrated that macrophages and T lymphocytes comprise the major cellular infiltrate in rejected corneal allografts [57]. In addition, $MHC\ II^+$ dendritic cells have been found in the basal layer of the epithelium and in the stroma during rejection [55]. More extensive investigations have been performed in rodent models by virtue of its similar expression of MHC molecules to the human cornea. In the rat model of corneal transplantation, large numbers of macrophages, T cells ($CD4^+ > CD8^+$), natural killer (NK) cells, and neutrophils were detected in graft tissue. Furthermore, $CD4^+$ and $CD8^+$ T cells have also been found in the aqueous humor [58].

4.2.3.2 Molecular Mediators

Adhesion molecules, cytokines, and chemokines construct a functional network and control the amplitude and duration of the allograft response.

Adhesion molecules play an essential role in corneal allograft rejection, especially in cell recruitment (Table 4.2). Clinical studies demonstrated that ICAM-1 was expressed on corneal epithelial cells, keratocytes, corneal endothelium, and vascular endothelial cells, particularly at the site of severe inflammation. E-selectin was present on endothelial cells of vessels in the stroma, which were characterized by dense infiltration with T cells and macrophages. VCAM-1 was predominantly expressed on inflammatory cells of the macrophage/monocyte lineage. LFA-1 has also been found in rejected grafts [130].

Fas ligand (FasL, CD95L) is a transmembrane protein and the interaction between Fas^+ cells and $FasL^+$ cells can lead to apoptosis of Fas^+ cells. Accordingly, the ocular immune privilege has been partially attributed to the abundant expression of FasL on cells that line the anterior chamber of the eye. The incidence of corneal graft rejection

Table 4.2 Effects of molecular mediators on experimental corneal transplantation. *LCs* Langerhans cells, *IL* interleukin, *TNFR* tumor necrosis factor receptor, *ACAID* anterior chamber-associated immune deviation, *IFN* interferon, *MHC* major histocompatibility complex, *NK* natural killer, *VEGF* vascular endothelial growth factor

Molecular mediator	Effects	Potential preventive/therapeutic application
IL-1	1. Induces migration of LCs to the central cornea 2. Induces apoptosis in the corneal endothelium 3. Stimulates neovascularization 4. Upregulates expression of ICAM-1, IL-6, IL-8, MCP-1, RANTES, and costimulatory factors	Topical application of IL-1 receptor antagonist (IL-1ra) significantly promotes corneal allograft survival in both normal-risk and high-risk grafts
TNF-α	1. Mediates migration of LCs 2. Induces RANTES, MIP, MCP secretion, ICAM-1 expression, IL-10 synthesis 3. Induces apoptosis through either TNFRI or TNFRII 4. Required in the induction of ACAID	1. Topical administration of soluble TNFR-I enhances acceptance of corneal allograft and decreases expression of RANTES and MIP1-β 2. Blockade of TNF-α by ex vivo gene transfer of soluble TNFR-Ig only marginally enhances allograft survival
TGF-β	1. Plays a key role in ocular immune privilege 2. Downregulates IFN-γ-induced expression of MHC–peptide complex, IL-6, CXCL-1, MCP-1, G-CSP, IGFBP-5 3. Inhibits expression of IL-2 receptor 4. Inhibits lymphocyte proliferation	
IL-10	1. Supports induction of ACAID 2. Inhibits Th1-type cytokines	Ex vivo gene transfer enhances allograft survival
IL-2	1. Induces corneal neovascularization 2. Promotes T cells proliferation	Administration of IL-2R MAb delays allograft rejection
IL-12	1. Induces secretion of IFN-γ 2. Inhibits angiogenesis of the cornea 3. Promotes proliferation of NK and T cells, induces Th1 cells differentiation	Inhibition of IL-12 by ex vivo gene transfer significantly prolongs corneal allograft survival
INF-γ	1. Promotes production of IL-1 2. Induces ICAM-1 expression 3. Selectively inhibits proliferation of Th2 cells	Depletion of INF-γ results in acceptance of MHC-matched, minor H-mismatched, but not of MHC-mismatched grafts
VEGF	Promotes hemangiogenesis and lymphangiogenesis	Neutralizing VEGF promotes graft survival in normal-risk grafts
Adhesion molecules	1. Participate in alloantigen recognition, T cell activation, and clonal expansion 2. Recruit inflammatory and immune cells	1. Depletion of LFA-1, VLA-4 alone or both ICAM-1 and LFA-1 or VLA-4 and LFA-1 enhances allograft survival 2. ICAM-1 MAb prolongs murine heterotopic corneal allograft survival
Chemokines	1. Induce migration of LCs 2. Mediate migration of alloantigen-primed T cells	IL-1 or TNF-α-induced recruitment of MHC class II^+ LC was significantly decreased in CCR5 knockout mice

increases markedly when graft donors are Fas ligand-negative (gld) mice [116]. Moreover, clinical studies demonstrated that the level of sFasL in the aqueous humor is upregulated in patients, particularly in those undergoing endothelial immune rejection [95].

Cytokines are thought to participate in many processes including immune and inflammatory responses. They recruit leukocytes, induce MHC antigen expression, promote the synthesis of other cytokines, activate T cells and participate in tissue destruction (Table 4.2). In rodent models, T cell-specific effector cytokines such as IFN-γ, IL-4, and IL-13 are only detected in allografts undergoing rejection. TNF-α has been detected in the rejecting grafts, the aqueous humor and the serum of the recipients [84]. Low levels of IL-2 mRNA were detected in both allogeneic and syngeneic recipients after grafting, but expression levels rose upon rejection. IL-1α, IL-1β, TNF-β, IL-5, IL-6, IL-10, and IL-12 are absent or expressed at low levels in normal corneas, but are upregulated immediately after transplantation and continued to rise in rejected allografts. Anti-inflammatory proteins such as TGF-β 1/2 and IL-1RA are constitutively expressed on normal corneas, remain high after transplantation, and increase 5- to 10-fold upon rejection. Chemokines such as RANTES, MCP-1, MIP-1α, MIP-1β, and MIP-II are present in normal cornea. There is an early peak of expression after transplantation and a second peak during rejection [52].

Much attention was given to IL-1 and TNF-α, as important proinflammatory cytokines and critical mediators in corneal immune response. Various stimuli can promote their secretion by corneal cells and they were up-regulated immediately after transplantation. They recruit APCs, upregulate the expression of other cytokines, adhesion molecules and costimulatory factors and induce chemokine secretion. In addition, studies showed that TNF-α is required in the induction of ACAID. Depletion of functional TNF-α in the anterior chamber blocked the induction of ACAID [25]. Both IL-1 and TNF-α are potential chemoattractants for LCs and can induce the migration of LCs to the central cornea, which may subsequently initiate an immune response. TNF-α is thought to play a more important role during this process. It could be shown that TNF-α can induce the recruitment of LCs in the absence of functional IL-1R, whereas IL-1 is unable to do this in the absence of TNF-αR. It is likely that LC migration induced by IL-1 is mediated by TNF-α [23].

IL-1 receptor antagonist (IL-1ra) is a natural inhibitor of IL-1 and can block the activity of IL-1 through high-affinity binding to IL-1 receptors. Unlike IL-1, IL-1ra is constitutively expressed on normal corneas [103] and remained at high level after transplantation. Based on these findings and its function, IL-1ra is thought to be involved in ocular immune privilege. Topical application of IL-1ra can promote corneal allograft survival in both normal-risk and high-risk grafts, and is accompanied by a decrease in LC migration and angiogenesis [136].

TGF-β, secreted by ocular cells, is considered to be an immunosuppressive cytokine and plays a key role in ocular immune privilege. Conventional APCs such as peritoneal exudates cells(PEC) treated with TGF-β can acquire the ability to induce ACAID [132]. TGF-β preferentially induces APCs to secrete IL-10, subsequently resulting in antigen-specific suppression of DTH. In contrast, APCs preferentially secrete IL-12 in the absence of TGF-β and elicited DTH [24].

4.2.4 Th1/Th2 Paradigm

It is well established that corneal allograft rejection occurs via a T cell-mediated immune response. Based on the cytokines that they produce, $CD4^+$ T cells are divided into two distinct subsets: F and Th2 cells. Th1-type cells predominantly produce interleukin-2 (IL-2) and interferon-γ (IFN-γ), which mediate cellular immunity involving DTH and CTL. Th2-type cells produce IL-4, IL-6, IL-10, and IL-13, which may mediate graft tolerance [61] and humoral immunity.

In the past, considerable evidence has accumulated to suggest that the immune response to corneal allograft is mainly mediated by DTH-mediating Th1 cells. This is based on:

1. A predominant expression of Th1-type cytokines in the aqueous humor and the graft during rejection.

2. A preponderant lymphocytic rather than eosinophilic infiltrate in rejected grafts.
3. Prolonged allograft survival following $CD4^+$ T cell depletion by gene knockout or treatment with anti-CD4 antibody [83, 137].
4. Unabated allograft rejection in the absence of $CD8^+$ T cells or perforin [36, 49].

The Th1-type and Th2-type cytokines mutually regulate each other through their cytokine pattern. IFN-γ selectively inhibits proliferation of Th2 cells, whereas IL-10 inhibits cytokine synthesis by Thl cells. In addition, the cytokine pattern changes depending on the local cytokine milieu. For instance, IL-4 promotes Th2 differentiation whereas IL-12 advances Th1 development. Accordingly, the immune response might be activated predominantly through one direction, either Th1-type response or Th2-type response. This has led to the hypothesis that prevention of Th1-mediated processes such as allograft rejection could be achieved by inducing a Th2 response. We previously reported the first evidence of the effect of coexpression of IL-4/IL-10 in an experimental PKP model [85]. Whereas IL-4 gene transfer alone did not demonstrate a positive effect on graft outcome, combined treatment using IL-4/vIL-10 gene transfer was able to prolong allograft survival [86]. In addition, Klebe et al. [53] also demonstrated the enhancement of allograft acceptance by ex vivo transfer of the IL-10 gene to the grafts in a sheep model. Strikingly, a more recent study modulated IL-12 by its natural inhibitor P40 IL-12 and displayed a salutary effect on corneal allograft survival [54].

However, corneal allografts can undergo rejection in IFN-γ KO mice in the manner of a Th2-mediated process, which is characterized by a preponderant cellular infiltration with eosinophils and upregulation of Th2 cytokines [33]. Interestingly, similar histopathological features have been observed in rejected grafts of patients with atopic dermatitis and keratoconus who were thought to have a Th2 immune bias [32]. Strikingly, more recent studies demonstrated that mice with a Th2 immune bias induced by atopy more easily reject allografts [8]. In fact, both Th1- and Th2-type cytokines were expressed at high levels when rejection occurred. It is therefore likely that Th1 and Th2 cells together play a critical role in corneal allograft rejection, and corneal alloimmunity is not a simple switch between a Th1 and Th2 response.

4.2.5 Role of Draining Cervical Lymph Nodes in Corneal Allograft Rejection

The successful outcome of corneal transplantation is, in part, attributed to the natural lack of lymphatic drainage of the cornea. However, functional afferent lymphatic drainage from the uveoscleral route or conjunctiva to the ipsilateral submandibular lymph node, the place that is also a primary location for initial antigen-specific T cell expansion, has been confirmed [41]. Several findings support assumption that CLNs play a critical and necessary role in allosensitization and corneal graft rejection.

1. There is consensus that a vascularized corneal graft bed bears a higher risk of rejection. In fact, the existence of lymphatic vessels in vascularized human corneas has been demonstrated [21].
2. Lymphatic vessels can be detected as early as 3 days after corneal transplantation in a normal-risk animal [22].
3. APCs derived from both donor and recipient could traffic to local lymph nodes [62].
4. In a rodent model, ipsilateral cervical lymphadenectomy performed before transplantation resulted in an astonishingly indefinite period of allograft survival, which is associated with suppressed induction of allospecific DTH reactivity [138].

Taken together, the generation of allospecific DTH is dependent on functional lymphatic flow to the draining CLNs.

4.2.6 Role of Cytotoxic T Lymphocyte Response in Corneal Graft Rejection

The precise role of CTL in allograft rejection is still controversial. The cornea has the ability to

stimulate CTL activity based on the following findings.

1. Not only $CD4^+$ T cells but also a large number of $CD8^+$ were detected during the rejected cornea tissue.
2. $CD8^+$ T cells can be activated via the direct pathway [13].
3. Vigorous CTL activity has been observed when the cornea is placed heterotopically.

However, it is clear now that CTL is not required for graft rejection [13, 36]. Depletion of $CD8^+$ T cells or perforin by gene destruction cannot impede the progress of rejection. Transplants exhibit the same incidence, tempo, and even the same histopathologic features as controls and allograft rejection is associated with a strong DTH response [36, 49], even though CTL activity can still be found in local tissue, lymph node, and spleen, especially in high-risk animal models [26]. Interestingly, although $CD4^+$ T cell KO mice shared enhanced allograft survival, delayed rejection occurred 8 weeks after transplantation. Moreover, 14–30% of corneal allografts were disparate at H3a minor-H alloantigens, which are exclusively recognized by $CD8^+$ T cells, did undergo rejection [34]. These findings provide direct evidence that given sufficient time, a donor-specific CTL response can eventually happen [137].

Summary for the Clinician

- Experimental keratoplasty models have contributed in great detail to our understanding of the underlying immune mechanisms following corneal grafting.
- Histocompatibility antigens can be presented to the recipient by both the direct and indirect pathways of antigen presentation.
- The indirect pathway of antigen presentation is probably the driving force in both normal-risk and high-risk recipients, while the direct pathway may play a role in particular in the high-risk setting.
- Considerable evidence favors the hypothesis that immature dendritic cells bear tolerogenic properties whereas mature ones have an immunogenic character.
- Blockade of costimulatory molecules during antigen presentation is an effective strategy for preventing graft rejection in solid organ transplantation and showed some effect in corneal transplants.
- The existence of an ocular–lymphatic axis is important for corneal alloimmunization, while an ocular–splenic axis is crucial for immune tolerance. This finding supports the assumption that both local and systemic immune mechanisms are involved in the afferent and efferent arc of the immune response in keratoplasty with consequences for prevention and therapy.
- The specific corneal allograft immune response is mediated by $CD4^+$ T cells, either through the Th1 or the Th2 pathway.
- In contrast to solid organ transplantation, a CTL immune response is not necessary for corneal allograft rejection.

4.3 Strategies for the Prevention of Allograft Rejection

Several options are used to reduce the risk of corneal allograft rejection. Most are derived from other fields of transplantation medicine.

Two major strategies are used to improve corneal graft survival:

1. Decrease in the recipient's sensibility by reduction of antigen difference between donor and recipient (histocompatibility complex [HLA] matching);
2. Reduction of the recipient's afferent or efferent immune response by pharmacological modulation.

4.3.1 Use of HLA-matched Transplants

Based on the immunobiology of the cornea, rational arguments can easily be generated in favor of HLA matching in keratoplasty. HLA class I antigens are expressed by the corneal epithelium, keratocytes, and endothelium, whereas HLA class II molecules are mainly present on dendritic cells and in the superficial epithelium. However, their expression can be induced by inflammation due to infection, rejection or even the surgical trauma itself [131]. As a consequence of HLA antigen distribution in the donor tissue, graft rejection may occur in all three layers, either independently from each other or in combination [1, 80].

In clinical practice, HLA matching is still widely neglected, even when growing evidence shows a benefit for graft outcome in corneal transplantation (Table 4.3). Whereas mainly in Europe several studies support a significant benefit of HLA-A, -B, and -DR matching, this is not generally accepted elsewhere [29, 101, 128]. Interestingly, the prospective randomized CCTS study demonstrated no effect for either Class I or Class II matching even when that same study demonstrated an increased risk of graft rejection in patients with lymphocytotoxic antibodies to HLA class I and II antigens [20]. However, the validity of the this study has been discussed because of high error rates in the matching procedures and a concomitant high dosage of topical corticosteroids [42]. More conflicting results derived from other, well-designed studies that came to the conclusion that HLA matching may even have a negative outcome on graft survival [16, 125]. The fact that certain HLA mismatches differ in immunogenicity and may have an impact on graft survival has been known for sometime in kidney transplantation and in keratoplasty, but have only recently attracted adequate attention. For HLA class I/II loci, the structural basis of this phenomenon has been established [5, 51, 69, 125]. Therefore, in order to select the optimal donor for this category of patients, it will be important to take advantage of the differential immunogenicity and thus differential importance of mismatched HLA antigens. Indeed, it has been possible to define "acceptable or permissible mismatches" with low immunogenicity, which are associated with good graft survival, versus "taboo mismatches" with high immunogenicity and poor graft survival [12]. It can be expected that further experience in this direction will allow new strategies for future HLA matching that will not only suit a rare number of patients with frequent haplotypes, but also provide an acceptable waiting time for the great percentage of most other patients. Recently, a computer-based approach, "HLAMatchmaker," to the histocompatibility algorithm has been introduced, which respects this problem. It can identify immunologically acceptable mismatches and reduce the time on the waiting list substantially [11].

Yet some shortcomings remain. A major limitation of HLA matching in keratoplasty is adequate logistics, which are not easily available except in Europe. A cost–benefit analysis to balance the cost of reshaping existing eye bank procedures seems to be necessary to bring more widespread sharing of corneas to clinical practice. Monitoring of typing procedures has to be standardized and quality control mechanisms have to be instilled.

In addition, experimental studies demonstrate that even in fully compatible MHC class I and II antigen inbred strains 30–50% of allografts were rejected [102]. This may indicate that factors such as minor histocompatibility antigens, which are still underestimated, may play a role. At present, these antigens are not sufficiently characterized in humans.

Table 4.3 Monocentric clinical trials: histocompatibility complex (HLA) matching. *IR* immune rejection, *irrev.* irreversible

Authors	Number of patients	Study design	Follow-up (yrs)	Prognosis	Assayed antigenes/ matching priorities			Criteria	HLA-typing	Results/Comments
					HLA class I	HLA class II	other			
[7]	303	Retrospective	4	High risk	A, B			Irrev. IR	Serological	Significant beneficial effects of HLA-A and B matching on graft survival; superior effect of HLA split matching at 3–12-year follow-up
[96]	398	Retrospective/extensive standardization of control groups	3	Normal risk	A, B	DR		IR graft failure	Serological (HLA class 1) DNA (HLA class 2)	Combined HLA-A, B, and DR matching reduces the incidence of rejection and improves graft prognosis significantly
[51]	459	Retrospective	3	Normal and high risk separate	A,B	DR		IR	Serological	HLA-A, B, and DR matching lowers significantly rate of immune reaction for normal- and high-risk patients; no significant benefit of HLA split matching
[6]	64	Case-control trial	3	Normal and high risk separate	A, B	DR		IR	DNA (retrospective)	Significant beneficial effects of HLA-A, B, and DR matching on rate of immunological rejection for high-risk patients
[128]	1681	Large homogenous study 1976–1996	5	Normal and high risk separate	A, B	DR		IR irrev. IR	Serological	Significant beneficial effects of HLA-A, B, and DR matching on graft survival for both groups
[99]	693	Retrospective 1987–1993	3	Normal and high risk separate	A, B		Lewis-antigens	IR	Not known	Significant beneficial effect of HLA-A, B, and Lewis antigen matching on rate of immune reaction for normal-risk patients

Summary for the Clinician

- Major histocompatibility complex (HLA) matching should be considered as a rational procedure in addition to immunotherapy in high-risk patients. It is the only way of improving corneal graft prognosis that is free of side effects, is long lasting, and is independent of patients' compliance.
- Even though normal-risk recipients have also profited from HLA matching, most centers do not use typed donor material in a first graft.
- The use of proper HLA typing techniques, adequate matching programs, and sufficiently large donor pools are necessary to take advantage of recent developments in this field.
- New algorithms for histocompatibility determination have been introduced that respect the fact that HLA mismatches differ in their effect on graft rejection.

4.3.2 Immunmodulatory Agents in Keratoplasty

No generally accepted "guidelines" on the prevention and treatment of corneal allograft rejection are available. Several surveys of corneal transplant societies indicate that a broad pattern of clinical practices exists [9, 98]. It can be agreed, however, that a certain subpopulation of patients can be classified as high-risk individuals that should be handled differently. Experimental as well as clinical data show that the immune privilege of the cornea can be easily eroded. Subsequently, the common laws of transplantation also apply to keratoplasty.

Well-known risk factors for corneal graft rejection include:

1. More than 2/4 vascularization of the recipient's cornea
2. Preoperative inflammation of the anterior segment
3. Keratoplasty à chaud
4. Anterior synechiae
5. Large graft (>7.7 mm) diameter
6. Grafts near the limbus
7. Multiple grafts

Identification and proper handling of these patients is essential to provide the optimal result. Pharmacotherapy for prophylaxis and treatment of corneal graft rejection is warranted in these high-risk patients. Even if recipients often get HLA matched corneas, immunomodulatory agents are applied in addition.

As in other fields of transplantation medicine, treatment goals include:

1. Induction of donor-specific tolerance;
2. Selective inhibition of sensitized immune cells;
3. Unaffected physiological immune response of the donor;
4. No undesired side effects;
5. The substance should be applicable topically.

In patients without risk factors for transplant rejection, topical postoperative treatment with corticosteroids is considered sufficient. Patients having risk factors for corneal graft rejection usually need additional treatment.

4.3.2.1 Care of Normal-Risk Keratoplasty Patients

Whereas most investigators deal with the prevention of allograft rejection in high-risk patients, only a few studies focus on postoperative care in "normal" recipients. Commonly, topical postoperative treatment with corticosteroids is considered sufficient.

Topical corticosteroids have been used as standard treatment for the prevention of corneal allograft rejection for over 40 years [107]. The main advantages include immediate and potent anti-inflammatory effects, favorable pharmacological properties, and low price. Steroids rapidly permeate the cellular membrane and act intracellularly through glucocorticoid-responsive elements that regulate the expression of more than 200 different genes. Biological effects include downregulation of interleukin (IL)-1, IL-3, IL-6, and IL-8, which are secreted predominantly by antigen-presenting cells. In addition, reduction of IL-1 and IL-6 leads to inhibition of IL-2 secretion and subsequent T cell inhibition [92]. Important for the postoperative use of cortico-

steroids in corneal grafts is their broad spectrum of antiphlogistic properties. They reduce the permeability of vascular endothelium (blood–aqueous barrier, blood–retina barrier) and inhibit the migration of monocytes. Experimental data demonstrate a dose-dependent inhibitory effect on cytokine production. Already very low doses (10–100 nM) inhibit resting cells, while far higher doses are necessary to also block activated cells.

The pharmacological preparation of topically used corticosteroids is important for the intraocular effects of the drug. Prednisolone acetate penetrates the cornea very well even with an intact epithelium, whereas the topical application of prednisolone phosphate leads to intraocular availability only when the epithelium is abraded. The effects of different corticosteroids on the migration of leukocytes in the cornea vary widely [59]. It is important to emphasize that the biological effect of local steroid therapy is mainly anti-inflammatory. Although local steroids are commonly applied for postoperative treatment after keratoplasty, there are different opinions about dose and duration of treatment. There are no controlled clinical studies from which a gold standard could be derived. In normal-risk patients the topical application of 1% prednisolone acetate qid has been suggested for the first 2–4 weeks, reduced to 1 drop/day after 3 months and then continued up to the 12th postoperative month.

Systemic treatment with corticosteroids has not only antiphlogistic but also immunosuppressive effects. Suggestions for intensity and duration of the treatment vary as much as in the case of local treatment [122]. For the perioperative period some authors suggest 100–250 mg/day prednisolone intravenously. Prolonged monotherapy with systemic corticosteroids should be avoided because of the high potential for side effects. In a randomized prospective study there was no additional benefit for normal-risk patients who received systemic fluocortolone for a 3-week period plus topical prednisolone acetate compared with local treatment only [66].

It is of clinical importance that the inhibition of cytokine synthesis by corticosteroids is quickly reversible and the effect on T cells and antigen-presenting cells diminishes rapidly. Sudden termination of corticosteroid therapy can provoke rebound effects resulting in immediate adverse effects. Marked T cell proliferation after corticosteroid termination could be detected possibly because of the upregulation of T cell receptors. These findings indicate that steroid therapy should be terminated by tapering of the drug.

Well-known undesired side effects of local corticosteroids are altered wound healing, increased risk of infection, reactivation of herpes virus keratitis, secondary glaucoma, and cataract formation [107]. Whether topical steroid therapy alters re-epithelialization of the cornea is not proven [117]. However, prolonged wound healing after keratoplasty can be expected due to fibroblast inhibition and reduced collagen synthesis. The increase in intraocular pressure due to steroid response is a well-known side effect in up to 30% of patients. This is of particular importance in keratoplasty patients, since not only the secondary glaucoma may alter ocular function, but corneal graft endothelium is also susceptible to pressure rise. Often, incorrect pressure measurements after keratoplasty are another challenge in these patients. Several new agents are the focus of clinical studies to prove whether the rate of IOP increase can be reduced by the application of less pressure-altering steroids, or steroid receptor agonists (SEGRA) [88]. Cataract formation has been observed after a cumulative dose of 740 drops of 0.1% dexamethasone [64]. Systemic steroid therapy provokes all the side effects of local therapy and may lead additionally to severe metabolic alterations (Table 4.4).

Summary for the Clinician

- Corticosteroids remain the standard treatment in normal-risk patients; however, no definite scheme on the frequency and duration of therapy exists.
- A number of new steroids and steroid-like agents may become available that may reduce well-known side effects, in particular intraocular pressure rise.
- Reduced corticosteroid treatment might be possible in low-risk patients receiving HLA-matched corneal grafts without increasing the risk of rejection.

Table 4.4 Immunmodulatory agents in keratoplasty

Generic	Effect	Initial dosage	Maximum dosage	Initiation of effects	Common side effects	Control parameter
Azathioprine	Purine-metabolism	1 mg/kg/day	2.5–4 mg/kg/day	1–3 months	Leukopenia Thrombocytopenia Hepatitis	White blood cell count 4–6 weekly Liver enzymes
Cyclosporin A	T cell inhibitor	3-5 mg/kg/day	10 mg/kg/day	2–6 weeks	Renal function Arterial hypertension Tremor Hirsutism Gingival hyperplasia	Creatinine* level Monthly (*potentially C2 level) RR control White blood cell count
Leflunomide	Pyrimidine-synthesis	3 days 4×100 mg/day, afterwards 4×20 mg/day	20 mg/day	2 weeks	Cytopenia Arterial hypertension Diarrhea	
Methotrexate	Antimetabolite	7.5–12.5 mg/week	25 mg/week	2–12 weeks	Leukopenia Thrombocytopenia Gastrointestinal function Hepatotoxicity Lung fibrosis	White blood cell count 2–8 weekly Liver function
Mycophenolate Mofetil	Purine- synthesis	2×500 mg/day	2×1.5 g/day	2–12 weeks	Diarrhea Gastro-intestinal function Infection Neutropenia	White blood cell count Liver enzymes
Tacrolimus	T cell inhibitor	0.15–0.3 mg/kg/d	0.30 mg/kg/day	2–8 weeks	Bone marrow Depression Hamaturia Infection	White blood cell count 1–4 weekly Urine analysis

4.3.2.2 Care of High-Risk Keratoplasty Patients

4.3.2.2.1 Corticosteroids

Because of their rapid and broad anti-inflammatory effect, topical corticosteroids are also standard in high-risk patients. Frequency and duration of treatment are adapted to the individual clinical situation of the patient. For high-risk patients recommendations vary for prednisolone-acetate drops, from bid to up to 24 times per day [9, 98]. But even a high-dose local corticosteroid therapy cannot avoid graft rejection and undesired side effects may instead dominate. Therefore, alternative/additional therapeutic options are necessary.

The pathophysiologic basis of modern immune pharmacology is focused on T cell modulation and the interaction with antigen-presenting cells. Identification of pathophysiologic pathways such as costimulatory signals, T cell receptor and calcineurin pathways, and cytokine signaling have also resulted in more tailored immunomodulation.

Currently, a broad spectrum of immunomodulatory agents is available (Fig. 4.5). Because ophthalmologists may not be familiar with systemic immunosuppressive treatment, the main effects and side effects are briefly summarized and reflected in light of their use in corneal grafting.

4.3.2.2.2 Antiproliferative Agents

Proliferation-inhibiting substances include azathioprine, methotrexate, and corticosteroids.

4.3.2.2.3 Azathioprine

Azathioprine is a prodrug metabolized to its active form 6-mercaptopurine and interferes with DNA synthesis. It inhibits T cell and, to a lesser extent, B cell functions. During the recent years, no large studies reporting the effect of azathioprine in keratoplasty patients have been published [64]. Due to the inhibition of cell proliferation, major side effects are a consequence of bone marrow suppression resulting in leukopenia and thrombocytopenia (Table 4.4).

4.3.2.2.4 Methotrexate

Low-dose methotrexate is another agent more frequently used in the management of chronic uveitis than in postkeratoplasty care. It blocks dihydrofolate reductase and thymidylate synthesis, which leads to inhibition of DNA replication and cell division. Its immunomodulatory effect is mediated by T and B cell inhibition. 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. Gastrointestinal side effects can be reduced by s.c. injection.

4.3.2.2.5 Mycophenolate Mofetil

Mycophenolate mofetil (MMF) was initially introduced for psoriasis some 30 years ago. Like methotrexate, MMF interferes with purine synthesis. When its immunomodulatory effects were revealed, it was approved for use in kidney and heart transplantation. After oral intake, MMF is transformed into MPA, the biologically active compound acting as a selective inhibitor of lymphocyte proliferation. In 2001 it was [94] demonstrated in a small randomized study that MMF was equally effective as CsA in high-risk keratoplasty patients. A recent survey of German transplant centers revealed good acceptance of this agent in high-risk recipients [9]. Side effects of MMF include gastrointestinal symptoms, an increase in serum levels of liver enzymes, anemia, as well as an increase in respiratory tract and urogenital tract infections. Interestingly, MMF also has antiviral activity, which supports corneal graft survival in patients with HSV-associated keratitis [65].

4.3.2.2.6 Calcineurin Inhibitors (Cyclosporin A, Tacrolimus)

Inhibition of calcineurin in the intracellular signaling pathway interferes with DNA transcription and thus leads to decreased function of immune

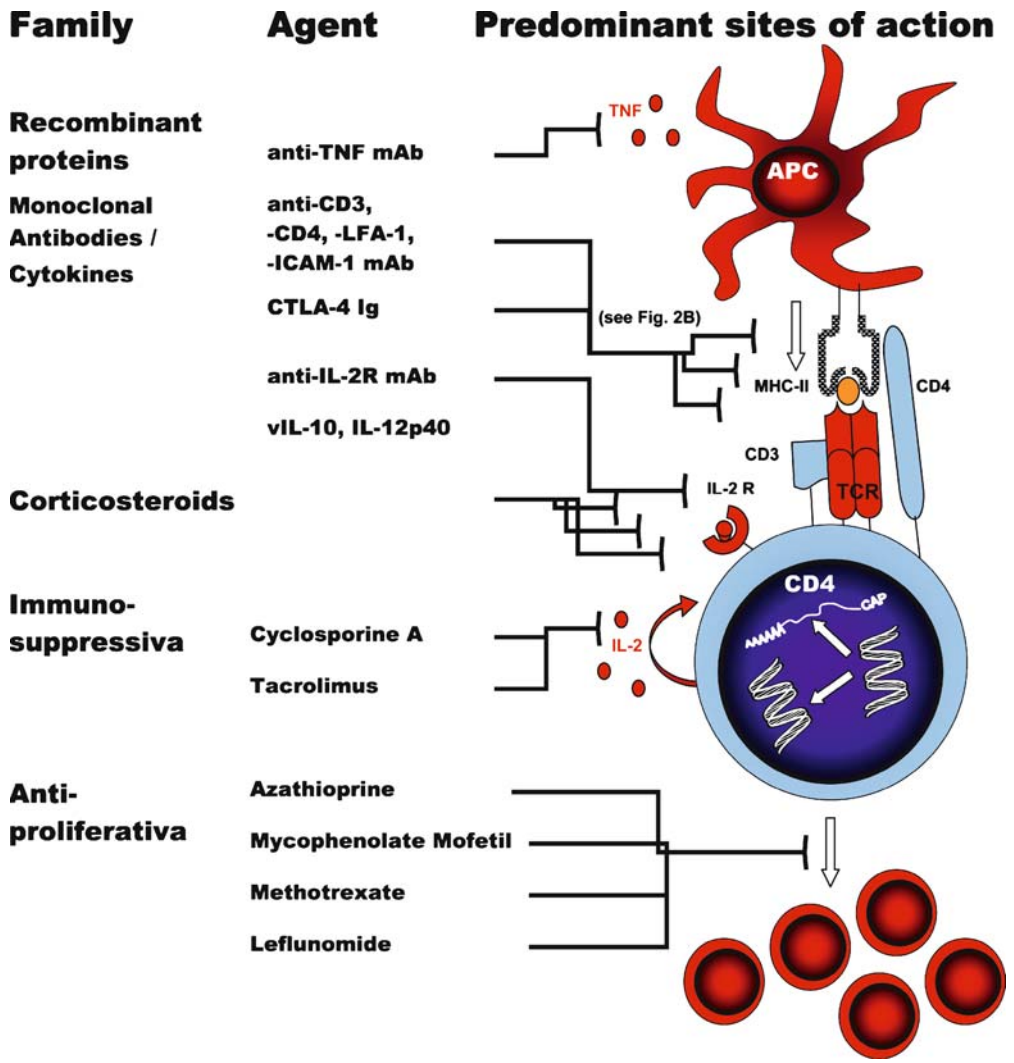


Fig. 4.5 Immunomodulatory agents in keratoplasty. Schematic representation of the sites of action of compounds that are currently used clinically (corticosteroids, immunosuppressive, and anti-proliferative agents), and of recombinant proteins as future therapeutics

cells. Calcineurin inhibitors are potent immunosuppressive drugs acting predominantly through inhibition of T cell activation. While FK506 is 50–100 times more potent than cyclosporin A (CsA), it still shares its toxicity and many of its side effects.

Cyclosporin A

The introduction of CsA and tacrolimus (FK506) was a major step forward to improving the prognosis of solid organ transplants. Therefore, treatment of high-risk keratoplasty patients had been started with high expectations.

As a lipophilic compound, CsA can pass the cellular membrane by diffusion and binds to spe-

cific receptors called cyclophilins. Cyclophilins have enzymatic activity and contribute to intracellular protein benching [108]. Target molecules of the CsA-cyclophilin complex are proteins that regulate the gene transcription in the nucleus (NF-AT, AP-3, NF-B), which are involved in the transcription of IL-2 genes [120]. In different tissues various types of cyclophilins exist indicating that the effect on IL-2 regulation might vary in different tissues. Until now, cyclophilins A, B, C, and S have been characterized. It could be shown that cyclophilin A is highly expressed in the corneal epithelium, but only weakly in the endothelium and in the uvea [82]. The regulation pathway of CsA activity is not completely revealed, but the main effect is the inhibition of cytokine synthesis, especially of IL-2 but also IL-4 and tumor necrosis factor. CsA acts dose-dependently on the afferent and efferent arc of the immune response. The MHC_{50} of CsA is 10–20 ng/ml for IL-2 production and 20–50 ng/ml for the proliferation of cytotoxic lymphocytes.

A number of experimental and clinical studies dealt with topically applied CsA, but pharmacological properties limit its clinical benefit. As a lipophilic compound CsA easily penetrates the corneal epithelium, but intraocular levels remain very low. Improved solvent preparations like cyclodextrin, azone, collagen lenses, and liposomes led to higher intraocular concentrations. Subsequent experimental studies using these preparations demonstrated a significant therapeutic effect [56, 67, 69]. In a retrospective clinical study a positive effect of topically applied CsA (2%) on graft survival in high-risk, but not low-risk patients could be seen [46]. No benefit of CsA could be found in a multicenter study that compared local CsA therapy with corticosteroids in high-risk recipients. Perry et al. [79] suggested that topical CsA (0.5%) application may substitute local steroids in secondary glaucoma responders, but at the same time an increased rejection rate in CsA-treated patients occurred.

Systemic application of CsA was more favorable. Several uncontrolled clinical studies showed a positive effect on the graft survival in high-risk cases [38, 39, 119]. There are a number of controlled and uncontrolled clinical studies that support post-transplant use of CsA (Table 4.4).

A prospective study showed that combined CsA treatment with topical dexamethasone was superior to topical corticosteroid treatment alone. However, different opinions exist on the duration of CsA application. Although a positive effect of short-term therapy (3 months) was reported in one study [68], there are later results showing best efficacy with 1 year of CsA treatment. The inefficacy of a short-term (3 months) CsA treatment was confirmed by others. In addition, some studies could not confirm the benefit of this approach at all [47, 89, 100].

Potential side effects of CsA that have to be carefully monitored include nephrotoxicity, an increase in arterial blood pressure and hepatotoxic effects. Patients frequently complain about hypertrichiasis. Beside the serum concentration of CsA that has to be monitored, serum creatinine, liver enzymes, and arterial blood pressure have to be controlled.

Taken together, CsA is the most frequently used immunomodulatory agent for the treatment of high-risk keratoplasty patients [89].

Tacrolimus (FK506)

The acting mechanism is similar to that of CsA, but inhibition of lymphocyte proliferation, for example, is 10- to 100-fold stronger than CsA [17]. Like CsA, FK506 is a lipophilic compound with similar limitations following local application, but intraocular drug levels are higher than with CsA [81]. A subsequent clinical study demonstrated that topical FK506 was at least as effective in preventing graft rejection as topical steroids in a prospective pilot study. However, local discomfort limited further use [97]. In another noncomparative case series tacrolimus was effective in the prevention of rejection in patients with high-risk corneal and limbal grafts [111]. A high rate of undesired side effects and narrow therapeutic index has limited the clinical use of FK506 in other fields of transplantation medicine. Predominantly reported side effects are nephrotoxicity and neurotoxicity [106].

Summary for the Clinician

- Current approaches in high-risk patients include the use of systemic immune modulators, e.g., calcineurin blockers (cyclosporin A, tacrolimus).
- Considerable evidence exiists that systemic treatment with cyclosporin A shows a significant benefit in high-risk recipients.
- A major limitation of these agents are side effects that have to be closely monitored by experienced experts.

4.3.3 Future Aspects: Immunological and Gene Therapy Approaches

The pathophysiologic basis of modern immune pharmacology is focused on T cell modulation and the interaction with antigen-presenting cells. Identification of pathophysiologic pathways such as costimulatory signals, T cell receptor antagonists and cytokine signaling has also resulted in a more tailored immune modulation, which is already being used in other fields of transplantation, but has hardly been approached in human keratoplasty.

4.3.3.1 Monoclonal Antibodies and Other "Biologicals"

Because of their high specificity and selective biological effects, monoclonal antibodies (mab) hold great promise as pharmacological agents. Treatment of allograft rejection was one of the first major fields for polyclonal and monoclonal antibodies. Understanding the immunobiology of transplantation led to the application of antibodies targeting structures including the T cell receptor, CD3 molecule, IL-2 receptor, and CD4 molecule of T helper cells [3, 83, 123]. Other strategies focus on costimulatory molecules, cell adhesion molecules (LFA-1, ICAM-1) or proinflammatory cytokines (TNF). In spite of a multitude of experimental studies in corneal transplantation, there are only a few clinical studies.

Ippoliti and Fronterre [48] injected anti-CD3 and anti-CD6 mab into the anterior chamber of patients with acute corneal graft rejection refractive to steroid treatment and were able to reverse rejection. In another study, a pan-T cell antibody (CAMPATH-1H), which is well known as a strong T cell-depleting agent, was successfully applied (systemically) in patients with graft rejection [70]. The only report on the preventive application of monoclonal antibodies was targeting activated T cells by using basiliximab, an anti-CD25 mab [104]. Perioperative application of systemically administered mab in combination with systemic CsA was well tolerated and led to rejection-free long-term survival in 5 high-risk patients. Recently, anti-TNF therapy (infliximab) demonstrated a favorable clinical course in patients with rheumatic corneal ulceration. Problems that limit the broader use of monoclonal antibodies include first-dose anaphylactic reactions, production of antibodies against the therapeutic antibody by the recipient, and high costs.

4.3.3.2 Gene Therapy

Another future option for prolonging corneal graft survival is to modify the recipient's immune response by gene transfer. The cornea, and in particular the corneal endothelium, as the principle target of allograft rejection is a suitable candidate for a gene-based immunomodulatory approach for several reasons:

1. Unlike other organs, it can be preserved for periods of several weeks. This allows time for ex vivo genetic modulation *before* transplantation.
2. The transparency of the cornea allows direct visualization of any significant consequences of gene transfer. Since maintenance of corneal transparency is essentially dependent on the normal activity of corneal endothelial cells, this makes it an attractive target for gene therapy strategy. Any alteration in this cell layer will have profound effects on the remaining tissue.
3. Corneal endothelial cells are readily accessible and hence amenable to gene transfer.
4. The corneal endothelium has a distinct advantage as a target for gene therapy. Transgene

expression in most organs is transient and is limited by cellular immune responses directed against transduced cells, viral proteins, and foreign transgene proteins [124]. In contrast, prolonged and persistent adenovirus-mediated transgene expression is observed in immune-privileged sites such as the eye and brain [40].

5. Because the anterior chamber of the eye is an immune-privileged site, the anatomic location of the corneal endothelium makes it an optimal site for gene transfer.

Several studies demonstrated successful gene transfer into ocular tissues by different vehicles like adenovirus, retrovirus and liposomes [83, 123]. Again, studies in experimental corneal grafts were promising and able to prolong transplant survival targeting different structures such as vIL-10, IL-12-p40 costimulatory signals or nerve growth factor [53, 54, 87], but were not successful in others [85, 91].

Summary for the Clinician

- New preventive and therapeutic options that are highly selective and specific include monoclonal antibodies and gene therapy.
- The unique situation of the corneal graft seems to be particularly attractive, since local mechanisms may not only be important for the induction of graft rejection, but also for local immunomodulatory approaches.
- Gene therapeutic approaches are particularly attractive for corneal transplants because of several exceptional characteristics such as long-term preservation of the transplant and easy access to the organ.

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Chapter 5

Autoimmune Keratitis

John D. Gottsch

5

Core Messages

- Autoimmune keratitis should be suspected in all cases of unexplained stromal ulceration.
- Corneal ulceration should be closely monitored for rate of progression in autoimmune keratitis. Rapidly progressive ulcerative disease needs prompt and appropriate immunosuppression.
- Ulcerative autoimmune keratitis can involve life- and sight-threatening complications that require expertise in the diagnosis, treatment, and monitoring of rheumatologic and vasculitic disease. Ophthalmic surgery may be required to retain integrity of the globe.
- Progressive corneal ulceration is unlikely to benefit from topical gluing alone.
- The majority of patients with collagen vascular stromal ulceration have a long history of disease. However, the general ophthalmologist may be presented with a patient with ocular symptoms and signs that represent the initial onset of a collagen vascular disease.
- Rheumatoid peripheral ulcerative keratitis carries an increased risk of a vasculitic complications and increased mortality.
- In Wegener's granulomatosis, if serial ANCA titers do not revert to normal, recurrent disease may be more likely.
- Trauma or surgery may induce autoimmune keratitis.
- Patients with scleritis should be followed closely for signs of corneal involvement which may suggest a life threatening vasculitic disease and increased risk of corneal perforation.

5.1 Introduction

Autoimmune keratitis encompasses a number of diseases whose etiologies are derived from a dysfunctional immune system, which can result in inflammatory destruction of the cornea. Clinical signs of autoimmune keratitis can at times be indistinguishable from those arising from infectious, allergic, and neurotrophic causes. Thus, the diagnosis of an autoimmune keratitis requires a thorough history, physical and ocular examination, and appropriate laboratory testing. Autoimmune keratitis can be a new sign in a patient with a long-standing history of a collagen vascular disorder. Importantly in some patients the signs of an autoimmune keratitis may be the initial presenting sign of a new disease. In either event, the early recognition by the ophthalmologist that corneal inflammatory disease could represent an autoimmune process is critical to minimizing corneal destruction and providing appropriate medical intervention in what could be a life-threatening systemic disease.

The designation of this group of disorders as autoimmune keratitis is based on the presumption that in susceptible individuals exogenous agents (virus, bacteria) or endogenous antigens can initiate an aberrant immune response. By the mechanism of molecular mimicry by way of cross-reactive epitopes, an inflammatory cascade can be initiated against a "self" antigen initiating a destructive local process [22]. The evidence for this mechanism in the pathogenesis of these diseases is circulating autoantibodies, such as the rheumatoid factors (RFs) as autoantibodies against the Fc portion of IgG and the antinuclear cytoplasmic antibodies in Wegener's granulomatosis [18, 22, 28, 42]. Evidence has also accumulated of autoimmunity to corneal antigens in Wegener's granulomatosis and Mooren's ulcer.

5.2 Background

5.2.1 Differential Diagnosis of Patient with Stromal Thinning Ulceration

It is not uncommon that patients will present with unexplained corneal infiltrates leading to epithelial and stromal ulceration. The differential diagnosis of stromal ulceration includes infectious, allergic neurotrophic, and autoimmune causes. A careful history can help to narrow the likely cause as recent trauma or contact lens wear can help identify an infectious etiology. A history of atopy with itching and a mucoid discharge may point to an allergic etiology. A past history of HSV or HZO may indicate a post-infectious inflammatory keratitis or a neurotrophic cause. Fifth and/or seventh nerve palsy(ies) may lead to a diagnosis related to neurotrophic disease.

Autoimmune keratitis can be a consequence of collagen vascular and primary vasculitic diseases such as rheumatoid arthritis, Wegener's granulomatosis, polyarteritis nodosa, relapsing polychondritis, microscopic polyangiitis and Churg Strauss syndrome (Table 5.1). Mooren's ulcer is an autoimmune disorder that involves the cornea exclusively, as no systemic disease is associated with the condition. Because the vasculitic causes of autoimmune keratitis have sight- and life-threatening consequences, it is imperative that they are ruled out first. Mooren's ulcer is a diagnosis of exclusion.

Table 5.1 Autoimmune keratitis

Collagen vascular disorders
Rheumatoid arthritis
Sjögren's syndrome, primary or secondary
Wegener's granulomatosis
Systemic lupus erythematosus
Polyarteritis nodosa
Relapsing polychondritis
No systemic involvement
Mooren's ulcer

5.2.2 Laboratory Tests

Essential laboratory tests for patients who present with stromal ulceration include culture and smears to rule out an infectious etiology. Bacterial, viral, fungal, and acanthamoebal keratitis have all presented with features of ulcerative keratitis with infiltrates and suppuration.

In patients who have an underlying systemic autoimmune disease and their keratitis is suspected to be related to an exacerbation or a new onset of vasculitis, a thorough history and physical examination is an important requirement to determine if there are any other extraocular manifestations of the disease. Directed laboratory tests can be helpful to support a diagnosis of systemic autoimmune disease with or without ongoing vasculitis (Table 5.2). A sedimentation rate, C-reactive protein (CRP), rheumatoid factor, ANA, ANCA, antibodies to Ro(SS-A) and/or La(SS-B) can be useful when combined with physical findings and biopsy results to determine if an autoimmune disease with or without a vasculitic process is ongoing and what specific disease may be involved.

Summary for the Clinician

- As stromal keratitis of an autoimmune origin can have the same clinical appearance as an infectious process, cultures and smears are obligatory.
- Directed laboratory tests can help support a diagnosis of keratitis related to an underlying systemic autoimmune disease.

Table 5.2 Relevant laboratory tests in autoimmune keratitis. *CBC* complete blood count, *ESR* erythrocyte sedimentation rate

CBC with differential
ESR
Rheumatoid factor
Anti nuclear antibody
Anti nuclear cytoplasmic antibodies
Anti Ro(SS-A) and/or La(SS-B)

5.3 Autoimmune Keratitis

Autoimmune keratitis most commonly occurs in patients with collagen vascular diseases with rheumatoid arthritis as the most frequent cause. The cornea can be involved directly by peripheral ulcerative keratitis (PUK) or paracentral keratitis (PCUK). Indirectly the cornea can be affected by an adjacent autoimmune scleritis by stromal thinning, PUK, and stromal keratitis by direct extension (Fig. 5.1).

5.3.1 Mechanisms of Autoimmune Keratopathy

There are a number of clinical presentations of autoimmune keratitis ranging from mild infiltrates to massive necrotizing ulceration. Several different autoimmune pathways may play a role in these various presentations. There is ample evidence with fluorescein angiography of the anterior segment in patients with scleritis and associated corneal diseases with PUK that vaso-occlusive disease occurs in the arteriolar venular capillary network of the episcleral and conjunctival vessels [60, 61, 63, 64]. Histopathologically, microangiopathy has been confirmed in scleritis patients, which may correlate with an ongoing systemic vasculitic process [12, 37, 44, 46, 65]. The lack of a blood supply to the limbus has been hypothesized to lead to an influx of inflammatory cells into the cornea and the release of enzymes and cytokines, which contribute to collagenolysis and subsequent stromal ulceration [43]. Other mechanisms that could play a role in autoimmune keratitis are the presence of sequestered corneal antigens, which, when exposed by trauma, infection, or surgery to the afferent arc of the immune system provoke a self-perpetuating destructive inflammatory reaction to the cornea.

In patients with sclerokeratitis associated with rheumatoid arthritis, interstitial collagenase (MMP1) was found to be abundantly expressed by macrophages and keratocytes. The tissue inhibitors of metalloproteinases (TIMPs) were found to be decreased in affected corneas. These findings suggest that in immune-related corneal diseases the degradation of corneal matrix proteins by the upregulation of collagenases such as MMP1 and the relative imbalance or lack of sufficient quantities of their inhibitors such as TIMP may play a role [43]. In addition, corneal matrix metalloproteinase-2 (MMP-2) was found to be overexpressed in corneas with PUK [49]. Future therapies may be specifically directed at these proteinases.

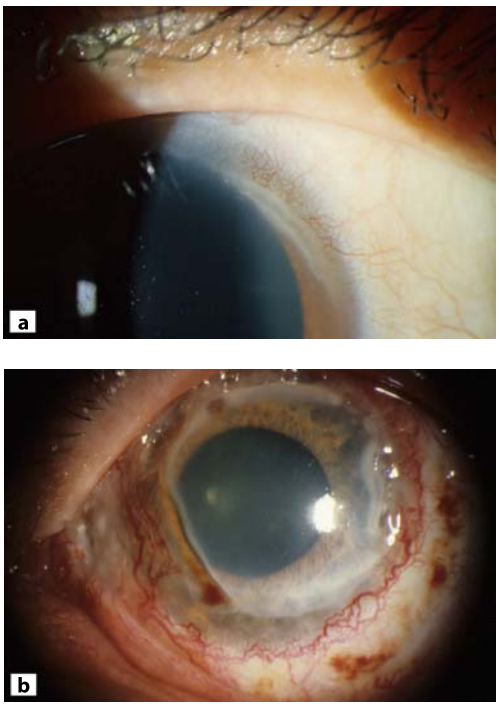

Fig. 5.1 **a** Mild recurrent infiltrative disease in rheumatoid arthritis-responsive topical steroids. **b** Severe peripheral ulcerative keratitis (PUK) that responded to systemic immunosuppressive therapy with cyclophosphamide

5.3.2 Rheumatoid Arthritis

Corneal disease secondary to rheumatoid arthritis is the most common autoimmune disease encountered by the ophthalmologist. The great majority of patients who present with rheumatoid keratitis have a long-standing history of rheumatoid arthritis. However, not infrequently the ophthalmologist will encounter the patient with keratitis as the presenting sign of a rheumatoid disease.

Rheumatoid arthritis is a systemic autoimmune disease of uncertain etiology that affects 1–3% of the population [9]. The disease is presumed to arise in genetically susceptible individuals who, with exposure to unknown antigens, develop, by way of the mechanism of molecular mimicry, immune disease against the self [9, 22]. Tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) both play a large role in the inflammatory process, leading to destruction of synovial joints [9, 65]. A diagnosis of RA rests on seven criteria established by the American Rheumatism Association. Four of the following seven criteria are diagnostic: arthritis, usually in the hands and feet; rheumatoid nodules; the presence of morning stiffness and fatigue; radiographic features of the disease; and positive rheumatoid factors. Increased rheumatoid factor is present in 70–90% of patients.

Extra-articular manifestations of RA can occur and need to be understood by the ophthalmologist as they can be responsible for life-threatening vasculitic and sight-threatening corneal destructive disease. High titers of RF suggest an increased risk of extra-articular disease including vasculitis [44, 55–58]. The development of necrotizing scleritis or peripheral ulcerative keratitis carries a decreased life expectancy due to the perpetuation of vasculitic lesions in vital organs such as the lungs, heart, and brain [15, 44, 55–58].

5.3.3 Rheumatoid Peripheral Ulcerative Keratitis

5.3.3.1 Diagnosis

Peripheral ulcerative keratitis (PUK) is most commonly associated with rheumatoid arthritis, although other, less frequently encountered vasculitic disorders such as Wegener's granulomatosis, polyarteritis nodosa, relapsing polychondritis, and primary Sjögren's syndrome must be considered as differential diagnoses [23, 38, 48, 65]. As previously discussed, the presence of corneal epithelial ulceration in a crescentic pattern with usually mild stromal infiltrate and thinning in a patient with a history of rheumatoid arthritis suggests disease exacerbation and the possibility of vasculitis and increased mortality risk [15, 47, 48, 55–58]. Although classic PUK has epithelial and stromal ulceration that is deep and extensive, many ulcers involve only mild infiltration with minimal epithelial ulceration and little detectable stromal thinning. For the ophthalmologist involved, the rate of ulcerative progression must be ascertained and documented. An assessment of the medical condition of the patient with a thorough physical exam including dermatologic evaluation for signs of vasculitis (ulcers, purpura, and petechiae) is needed. Appropriate laboratory testing is helpful to determine if a vasculitic component of the disease is present. If so, more aggressive systemic immunosuppression is warranted. If the systemic disease is quiescent and the disease ulcerative process is slow, a more cautious approach to treating the disease locally with topical steroids and cyclosporine may be appropriate.

5.3.3.2 Treatment

Slowly progressive ulcers maybe amenable to topical corticosteroids and cyclosporine. Patients with minimal disease who are placed on topical medications should be followed daily to determine that there is an appropriate clinical response with improvement. Resolving ulcers can most easily be monitored by the extent of epithelial ulceration as measured by fluorescein staining with a slit lamp micrometer. Gradual decreases in the extent of stromal ulceration with the surrounding infiltrate will also be noted, but are more difficult to confirm quantitatively.

Rapidly progressing ulcers can proceed to perforation and loss of integrity of the eye. As systemic immunosuppression may take days to weeks to moderate and turn around an aggressive ulcerative process, a delay should be recognized as probable and factored in the treatment decision-making process [8, 15, 27, 30, 39, 50, 53]. Combination therapy by utilizing faster acting systemic corticosteroids with a slower onset immunosuppressive agent may be the best course of action in these progressive ulcers that move toward perforation. Pulsed intravenous immunosuppressive therapy has been proposed as a treatment modality in scleral and corneal ulceration,

which may improve efficacy and avoid some of the complications of oral administration [39].

A number of immunosuppressive agents have been used successfully in PUK associated with rheumatoid arthritis [8, 15, 27, 30, 39, 50, 53]. Systemic steroids can be useful in some cases, but patients who do not respond within days and who continue to worsen should be considered for aggressive immunosuppression. Cyclophosphamide, azathioprine, cyclosporine, methotrexate, and infliximab have been successfully used in nonresponsive cases.

The use of systemic immunosuppressive agents requires a complete history and physical examination to ensure that no contraindications exist such as infections or a systemic susceptibility to toxicity or medical complications such as heart failure, pneumonitis, nephritis, or colitis [10, 13, 25, 27]. Complete monitoring of the immune status of the patients and routine screening for drug toxicities are mandatory during the course of treatment.

5.3.4 Rheumatoid Paracentral Ulcerative Keratitis

Although peripheral ulcerative keratitis is a well-recognized complication of rheumatoid disease, paracentral rheumatoid corneal ulceration is a unique clinical presentation (Fig. 5.2). Whereas PUK is understood to represent worsening systemic disease in rheumatoid arthritis, PCUK may not carry the same prognosis. PCUK presents usually in a setting of a quiescent eye with little evidence of infiltrate and with a sharply marginated ulcer that can quickly perforate [4, 16, 32]. Because of the different clinical presentations and the response to treatments between PUK and PCUK, the etiologies of these two types of ulcerative keratitis likely differ.

In a study, 8 eyes in 6 patients who presented with rheumatoid arthritis and a history of recurrent corneal ulcerative disease, and who had previously been treated with systemic immunosuppression, corneal gluing, and keratoplasty, presented with PCUK [32]. Using topical cyclosporine in 5 eyes, there was a rapid response with resolution of keratolysis and re-epithelialization of the ulcer. In one case of PCUK without a previous history of any previous treatment, the ulcer quickly resolved with topical cyclosporine.

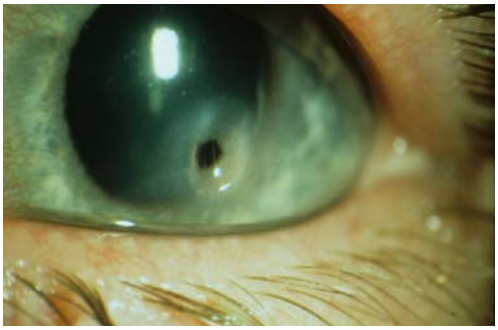

Fig. 5.2 Paracentral ulcer demonstrating sharply marginated ulceration with minimal infiltrate

In another report of 7 eyes in 5 patients with PCUK with collagen vascular disease, immediate reversal of the ulcerative process with rapid re-epithelialization was noted with topical cyclosporine [16]. An early clinical sign that was noted in all cases was vascularization of the cornea, which spread centrally leading to resolution of infiltrates and ulceration. The intensity of the vascularization appeared to be greatest in those eyes in which a previous patch graft had been placed, which was necessary for tectonic support from a previously perforated ulcer.

5.3.5 Scleritis-Associated Peripheral Keratopathy

After dry eye or keratitis sicca, scleritis is the most common ocular manifestation of rheumatoid arthritis. Along with peripheral ulcerative keratitis, scleritis is a worrisome clinical sign that may signify a deteriorating systemic disease with life-threatening vasculitis [37, 44]. In a study of corneal findings in patients with keratopathy associated with scleritis, three types of corneal disease were noted, peripheral corneal thinning, stromal keratitis, and peripheral ulcerative keratitis [44]. Patients with keratopathy had more necrotizing scleritis (57%), and more potentially life-threatening disease associations such as rheumatoid arthritis and Wegener's granulomatosis (87%), than patients with scleritis alone. Patients with PUK had the worst prognosis for

visual outcome due to corneal perforations and decreased life expectancy due to worsening systemic disease [44].

5.3.6 Postsurgical Ulcerative Keratitis

Both peripheral and paracentral ulcerative keratitis have been reported in postsurgical patients with rheumatoid arthritis [2, 31, 35, 45]. PUK after clear corneal cataract extraction has been reported as the presenting sign in a patient newly diagnosed with rheumatoid arthritis (Fig. 5.3) [2]. Vaso-occlusive disease with microangiopathy of the episcleral and conjunctival vessels may be set in motion by surgical trauma in patients susceptible to vasculitic disease [44]. Most cases have been reported in patients with rheumatoid arthritis. Peripheral ulcerative keratitis after extracapsular cataract surgery in patients with rheumatoid arthritis has been reported [45]. In another series of 11 eyes in 8 patients with postsurgical corneal ulceration from extra- and intracapsular cataract extraction, 10 eyes had a central ulcer [35]. One patient presenting with PUK in the setting of active rheumatoid arthritis responded well to cyclophosphamide [35]. In the patient with PUK after clear cornea cataract extraction as the first manifestation of rheumatoid arthritis, an immediate resolution was obtained with systemic prednisone [2].

5.3.7 Sjögren's Syndrome

Sjögren's syndrome is a common autoimmune disease that primarily affects women. As a primary disease there is progressive lymphocytic infiltration of the lacrimal and salivary glands leading to dry eyes and dry mouth [11]. Secondary Sjögren's has, in addition to the lacrimal and salivary gland involvement, an association with systemic autoimmune disease, most commonly rheumatoid arthritis. In most patients with Sjögren's syndrome severe keratitis sicca can develop. Autoimmune keratitis may develop in Sjögren's syndrome, usually in the form of a paracentral ulcer (Fig. 5.4) [16, 32]. Treatment with topical cyclosporine was beneficial in both series.

The diagnosis of primary Sjögren's syndrome can be made if four of the following criteria are present or three of the last four objective criteria (3–6):

1. Symptoms of a dry eye;
2. Symptoms of a dry mouth;
3. Signs of a dry eye (decreased Schirmer test or positive Rose Bengal staining);
4. Lymphocytic infiltrate of minor salivary glands on biopsy;
5. Decreased salivary gland flow;
6. Antibodies to Ro(SS-A) and/or La(SS-B).

In secondary Sjögren's there is a defined autoimmune connective tissue disease along with crite-

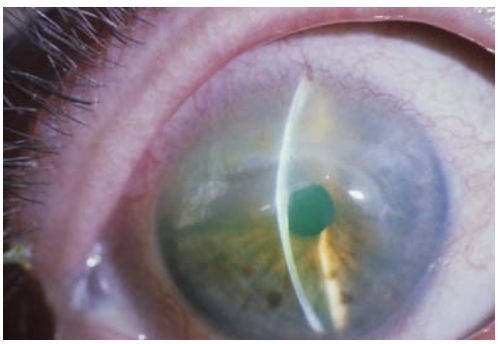

Fig. 5.3 Peripheral ulcerative keratitis following clear corneal cataract extraction in a patient with newly diagnosed rheumatoid arthritis

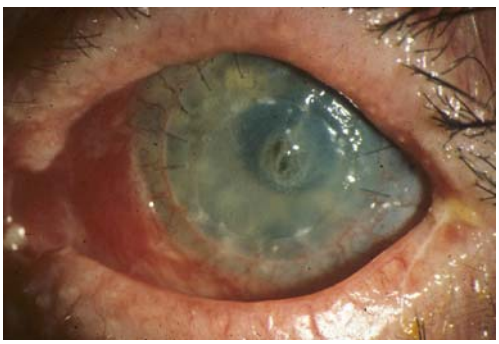

Fig. 5.4 Recurrent paracentral ulcer in a patient with primary Sjögren's syndrome who has had a previous patch graft. Paracentral ulcer resolved with topical cyclosporine

ria 1 or 2 from above and two of the three criteria from 3, 4, and 5.

5.3.8 Wegener's Granulomatosis

Wegener's granulomatosis is a necrotizing granulomatous vasculitis that usually affects the upper and lower respiratory tracts first and can later expand into a generalized vasculitic disease in the latter stages [24, 33]. If untreated, the disease carries a high mortality rate [15, 24, 33, 41].

The diagnosis can be made by a biopsy of involved tissues where inflammation and necrosis of small and medium vessels with granuloma formation can be demonstrated. Laboratory tests may reveal increased sedimentation rate and positive rheumatoid factor [33, 65]. The most sensitive and specific laboratory tests are those for antineutrophil cytoplasmic antibodies (ANCAs) [29, 33]. Diffuse cytoplasmic staining to antibodies to neutrophil serine proteinase is called the cANCA test. Perinuclear staining of lysomal enzymes is the pANCA test. The cANCA is specifically for Wegener's granulomatosis while the pANCA is not as specific as it can be positive in polyarteritis.

The eye is frequently involved in Wegener's granulomatosis with up to 60% of affected individuals having orbital inflammation, retinal vasculitis, and ischemic optic neuropathy [24]. Anterior findings include scleritis, episcleritis, conjunctivitis, peripheral ulcerative keratitis (Fig. 5.5), and most commonly secondary Sjögren's syndrome and keratitis sicca.

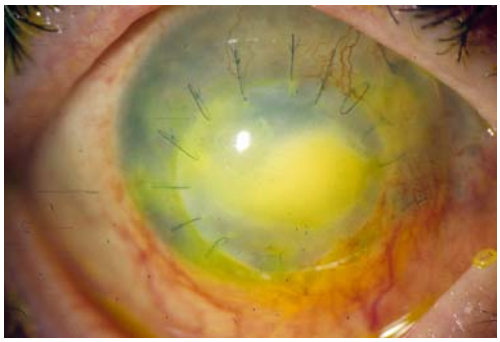

Fig. 5.5 Lamellar patch graft for paracentral ulcer in a patient with Wegener's granulomatosis. Note sutures for patch graft supero-temporally where original PUK and perforation occurred. Ulceration with re-epithelialization occurred with cyclophosphamide

In a series of 8 patients with ocular involvement in Wegener's granulomatosis, 5 patients had PUK associated with scleritis [41]. The other three had scleritis alone. In those patients who had ANCA levels that did not revert to normal, 4 of the 5 suffered a relapse of their disease. Thus, ANCA levels that do not revert to normal after immunosuppression may be an indicator of a future relapse [41]. In addition, systemic disease relapses are associated with rises in ANCA levels [29]. Thus, serial ANCA levels once a month are of value at least in the first year to help anticipate reactivation of the disease [29]. In those with eye complications of Wegener's granulomatosis, there may be autoantibodies to the corneal protein cytokeratin [42]. These antibodies may provide further insight into the etiology and prognosis of the disease.

5.3.9 Polyarteritis Nodosa

Polyarteritis nodosa is primary vasculitis involving medium-sized arteries and a tendency to form microaneurysms [1, 44, 51, 65]. The lungs are not involved and granulomatous inflammation is absent as well as anti-neutrophil cytoplasmic antibodies, in contrast to Wegener's granulomatosis. In a report of 5 cases of polyarteritis nodosa, 1 presented with PUK [1]. PUK has also been reported in other primary vasculitides, microscopic polyangiitis (MPA), and Churg-Strauss syndrome (CSS). MPA is small vessel vasculitis featuring necrotizing glomerulonephritis and respiratory involvement. CSS is characterized by eosinophilia with granulomatous inflammation involving the respiratory tract and necrotizing vasculitis of small to medium vessels as well as asthma [1, 44, 65].

5.3.10 Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a connective tissue disease that can affect numerous organs such as the heart, kidney, and nervous

system. SLE can cause arthritis, multiple skin disorders such as discoid lupus and a malar rash. The most common ocular problem in SLE is a dry eye, whereas PUK is uncommon [44, 51, 65]. A diagnosis of SLE centers on identifying features of the disease by clinical examination, by histopathology, and by laboratory tests for circulating antibodies (ANA).

5.3.11 Relapsing Polychondritis

Relapsing polychondritis is an uncommon autoimmune disease of cartilaginous tissues [26, 51]. As there is no one specific laboratory test to establish the diagnosis, the disease is diagnosed by histopathologic examination and clinical signs of inflammation of nasal cartilage, auricles, or cartilage of the respiratory tract. Ocular complications can occur in up to 60% of affected patients. In one study about 40% were affected with episcleritis and about 15% had scleritis. Rarely, in less than 5%, did PUK occur [26].

5.3.12 Mooren's Ulcer

A diagnosis of Mooren's ulcer is made by excluding other causes of peripheral ulcerative keratitis. Diseases involving a systemic vasculitic process such as rheumatoid arthritis and Wegner's granulomatosis need to be ruled out. Mooren's ulcer has also been found to have an association with hepatitis C in patients and who have been found to have a favorable response to interferon [40].

Mooren's ulcer has characteristic clinical findings, which include ulceration of the cornea several millimeters in from the limbus without scleral involvement [6, 7, 67]. Extension not only occurs circumferentially but also centrally. The ulcer appears to affect primarily the corneal stroma and as the ulcer extends centrally into the stroma, there often occurs an overhanging edge of epithelium that does not appear to be involved in the ulcerative process. Mooren's ulcer can become quite extensive without proceeding to corneal perforation. This may occur because the disease appears to primarily affect the corneal stroma. Indeed one proposed treatment for Mooren's ulcer is to excise remaining central corneal stromal tissue [36].

Mooren's ulcer is more common in Africa and India and less common in the northern hemisphere [52, 65]. The geographic localization of the disease in certain populations has suggested that there may be an underlying genetic component, which could be a risk factor for the disease. Both HLA-DR17(3) and/or DQ2 as HLA types have been identified as associations with Mooren's ulcer [52].

Evidence that a stromal antigen could be responsible for the development of Mooren's ulcer was found in a young patient after penetrating keratoplasty [17]. In this study, the serum from the patient demonstrated circulating antibodies to a stromal protein fraction. A cell-mediated immune reaction to the same protein fraction was found in a lymphocyte transformation assay. Autoimmunity to the stromal antigen was found in a series of patients with Mooren's ulcer [18] and later the antigen was identified as calgranulin C [19, 34]. Calgranulin C is a member of a family of calcium and zinc binding proteins and is expressed in neutrophils and in keratocytes [20]. Calgranulin C has been found in parasite extracts and has been shown to have filariacidal and filariastatic activity [21], and has been found to bind to paramyosin, the thick filaments of parasite muscles [5]. Because helminthic infections have been associated with Mooren's ulcer, it has been hypothesized that the paramysosin–calgranlulin C complex on helminths could initiate an autoimmune response to naturally occurring calgranulin found in the corneal stroma, especially with trauma, surgery, and infections [3].

The treatment of Mooren's ulcer may be dependent on its clinical presentation. Unilateral and bilateral slowly progressive disease has been reported to respond to topical and surgical therapy [7]. In general, patients with Mooren's ulcer can be approached differently from those with collagen vascular PUK, as progression is typically slower than with collagen vascular-related PUK. Because patients with Mooren's ulcer have been evaluated for collagen vascular disease and other causes of PUK with underlying vasculitis, the urgency to use systemic immunosuppression as a life-saving measure is not present. Thus, a stepladder approach to the disease can be used,

beginning with topical therapy and progressing through to systemic immunosuppression [7]. Increasing progression of the disease warrants greater immunosuppression and surgical intervention. Conjunctival peritomy has been proposed to be useful for some more recalcitrant ulcers that do not respond to topical therapy [6, 7]. However, more aggressive forms of Mooren's ulcer, those with bilateral simultaneous disease, are not likely to respond to these therapies and require systemic immunosuppression [7, 13, 14, 62, 65]. Cyclophosphamide and methotrexate have had varying success at bringing these severe ulcers under control (Fig. 5.6) [13].

Balancing saving vision and avoiding complications from immunosuppressive therapy can present difficult choices for the patient and the treating physician. When the disease is unresponsive to full systemic immunosuppression and the patient suffers severe complications from therapy, abandoning treatment and letting the disease take its natural course needs consideration.

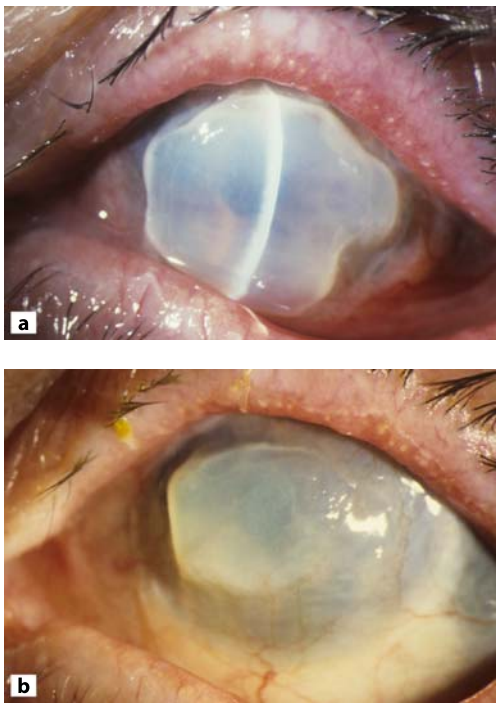

Fig. 5.6 **a** Severe progressive bilateral Mooren's ulcer. **b** Cornea in Fig. 5.1a after 4 months of treatment with cyclophosphamide

Summary for the Clinician

- Corneal disease secondary to rheumatoid arthritis is the most common autoimmune disease presenting to the ophthalmologist.
- Peripheral ulcerative keratitis is most commonly associated with rheumatoid arthritis and carries with it an increase in mortality.
- Paracentral ulcerative keratitis associated with rheumatoid arthritis may be amenable to cyclosporine topical therapy.
- Wegener's granulomatosis is a necrotizing vasculitis that involves the eye in 60% of affected individuals. The disease carries a high risk of mortality if untreated.
- Mooren's ulcer is a diagnosis of exclusion when no underlying systemic autoimmune disease can be identified.

5.4 Systemic Immunosuppression in Autoimmune Keratitis

Systemic immunosuppression in autoimmune keratitis is reserved for cases with progressive ulceration that is not likely to not respond to topical or systemic corticosteroids. The use of immunosuppressive agents requires a thorough medical examination, discussion of pregnancy status and plans, and laboratory tests to rule out underlying infectious diseases such as tuberculosis, to assess the patient's baseline immune status, and to ensure that the patient has appropriate immunizations such as for influenza [10, 25, 27]. Follow-up requires appropriate monitoring and recording of laboratory testing.

5.4.1 Classification of Immunosuppressive Drugs

Immunosuppressive drugs can be classified into four categories: antimetabolites (azathioprine, methotrexate), alkylating agent (cyclophosphamide), T-cell inhibitor (cyclosporine), and an-

tibodies to proinflammatory cytokines (infliximab). Each drug has its specific indications and potential complications [10, 25, 27].

5.4.1.1 Antimetabolites

Azathioprine blocks cell division and hence cell proliferation by inhibiting purine synthesis and ultimately DNA replication. The drug is effective at inhibiting the proliferation of T and B cells. Thus, a side effect can be related to myelosuppressive effects such as leukopenia and anemia.

Methotrexate is a folate analog and also inhibits DNA synthesis by inhibiting the DNA-dependent folate metabolic pathways. The drug also inhibits cell proliferation, especially those involved in the inflammatory cellular response. Side effects include heptocytoxicity as almost a third of patients will have elevated liver function tests, pneumonitis, cytopenia, and gastrointestinal complaints. Liver studies are required for therapy with studies to rule out hepatitis B. Patients on methotrexate are discouraged from drinking alcohol.

5.4.1.2 Alkylating Agents

Cyclophosphamide is a nitrogen mustard alkylating cytotoxic medication that cross-links purines in DNA resulting in cytotoxicity to both dividing and resting lymphocytes. The most common side effect with cyclophosphamide is bone marrow suppression. Hemorrhagic cystitis is also a common complication and patients are encouraged to drink several liters of water per day.

5.4.1.3 T Cell Inhibitors

Cyclosporine inhibits proliferation of immunocompetent T cells. The most common side effect with cyclosporine is nephrotoxicity and hypertension. A patient's blood pressure should be closely monitored while on cyclosporine.

5.4.1.4 Proinflammatory Cytokine Antibodies

Infliximab is a member of a new class of immunosuppressive drugs that utilize antibodies to the proinflammatory cytokine tumor necrosis factor alpha (TNFα) [54]. TNFα initiates the production of matrix metalloproteinases that have been hypothesized to be involved in the degradation of the epithelial basement membrane and the stroma. Contraindications to TNF antagonists include tuberculosis and congestive heart failure.

Summary for Clinicians

- A number of immunosuppressive drugs are available for the treatment of autoimmune keratitis. Before use in a patient with autoimmune keratitis, each drug should be carefully evaluated for its specific indications and potential complications.
- Patients on immunosuppressive therapy for autoimmune keratitis need periodic follow-up with appropriate ancillary testing to ensure no developing complications.

5.5 Surgical Intervention in Autoimmune Keratitis

5.5.1 Gluing

When considering the management of an impending or frank perforation in autoimmune keratitis, it is of paramount importance to have an understanding of the temporal progression of the disease. In a rapidly progressing ulcer, it is unlikely that temporizing measures such as corneal gluing are going to be of benefit. Corneal gluing may block a view of the ulcerative progress so that a determination of the response to treatment is inadequate. In no circumstance is corneal gluing alone an adequate treatment for a rapidly progressing autoimmune ulcerative disease (Fig. 5.7).

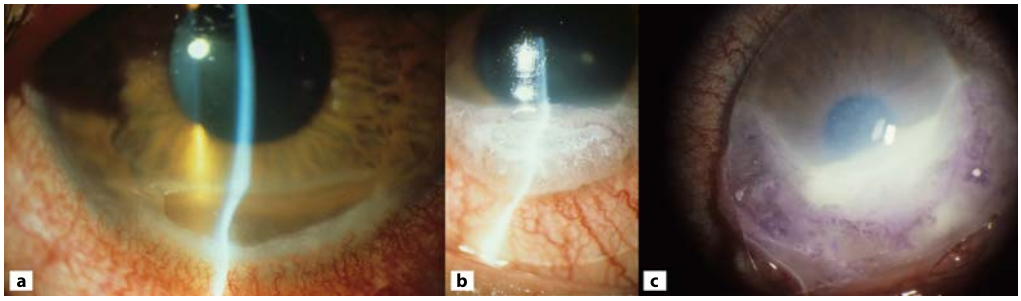

Fig. 5.7 a Patient with rheumatoid arthritis with PUK demonstrating little infiltrate in the bed of the ulcer with some peri-limbal vascular hyperemia. **b** Systemic steroids and cyclophosphamide begun with gluing. **c** Progression of the ulcer despite gluing within 1 week

5.5.2 Corneal Patch Grafting

If a there is a frank perforation of the cornea in PUK, the integrity of the globe must be restored. If there is progressive disease with likely progression of the perforation, the anticipated enlargement in the ulcer needs to be considered in the decision-making process for a surgical repair. In ulcers in which there is no response to systemic steroids and combination therapy is initiated with immunosuppressive agents, there can be up to a 2-week delay before the ulcerative process is brought under control.

Several approaches can be used to surgically repair corneal perforation. For paracentral ulcers, lamellar patch grafting can be useful for restoring integrity to the eye (Fig. 5.8) [4]. Full thickness penetrating keratoplasty is an option but trephination is more difficult in an eye that is soft from a perforation.

Perforations from PUK can be the most difficult repair. Because the ulceration is at the limbus, a repair can induce astigmatism and affect vision. Crescentic repairs can be fashioned, but require free handing the curves necessary to fit the inside and outside aspects of the ulcer. A trephine with a smaller diameter and smaller radius of curvature can be fashioned to delineate the inside curve and a larger trephine can cut the outside diameter.

If appropriate immunosuppression has begun and the ulcer is brought under control, a perforation can be closed with a partial thickness lamellar graft. The surrounding tissue of the ulcer is dissected to about one-third depth and a lamellar patch graft can be sutured in position so that edges are apposed and a smooth corneal surface is achieved.

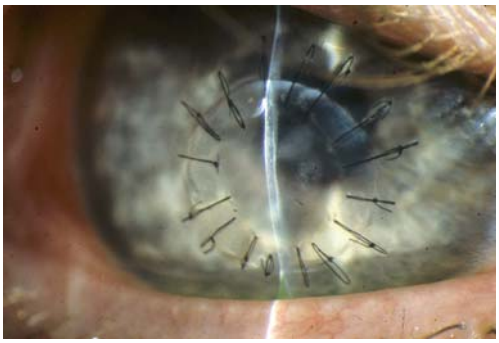

Fig. 5.8 Lamellar patch graft of the eye in Fig. 5.2

5.5.3 Ulceration in the Setting of a Patch Graft

For patients with a patch graft who continue to have an underlying autoimmune process, the new graft can become involved with a suppurative infiltrate. Reperforation can occur unless adequate immunosuppression is achieved. Paracentral ulceration of a patch graft has been reported to have been successfully treated with topical cyclosporine resulting in resolution of the suppurative infiltrate and ulceration that is notable by intense vascularization [16].

Summary for Clinicians

- Corneal gluing should not be used as the sole treatment in a rapidly advancing immune ulcer.
- Ulceration in the setting of a patch graft for autoimmune keratitis may respond to topical cyclosporine therapy.

5.6 Conclusions

Autoimmune keratitis can present with a number of clinical profiles and with a number of underlying autoimmune systemic diseases. Autoimmune keratitis should be suspected in all cases of unexplained stromal ulceration and should be closely monitored for rate of progression if autoimmune keratitis is identified. Rapidly progressive ulcerative disease needs prompt and appropriate immunosuppression.

Most patients with collagen vascular stromal ulceration have a long history of disease. However, the general ophthalmologist may be presented with a patient with ocular symptoms and signs that represent the initial onset of a collagen vascular disease.

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Chapter 6

Recent Developments in Herpes Stromal Keratitis

6

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Core Messages

- Immune responses are typically accompanied by some degree of inflammation and angiogenesis.
- Inflammation and neovascularization promote scar tissue formation.
- Because of its requirement for clarity, the cornea has a low tolerance for scar tissue.
- The cornea has acquired mechanisms to inhibit inflammation and neovascularization, but these are rapidly overcome following HSV-1 infection.
- A hallmark of the herpesvirus family is its ability to cause latent infections.
- Following primary infection, HSV-1 establishes a latent infection in sensory neurons within sensory ganglia.
- Latency is characterized by the retention of the viral genome in neuronal nuclei without the production of infectious viral particles.
- Our understanding of HSV-1 latency is evolving. Recent findings suggest that some viral lytic genes might be expressed during latency without leading inexorably to virion formation. Moreover, preventing full reactivation in these neurons might depend on the constant vigilance of the host immune system.
- Recurrent herpes keratitis in humans results from reactivation of HSV-1 from latency in neuronal nuclei, transport down nerve axons, and release at the corneal surface. This can lead to recurrent bouts of inflammation and progressive scarring.
- Although humans are the natural host of HSV-1, the virus can cause disease including keratitis in other species.
- T lymphocytes are divided into $CD4^+$ T cells (often referred to as T helper [Th] cells) and $CD8^+$ T cells (cytotoxic T lymphocytes [CTL]). $CD4^+$ T cells are further divided based on the pattern of cytokines they produce into Th1 (interferon gamma, TNF, and IL-2 involved in cellular immune responses) and Th2 (IL-4, IL-5, IL-6, and IL-10 involved in antibody and allergic responses).
- Molecular mimicry is a term that refers to autoimmune responses induced by peptide sequences (epitopes) in microbial proteins that are shared by self molecules. T cells that respond to these epitopes in the context of an infection can then attack normal tissue.
- T cells can be stimulated specifically through their T cell receptor (TCR), or nonspecifically by cytokines in a process called "bystander activation."
- Whether the $CD4^+$ T cells that mediate herpes stromal keratitis (HSK) are stimulated by HSV-1 antigens through their TCR, through bystander activation, or by molecular mimicry remains unresolved.
- Dendritic cells (DC) are professional antigen-presenting cells that are critical for the initial activation of naïve T cells in lymphoid organs, and can also re-stimulate effector T cells when they infiltrate sites of infection.

Core Messages

- The cornea is normally devoid of DC, but a type of DC called Langerhans cells is present at the corneal limbus and migrates into the central cornea following infection or trauma.
- Chemokines are small molecules that orchestrate the infiltration of leukocytes into infected or inflamed tissue. Because the receptors for these chemokines are differentially expressed on leukocyte populations, different chemokines attract different leukocyte populations.

6.1 Introduction

Clear vision requires a clear cornea. Corneal clarity is critically dependent on an optimal arrangement of collagen fibrils in the corneal stroma, the absence of blood vessels, and a relative lack of hematopoietic cells within the corneal stroma. Unfortunately, angiogenesis and edema, which typically accompany inflammatory responses, can transiently disrupt corneal clarity through neovascularization and disorganization of collagen fibrils. Moreover, inflammatory mediators can effect changes in the keratocytes that produce the extracellular matrix of the corneal stroma, leading to scarring and permanent loss of vision.

The cornea is considered an immune privileged tissue because it has acquired a variety of mechanisms to inhibit immunoinflammatory responses. For instance, the corneal endothelium is bathed in aqueous humor, which contains immunosuppressive factors including transforming growth factor β (TGF-β) and alpha-melanocyte-stimulating hormone (alpha-MSH) (reviewed in [34]). Corneal endothelial and epithelial cells express Fas ligand (CD95L), which can activate the apoptotic machinery of infiltrating inflammatory cells by signaling through Fas, leading to their destruction. Additionally, the lack of blood and lymphatic vessels and antigen-presenting cells (APC) in the normal cornea limits antigen presentation to the immune system. Together, these features of the cornea inhibit immunoinflammatory reactions by limiting the afferent delivery of antigens from the cornea to the lymphoid organs and by neutralizing T cell effector mechanisms within the cornea.

HSV-1 corneal infection in mice causes a transient epithelial lesion, resulting from HSV-1 replication in and destruction of epithelial cells. These lesions typically heal within 1 week of infection concomitant with elimination of replicating virus from the cornea. The high regenerative capacity of the corneal epithelium permits rapid healing of these lesions with no permanent visual compromise. However, within a week of infection herpes stromal keratitis (HSK) develops and is characterized by neovascularization and corneal opacity. Thus, all of the characteristics that confer immune privilege on the cornea are overcome following HSV-1 corneal infection.

Summary for the Clinician

- Corneal immune privilege as defined by increased rate of graft acceptance in the absence of tissue typing or systemic steroid treatment is rapidly lost when the cornea is brought in direct contact with the host immune system through neovascularization.
- This is exemplified by the loss of immune privilege and enhanced rate of rejection of corneal grafts placed in a high-risk (vascularized) graft bed, and by the progressive inflammation that develops in HSV-1-infected corneas in conjunction with neovascularization.

6.2 HSV-1 Latency and Recurrent HSK

While replicating in epithelial cells on the surface of the cornea, HSV-1 gains access to the termini of sensory neurons, and is transported by retrograde axonal transport to the neuronal nuclei within the ophthalmic branch of trigeminal ganglia (TG). In mouse models, transport of the virus from the cornea to the TG occurs within 2 days of cornea infection and is followed

by a brief period of viral replication and apparent horizontal spread within the TG. Following primary infection in mice, virus replication and spread within the TG is controlled, initially by an innate immune response (reviewed in [25]). However, mice with T cell deficiencies or that are deficient in the production of certain cytokines fail to fully control HSV-1 replication within the TG, and the virus is transported to the brain resulting in lethal encephalitis (reviewed in [9]). Control of virus replication in the TG does not result in eradication of the virus. Instead, a latent infection is established, which is characterized by the retention of functional viral genomes as episomal structures within the nucleus of sensory neurons, in the absence of infectious virus. After the establishment of latency, latent viral genomes are retained within neurons for the life of the individual. However, in some individuals HSV-1 periodically reactivates from latency in a limited number of infected neurons. The virion components are then transported down the nerve axons for assembly and release at the cornea. Thus, recurrent herpetic corneal disease usually results from reactivation of a latent virus in sensory ganglia rather than from re-infection from an external source. Intermittent shedding of the virus in the cornea can give rise to recurrent bouts of HSK, with progressive scarring, leading to visual compromise.

Although HSV-1 latency has long been considered a silent infection that is ignored by the host immune system, several recent studies challenge this view (reviewed in [9]). In mice and humans, $CD8^+$ T cells surround latently HSV-1 infected neurons in the latently infected TG. In mice these cells maintain an activation phenotype, form an apparent immunologic synapse with neurons, and can inhibit HSV-1 reactivation from latency in *ex vivo* TG cultures. These observations combined with the close association between stress-induced compromise of T cell function and HSV-1 reactivation from latency (reviewed in [33]) suggest a model in which HSV-1 latency is maintained at least in part through the constant vigilance of HSV-1-specific $CD8^+$ T cells that are in close apposition to latently infected neurons.

Summary for the Clinician

- The reason why HSV-1 typically recurs at the same anatomical site such as the cornea is that the source of virus for each recurrence is the sensory neurons that harbor latent viral genomes and innervate the infected tissue.
- The maintenance of viral latency appears to involve a tripartite interaction among the virulence properties and copy number of the latent viral genome, the characteristics of postmitotic neurons that may inhibit viral gene expression, and the host immune system that tends to be compromised by the stressful stimuli that are often associated with HSV-1 reactivation from latency.

6.3 Murine Model of HSK

Murine models have provided most of our current understanding of immunologic involvement in HSK. Typically, animal models fail to mimic every aspect of human disease, and the murine model of HSK is no exception. In general, HSK has been studied in mice following a primary infection, whereas most human HSK represents recurrent disease. However, the importance of this difference is not entirely clear. In mice HSK begins approximately 7 days after HSV-1 corneal infection, a time when replicating virus has been cleared from the cornea. In one study, mice that were constantly depleted of T lymphocytes retained clear corneas for 30 days after HSV-1 corneal infection. However, when T cell depletion was discontinued 30 days after infection and T cells began to reappear in the lymphoid organs of the mice, HSK promptly developed [19]. Such observations raised questions regarding the source of HSV-1 antigens that stimulate the immunopathology associated with HSK. It is possible that HSK in mice, as in humans, results from a virus that travels a round trip from the cornea to the TG and back again, although this concept remains controversial [4, 32].

The mouse clearly does not provide a comprehensive model of human HSK. Mice do appear

to provide a good model for human necrotizing HSK, which is characterized by neovascularization and opacity. However, perhaps the most common form of HSK seen in the clinic is disciform disease. Neovascularization, an essential component of necrotizing HSK, is conspicuously absent in disciform HSK, and the immunopathology is thought to be quite different in the two forms of HSK. Despite these shortcomings, the mice have provided most of our current knowledge of the immunopathology associated with HSK.

Herpes stromal keratitis begins approximately 7 days after HSV-1 infection of mouse corneas, progressing through 21 days post-infection (dpi), with corneal opacity and neovascularization persisting through at least 40 dpi. Seminal studies by Metcalf et al. [29] demonstrated that T cells play a critical role in HSK, since following corneal infection, T cell-deficient nude mice failed to develop disease. Subsequent studies showed that $CD4^+$ T cells orchestrated HSK following corneal infection of BALB/c mice with the RE strain of HSV-1 [18, 30]. Several studies have established that in HSV-1 infected corneas of Balb/c mice, $CD4^+$ T cells greatly outnumber $CD8^+$ T cells. The reason for the preferential infiltration or retention of $CD4^+$ T cells in the cornea is not clear. Involvement of the corneal microenvironment is suggested by equivalent infiltration of $CD4^+$ and $CD8^+$ T cells in the infected TG of mice that exhibit a predominantly $CD4^+$ T cell corneal infiltrate. Thus, even when corneal immune privilege is compromised, marked differences in the composition of the inflammatory infiltrate are observed when comparing the cornea with non-immune privileged tissues.

Summary for the Clinician

- There are several animal models of HSK in use. Some investigators favor the rabbit model because it features a cornea that is anatomically similar to the human cornea, and HSV-1 reactivates "spontaneously" from latency and is shed at the cornea as in humans. The mouse provides a model of necrotizing HSK (not disciform HSK) and because of the wealth of reagents available for dissecting the murine immune response is favored by those interested in defining the immunopathological mechanisms in HSK.

6.4 T Lymphocyte Involvement in HSK

In murine models, $CD4^+$ T cells appear to be the principle mediators of HSK, and they orchestrate disease primarily through the elaboration of Th1 cytokines (see below). However, cytokine production is tightly regulated and their production typically ceases within 2 h of removing T cell stimulation. Thus, $CD4^+$ T cells require constant stimulation to maintain cytokine-mediated inflammatory processes like HSK. The exact nature of the stimulus that maintains $CD4^+$ T cell activation within the HSK lesion remains contentious.

At least three hypothetical models for the activation of $CD4^+$ T cells in HSK have been advanced and supported by published data. These include:

1. Bystander activation of $CD4^+$ T cells by cytokines that are produced in the cornea in response to infection [10, 13, 14];
2. Autoimmune reactivity to corneal tissue resulting from molecular mimicry by a viral protein [1, 21, 31, 43];
3. Virus-specific activation.

Bystander activation of $CD4^+$ T cells as a mechanism for HSK development is supported by data using transgenic mice expressing the T cell receptor (TCR) specific for ovalbumin (ova). Despite a

lack of recognition of viral antigens by the CD4+ T cells of these mice, severe corneal inflammation developed following HSV-1 infection. CD4+ T cells expressing the ova-specific TCR were retrieved from the HSK lesions while CD4+ T cell depletion abrogated inflammation [12]. These studies provided the foundations of the principle that HSK-like disease can develop in the absence of HSV-1 reactive CD4+ T cells. However, important aspects of the model system employed hamper an accurate assessment of the participation of bystander-activated cells in HSK development. For instance, the mice utilized lacked an HSV-1-specific adaptive immune response, and thus the virus was never cleared from the corneas. Moreover, the mice died at a time when HSK was not fully developed. In fact, when HSV-1 replication was controlled in the corneas of these mice with anti-viral drugs, no inflammation developed, suggesting that bystander activation cannot fully account for the activation of CD4+ T cells in HSK [10]. Nevertheless, one cannot rule out a possible contribution of bystander activation of CD4+ T cells to chronic inflammation in HSK.

Possible involvement of an autoaggressive CD4+ T cell assault on corneal tissue during HSK was proposed by elegant studies from the laboratory of Harvey Cantor [1, 21, 31, 43]. In this model, the HSV-1 UL6 coat protein and a normal corneal protein were found to contain a common epitope. Their observations suggested that CD4+ T cells reactive to the UL6 epitope (and cross-reactive to a corneal protein) are generated during infection. These autoreactive CD4+ T cells then infiltrate the infected cornea and mediate tissue destruction. Perplexingly, this autoimmune involvement in HSK occurred after corneal infection with the KOS, but not the RE strain of HSV-1. Moreover, a study by another group failed to confirm this cross-reactivity, or the capacity of UL6-specific CD4+ T cells to regulate HSK [11]. Additionally, characterization of the specificity of T cell clones isolated from human corneas with HSK has not revealed reactivity to either the UL6 or corneal antigens [41]. Consequently, the proposal of molecular mimicry-induced autoimmunity in HSK remains controversial.

The final model, favored by our group, entails HSV-1-specific CD4+ T cells playing an indispensable role in the induction and progression of HSK. HSV-1 specific CD4+ T cell clones have been isolated from both human [41] and murine (our unpublished observation) corneas at various stages of HSK. Perhaps the best evidence for an involvement of HSV-1 specific T cells in HSK came from studies in which mice were tolerized to HSV-1 antigens. When HSV-1 injections were administered into the ocular anterior chamber, induction of an aberrant form of immunity occurred, which has been referred to as anterior chamber-associated immune deviation (ACAID) [42]. ACAID is characterized by preferential inhibition of CD4+ T cell functions such as delayed-type hypersensitivity and production of Th1 cytokines[35]. Induction of ACAID to HSV-1 antigens concurrently with HSV-1 corneal infection demonstrated protection of the cornea from HSK [26].

Summary for the Clinician

- Exactly how CD4+ T cells are activated in the cornea to regulate HSK remains uncertain.
- We favor the notion that reaction of HSV-1-specific CD4+ T cells to HSV-1 antigens in the cornea triggers inflammation, but bystander activation by cytokines and possibly some autoaggressive reaction to normally sequestered autoantigens in the inflamed cornea might contribute to the persistence of the inflammatory response.

6.5 Antigen Presentation in HSK

The requirement for constant CD4+ T cells stimulation for initiation and maintenance of HSK necessitates the presence of APCs within the cornea. While macrophage-like cells have been described in the normal mouse cornea, most lack detectable expression of MHC class II, and their APC function is debatable [7]. The presence of CD11c-positive dendritic cells (DCs) in the normal cornea is controversial, as the cells observed

were characterized as abnormal due to lack of detectable MHC class II [7, 15, 16]. Thus, regardless of the presence of a network of F4/80-expressing macrophage-like cells in the normal cornea, the concept that it is devoid of professional APCs remains tenable. However, the limbal region between the cornea and conjunctiva is densely populated with Langerhans cells (a type of DC) which, as studies have established, migrate into the central cornea subsequent to HSV-1 infection [20]. Several laboratories' studies have established that corneal DCs play an integral role in HSK. Studies in which Langerhans cells were eliminated from the surface of one eye via ultraviolet light exposure with an ensuing bilateral corneal infection exposed a DC role not only in the inductive phase of the T cell response in lymphoid organs, but also in the actual effector phase of the response in the infected cornea [20]. The latter conclusion was based on the observation that when Langerhans cells were present in the eye, HSK developed normally, but failed to develop in the cornea depleted of Langerhans cells. Replicating HSV-1 was eliminated normally (by 5 dpi) from these Langerhans cell-depleted corneas and subsequently the corneas appeared normal. Thus, ocular HSV-1 infection has the capability to drive DC infiltration into the normally devoid cornea and also drive the T cell effector response that instigates HSK.

The possibility that DCs might influence the preferential accumulation of $CD4^+$ over $CD8^+$ T cells arose from studies comparing the ability of two different laboratory strains (KOS and RE) of HSV-1 to induce HSK in A/J mouse corneas [18]. KOS HSV-1 infection resulted in a low incidence of HSK (50%), tended to be milder, and was characterized by a predominantly mononuclear infiltrate in which $CD8^+$ T cells outnumbered $CD4^+$ T cells by a 2:1 margin. In contrast, RE HSV-1-induced HSK occurred with much high incidence (80–100%), a predominantly polynuclear neutrophilic infiltrate, and a preponderance of $CD4^+$ over $CD8^+$ T cells. This study also revealed the important observation that KOS HSV-1 was a poor inducer of Langerhans cell migration into the cornea. Moreover, if Langerhans cell migration into the cornea was induced prior to infection, KOS HSV-1 induced a high incidence of HSK that was characterized by the immunopathology similar to that seen in HSV-1 RE infections. While not conclusive, these findings nevertheless suggest that $CD4^+$ T cell accumulation is favored by early infiltration of Langerhans cells into the HSV-1-infected cornea. How Langerhans cells mediate preferential $CD4^+$ T cell infiltration into the cornea is unclear, but likely involves a unique set of chemokines and/or homing receptors. Investigations exploring these possibilities are currently underway.

6.6 Cytokines and Chemokines in HSK

Following HSV-1 RE corneal infection, $CD4^+$ T cells regulate the migration of neutrophils into the cornea, damaging the corneal architecture and resulting in progressive opacity. Neutrophilic infiltration is regulated partially by the Th1 cytokines interleukin (IL)-2 and interferon (IFN)-γ, which $CD4^+$ T cells produce in the infected cornea [39, 40]. It is not entirely clear how these cytokines mediate neutrophilic infiltration. IFN-γ increases the expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) on corneal blood vessels, which seems to encourage neutrophilic extravasation from corneal blood vessels [39]. IL-2 appears to indirectly orchestrate neutrophil migration into the central cornea following extravasation into the perivascular space in the peripheral cornea, and also functions to regulate survival within the cornea [40].

Other cytokines with known roles in HSK include TNF-α, IL-1, IL-6, IL-12, and IL-17 [4]. Both TNF-α and IL-1 are pluripotent cytokines involved with several aspects of HSK [23], including regulation of the infiltration of T cells, neutrophils, and MHC II-positive DCs after HSV-1 infection. These cytokines seem to exert their function on infiltration indirectly by acting on both corneal cells (epithelial cells and stromal keratocytes) and infiltrating bone marrow-derived cells to induce their production of chemokines, and by activating the local vascular endothelium to increase leukocytic extravasation. Although the infiltrate seems important for initial viral clearance in the cornea, it may create a microenvironment that primes HSK onset. IL-17 is expressed in human corneas with HSK, and

the IL-17 receptor is constitutively expressed by corneal fibroblasts [28]. IL-17, TNF-α, and IFN-γ synergistically function to induce production of IL-6, the neutrophil-attracting chemokines macrophage-inflammatory protein (MIP)-1α and IL-8, and the DC chemokine MIP-3α by cultured human corneal fibroblasts. Moreover, IL-17 induces the production of the matrix metalloproteinase-1 (MMP-1) by corneal fibroblasts, which degrades the collagen matrix of the cornea, compromising corneal architecture. Therefore, the combined action of these cytokines creates a pro-inflammatory microenvironment that ultimately results in damage to corneal tissue leading to corneal opacity and loss of visual acuity.

Summary for the Clinician

- Persistent production of cytokines requires constant stimulation of T cells.
- Most studies support the hypothesis that the Th1 cytokines interferon gamma and interleukin-2 play an important role in regulating HSK.

6.7 New Experimental Approaches to Treating HSK

Neovascularization of the cornea is a hallmark of necrotizing HSK. A variety of factors produced in HSV-1-infected mouse corneas bind to receptors on the vascular endothelium of limbal blood vessels, inducing cell division and protrusion of vessels into the cornea. These factors also can facilitate movement of the vessels by degrading the corneal extracellular matrix. These factors drive rapid ingrowth of blood vessels into the previously avascular cornea, and this neovascularization appears to be a requisite step in the development of HSK in mice [44]. The proximal mediators of neovascularization in mouse corneas with HSK include vascular endothelial growth factor (VEGF), angiogenic chemokines, and MMP-9 [4]. The production of these angiogenic factors appears to be regulated by a variety of cytokines and chemokines present in the infected cornea. IL-1 and IL-6 play a prominent role as paracrine inducers of VEGF production in the cornea [3, 5, 6]. VEGF production begins in the cornea 1 day after infection, and continues well after viral clearance and throughout the course of HSK [3, 44]. The chronic presence of blood vessels in HSK lesions after viral clearance is likely due in part to VEGF production by infiltrating inflammatory cells [44].

The CXC chemokines that contain the ELR^+ motif also possess potent angiogenic properties and bind to the CXCR2 receptor. Among the ELR^+ chemokines that have been shown to be produced in corneas with HSK are IL-8 and the murine homologue MIP-2 [3]. In contrast to the potent angiogenic properties of ELR^+ CXC chemokines, those CXC chemokines lacking the ELR motif exhibit angiostatic properties. A role for the ELR^- CXC chemokines in regulating HSK was established in studies of the chemokine IFN-γ inducible protein-10 (IP-10; CXCL10) or the IFN-γ inducing cytokine IL-12. Both IP-10 and IL-12 were shown to inhibit neovascularization and HSK severity in infected corneas [27]. It should be noted, however, that another group obtained conflicting results, suggesting that IP-10 actually contributes to rather than inhibits HSK [8].

The above findings suggest that measures designed to inhibit neovascularization of the infected cornea or attenuate existing vessels might provide useful therapeutic options for HSK. The development of drugs capable of inhibiting angiogenesis is a high priority for cancer therapy, and efficacious drugs are likely to emerge in the near future.

In mice as in humans inflammation is ultimately ameliorated within HSK lesions. The mechanisms responsible for controlling HSK remain poorly defined. An association between diminished inflammation and the appearance of the Th2-associated cytokine IL-10 has been described [2, 17, 22, 24, 36]. IL-10 can antagonize the production and effects of the Th1 cytokines that appear to regulate HSK. An exciting new twist to this story emerged with the recent discovery of a subpopulation of T cells that possess regulatory functions (Tregs). In particular, a population of natural Tregs that express CD4 and the α chain of the IL-2 receptor (CD25) has been described to utilize IL-10 as one mecha-

nism to regulate Th1 responses [37]. These $CD4^+$, $CD25^+$ Tregs have been described in both mice and humans and have been associated with resistance to autoimmune diseases. Recently, Babu and colleagues [38] demonstrated that $CD4^+$ and $CD25^+$ Tregs inhibit the response of $CD4^+$ effector T cells in HSV-1-infected corneas, and reduce the immunopathology associated with HSK. The inhibitory effect of these natural Tregs was mediated at least in part through their production of IL-10. The potential therapeutic efficacy of these Tregs or the possibility of directly inducing IL-10 production within HSV-1-infected corneas offers additional exciting new approaches to treating HSK.

Summary for the Clinician

- Interfering with the T cell interaction with antigen-presenting cells such as macrophages and dendritic cells might prove useful in reducing the severity of HSK.
- Interfering with angiogenesis by inhibiting the activity of angiogenic factors such VEGF, certain chemokines, and metalloproteinase 9 (MMP-9) might provide useful therapy for some forms of HSK.
- Regulatory T cells (Tregs) capable of inhibiting Th1 cytokine responses are re-emerging as an important area of immunologic research with great potential for treating a variety of immunoinflammatory diseases, including HSK.

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Chapter 7

Genetic Insights into Uveitis

7

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Core Messages

- The association between HLA-A29 and birdshot chorioretinopathy may be the strongest association between an HLA antigen and a specific disease.
- The association between HLA-B27 and acute anterior uveitis has been known for more than 30 years, but the mechanism for that association is still uncertain.
- The gene responsible for a dominantly inherited form of uveitis known as Blau syndrome also influences susceptibility to Crohn's disease. This gene may unlock important clues to the pathogenesis of uveitis with respect to inflammatory responses to bacterial stimuli.
- The vast majority of immune-mediated forms of uveitis are complex, polygenic diseases. Genes that predispose to uveitis will likely have small independent effects on risk, but with a larger cumulative effect.

7.1 Introduction

7.1.1 Overview

From Mendel's first experiments with pea plants in 1866, the discovery of DNA by Miescher in 1869, the double helix structure by Watson and Crick in the 1950s, to the sequencing of the human genome in 2001, we have come a long way in understanding the genetic contribution to disease [32]. Inherited causes of diseases have long been known; the most famous may be the "royal disease," X-linked hemophilia, affecting the royal houses of Europe after intermarriages had occurred. Illnesses such as inflammatory eye disease are more challenging to assess at the gene level because they do not as clearly show accumulation in certain families. Still, an association between disease and genes (in addition to environmental influence) has always been suspected. Early clues that genes predispose to inflammatory disease were provided by the studies demonstrating that patients with ankylosing spondylitis and also acute anterior uveitis (AAU) were more often positive for human leukocyte antigen (HLA)-B27 than the normal population [9–11, 62]. There have been additional associations found between HLA genes and certain types of uveitis such as birdshot retinochoroidopathy (BSRC) and HLA-A29 or Behçet's disease and HLA-B51. Although we still do not know how the expression of certain HLA genes confers the risk of developing uveitis, we use it as a diagnostic and prognostic means. The successful sequencing of the human genome has opened up new doors for genetic research [32]. Also in 2001, the first genetic mutation found to cause an inherited (in this case autosomal dominant) form of uveitis, Blau syndrome, was discovered in the gene for CARD15 (or NOD2) [45]. Apart from giving insights into the pathogenesis of the disease, this knowledge hopefully will lead to new treatment possibilities.

In this review we discuss the different approaches to studying gene associations, provide an overview of the major genes that may influence susceptibility to uveitis, and discuss current and future developments in this field.

7.1.2 General Approaches to Genetic Study

Table 7.1 provides a short glossary of some common genetic terms. There are two general approaches to studying the possible genetic predisposition to disease: one is to look for polymorphisms or mutations in specific, so-called candidate genes based on the biological function of the gene and possible relevance to the disease mechanism. The second approach is possible due to the utility of genetic markers, which naturally occur throughout our genome. One type of marker is referred to as a microsatellite marker, and consists of specific short sequences repeated varying numbers of times (tandem repeat). Another type of genetic marker is a single nucleotide polymorphism (SNP). A SNP represents a base substitution present in a population with an incidence of at least 1%. More than 1.4 million SNPs have been found at about every 1–2 kilobases in the human genome (The SNP consortium, http://snp.cshl.org/). These or other markers are used in genome-wide scans to test for linkage to the disease phenotype. Regions identified can then be examined more closely. Researchers again can choose different methods for their study: case-control or pedigree studies. The traditional case versus control approach would be used for association studies such as in Brewerton's work testing whether patients with AAU were more likely to have a particular HLA allele than healthy donor controls [9]. Family pedigrees can be analyzed by linkage analysis or transmission disequilibrium test. The former would be used for large pedigrees with multiple affected family members or sets of affected siblings. The results are commonly reported as the logarithmic odds ratio (LOD). The latter test would be used in trios (affected individual and parents) to track the transmission of alleles from parents to their affected offspring.

In any case genetic studies will only be successful in identifying one or several involved genes if the phenotype of the disease has been properly defined. Clinicians and geneticists have to work together to obtain well-defined patient groups. Some types of uveitis, such as AAU, which is dis-

Table 7.1 Commonly used genetic terminology

Term	Definition/comments
Phenotype	Total physical appearance and constitution, or a specific manifestation of a trait of an individual. The phenotype is determined to some extent by the genotype.
Genotype	Specific combination of alleles of an individual carrier.
Complex trait	A phenotype that does not relate to a strict Mendelian inheritance pattern. Interaction between genes and/or environmental factors may be involved.
Allele	Individuals have two alleles of each gene, one inherited from each parent.
Linkage disequilibrium	Tendency of two or more alleles on one chromosome to be inherited together in a higher frequency than expected from a random association.
Logarithmic odds (LOD) score	A logarithmic scale used to quantify linkage by statistical estimation. Generally, a LOD score above 3.0 is considered significant.
Mutation	An alteration in DNA sequence with functional consequences (i.e., a polymorphism associated with a disease).
Polymorphism	A variation in DNA sequence.
Single nucleotide polymorphism (SNP)	A one-nucleotide change present in a population with an incidence of at least 1%
Haplotype	A group of SNPs that are transmitted together, i.e., as a "block"

tinctly characterized as anterior, unilateral uveitis with sudden onset and recurrence, are more suited to this than others. By further joint efforts in defining uveitis subsets and nomenclature we will hopefully facilitate genetic research in the future [70].

Summary for the Clinician

- Susceptibility to most forms of uveitis is influenced by genetics. The HLA genes are the most recognized set of genes that affect susceptibility to a variety of forms of uveitis.
- Despite the strength of genetic associations in some forms of uveitis, such as birdshot choroid retinopathy (BSCR), genotyping plays only a limited role in differential diagnosis.

7.2 Uveitis Associated with Human Leukocyte Antigen Genes

7.2.1 What Is the Human Leukocyte Antigen?

The human leukocyte antigen (HLA) is the genetic designation for the human major histocompatibility complex (MHC). The HLA genes are localized on the short arm of chromosome 6. They extend over some 4 centimorgans (cM) of DNA, about 4×106 base pairs (bp), containing more than 200 genes. The first human leukocyte antigens were discovered through antigenic differences between white blood cells from different individuals and were subsequently defined by antisera. The actual nomenclature combines serologic and genetic information. So for HLA-B*5101, HLA-B stands for the class I, B locus, where the gene product is encoded. The first two digits after the asterisk identify the serologic specificity whereas the last two digits indicate a unique DNA sequence.

Major histocompatibility complex genes are categorized into two major groups: class I and class II. MHC class I is expressed on all nucleated cells and is used to present self- and foreign antigen peptides to CD8+ T cells. The HLA class I molecule is a dimer. The light chain is β2 microglobulin, which is coded on chromosome 15 and is invariant. There are three highly polymorphic gene loci encoding the MHC class I α chains: HLA-A, -B, and -C with 1,325 different alleles identified to date (www.ebi.ac.uk/imgt/hla).

Major histocompatibility complex class II is primarily expressed on antigen-presenting cells (dendritic cells, macrophages, and B cells) as well as activated T cells and is recognized by CD4+ T cells. The class II region contains an equally polymorphic cluster of genes including the DR, DP, and DQ sub-regions. Each sub-region contains both the α- and β-chain genes that make up a specific MHC class II molecule. However, in many cases the HLA-DR sub-region contains an extra β-chain gene whose product can pair with the DRα chain, thus leading to even more variety of MHC class II molecules expressed on the cell surface. On the above-mentioned website 763 alleles for class II have been listed.

7.2.2 From HLA Association to Disease?

Even though the association between HLA and human disease has been recognized for decades, the exact mechanisms whereby HLA confers genetic risk of developing a disease are largely unknown. Epidemiological studies have shown that genetic factors alone are not sufficient. For example, the chance of an HLA-B27-positive individual with an affected relative developing spondyloarthritis is only 20% [72]. One hypothesis is that the disease can develop in genetically susceptible individuals if a second event, such as an infection or other environmental influence, takes place. The MHC is crucial for the T cell education that takes place in the thymus. Here T cell receptors are tested for their interaction with MHC loaded with self-peptides, the high avidity, autoreactive T cells are deleted and thus prevented from damaging peripheral organs. Another hypothesis for the pathogenesis of disease is that not all self-peptides are presented in the thymus, so that tolerance does not develop.

Certain mouse strains that do not express mRNA for interphotoreceptor retinoid-binding protein (IRBP) in the thymus are more susceptible to developing IRBP-induced experimental autoimmune uveitis (EAU) than mice expressing this antigen in the thymus [17].

In humans with uveitis, no antigens proven to cause or initiate the pathogenesis of the disease have been identified so far. Thus, it is more accurate to classify uveitis as an immune-mediated rather than an autoimmune disease. For a more detailed review of HLA and the mechanism of disease see Davey and Rosenbaum [14]. Nevertheless, some candidate auto-antigens have been identified in uveitis. For example, antibodies to β-B1 crystalline are elevated in patients with uveitis compared with healthy controls and this antigen has been shown to be expressed in retinal and uveal tissues [67]. Other candidate auto-antigens that may play a role in human uveitis are retinal proteins used to induce uveitis in the animal model EAU [15].

7.2.3 HLA-Associated Uveitis

Table 7.2 gives an overview of types of non-infectious uveitis that have been shown to be associated with HLA genes. We will describe only some of them in detail here.

7.2.3.1 Acute Anterior Uveitis

HLA-B27-associated AAU is the ideal form of uveitis for genetic studies because of its clearly defined phenotype: unilateral, anterior uveitis with a sudden onset of less than 3 months' duration and recurrence. This disease has been the subject of a recent genome-wide scan on 76 affected sibling pairs with AAU [43]. The results were compared with an analysis of 245 affected sibling pairs with ankylosing spondylitis, a form of arthritis frequently associated with AAU [74]. Again, strong linkage to the MHC region on chromosome 6 was observed, certainly due to the association with HLA-B27. Two other genetic regions showed significant linkage to AAU. One area at chromosome 1q25-31 coincides with a previously identified locus for ankylosing spondylitis [34]. The second area at the short arm of chromosome 9 (9p21-24) seems to be unique for the uveitis phenotype as it did not show any significant result on the ankylosing spondylitis scan [43, 74].

In addition to the MHC class I gene HLA-B27 [9], there have been reports of an association between MHC class II genes and AAU. In 1995 an association with HLA-DR8 (i.e., HLA-DRB1*08) and AAU, but not ankylosing spondylitis, was reported in a group of 42 Japanese patients [48]. This is similar to the results of a haplotype analysis on a north American Caucasian cohort in which an association with the haplotype HLA-DRB1*0801/DQA1*0401/DQB1*0402 was found in 72 unrelated patients with AAU [39].

7.2.3.2 Chronic Anterior Uveitis in Juvenile-Onset Arthritis

Juvenile idiopathic arthritis (JIA), formerly known as juvenile rheumatoid arthritis (JRA) is an idiopathic inflammatory form of arthritis occurring in children. This disease encompasses several clinical subgroups. The subgroup of early onset pauciarticular (EOPA) JIA, which mainly affects girls who are ANA+, has the highest prevalence of chronic bilateral anterior uveitis (CAU). An association with DRw5 (new nomenclature DR5) [20] and also with a split allele of DR5, DRB1*1104 [44] has been reported for north American cohorts containing mainly Caucasians of northern European ancestry. A study in southern Germany including 200 children confirmed this association of DRB1*1104 with EOPA-JIA, but did not find a significant difference between allele frequencies in children with or without CAU [23]. JIA has also been known to be associated with HLA-DR8, but even more recent studies have not examined this association with regard to the subset of JIA patients with uveitis [60, 65, 71].

7.2.3.3 Pars Planitis

Pars planitis is an intermediate uveitis, requiring the presence of pars plana exudates, or "snowbanks" to establish the diagnosis. Three independent studies in north American patients found an association between pars planitis and HLA-DR2

Table 7.2 Case-control studies demonstrating human leukocyte antigen (HLA) associations with non-infectious uveitis. *AAU* acute anterior uveitis, *AS* ankylosing spondylitis, *BSRC* birdshot retinochoroidopathy, *CAU* chronic anterior uveitis, *EOPA-JIA* early onset pauciarticular juvenile idiopathic arthritis, *TINU* tubulointerstitial nephritis and uveitis syndrome, *VKH* Vogt-Koyanagi-Harada's disease

Disease	HLA association	Patients	Controls	Comment	Reference
AAU	B27	50	50	55% of patients vs. 4% control subjects	[9]
	DR8	20	22	65% of patients with AAU vs. 4.5% of patients with AS only	[48]
	DRB1*0801/DQA1*0401/DQB1*0402	72	250	12.7% allele frequency in patients vs. 2.6% in controls (p=0.0001)	[39]
Behçet's disease	Bw51 (=B51)	184	130	61% of patients vs. 31% of controls	[52]
BSRC	A29	20	418	80% of patients vs. 7.4% of controls	[49]
	A29.2	33	27[a]	100% of BSCR patients, but only 11% of A29+ controls	[69]
CAU in JIA	DRw5 (=DR5=DRB1)	24	84	71% of patients with CAU and EOPA-JIA vs. 19% of controls	[20]
	DRB1*1104	164	218	31% of children with CAU vs. 12% of children with EOPA-JIA alone	[44]
Pars planitis	DR2	40	431	68% of patients vs. 28% of controls	[40]
	DR15 (=DRB1*1501)	28	50	64.3 % vs. 20%	[53]
		32	1983	46% vs. 23.6%	[57]
	DRB1*0802/DQA1*0401/DQB1*0402	79	204	Higher frequency in patients (p=0.00002; OR 2.8)	[3]
TINU	DQA1*01/DQB1*05/DRB1*01	18	N/A[b]	72% frequency in patients	[36]
VKH	DR4	72	130	88% of Japanese patients vs. 32% of controls	[51]
		48	100	65% of Mexican Mestizo patients vs. 25% of controls	[4]
	DRB1*0405	40	70	95% of Japanese patients vs. 26% of controls	[63]
		37	230	37% of Brazilian patients vs. 5% of controls	[21]

[a] Healthy control individuals were all HLA-A29 positive

[b] Not applicable because control data were from published allele frequencies

[40], more precisely with its subtype HLA-DR15 [53, 57]. A subgroup of pars planitis patients will develop multiple sclerosis. Interestingly, this HLA association has also been repeatedly described for multiple sclerosis [13, 24]. However, it has not been confirmed in a Scottish [22] or Mexican Mestizo cohort [3] of pars planitis patients. The latter found an increased frequency of the DRB1*0802/DQA1*0401/DQB1*0402 haplotype in their patient group [3].

7.2.3.4 Birdshot Retinochoroidopathy

The strongest HLA disease association ever reported is for HLA-A29 and BSRC [49], especially the HLA-A*2902 allele [69]. BSRC is a rare form of posterior uveitis that belongs to the broad group of white dot syndromes. Of interest is the suggestion of retinal soluble antigen (S-Ag) as the involved auto-antigen in that disease [49]. S-Ag is a major component of rod outer segments and can induce EAU in rodents and primates, mimicking many aspects of the human disease [15]. Bovine or human S-Ag when given in vitro to lymphocytes from birdshot patients induced proliferation, but not in lymphocytes from healthy controls [49]. Another report showed effective binding of two peptides derived from the carboxyl-terminal sequence of human S-Ag to HLA-A29 in vitro [8]. Furthermore, transgenic mice that overexpress HLA-A29 develop spontaneous retinopathy [68].

Despite the variety of forms of uveitis that are now known to be associated with HLA alleles, HLA typing only rarely contributes to clinical diagnosis. Even considering the strongest associations, HLA-B27 and HLA-A29, the clinician needs to recognize that the majority of individuals with these genotypes do not develop uveitis. HLA typing can be useful in a selected patient for whom a diagnosis such as ankylosing spondylitis or BSRC is being strongly considered, but has not been definitively established. Typing is not clinically indicated if the diagnosis is already established or if the diagnosis seems unlikely.

Summary for the Clinician

- Patients with acute anterior uveitis have about a 50% likelihood of being HLA-B27-positive.
- A patient with HLA-B27-associated anterior uveitis often has associated sacroiliitis that has not been previously diagnosed.
- HLA-B27-associated anterior uveitis is the most common cause of hypopyon-associated uveitis in North America.

7.3 Uveitis Associations with Non-HLA Genes

Even though associations between uveitis and particular HLA genes are among the strongest genetic risk factors known, there must be other genetic contributions to immune-mediated forms of uveitis. Evidence for this comes from various observations; the most convincing is that the majority of people with a "risk" HLA genotype do not develop the disease. This is well illustrated by HLA-B27, in which over 90% of Caucasian patients with ankylosing spondylitis are HLA-B27-positive. However, HLA-B27 is found in approximately 10% of all Caucasians, with only a small minority developing the disease.

The major tasks of our immune system include immune recognition and immune regulation. Immune recognition is critical for distinguishing and eliminating pathogens, whereas immune regulation plays a pivotal role in controlling inflammation and avoiding the detrimental effect of excessive immune response. Disruption of these important immune functions will lead to a number of complex autoimmune/inflammatory diseases such as Crohn's disease, Blau syndrome, ankylosing spondylitis, JIA, and sarcoidosis. Many of these diseases have clinical manifestations of uveitis.

It is accepted that uveitis and uveitis-associated diseases are complex, polygenic diseases. In other words, there is no obvious pattern of inheritance. Furthermore, within one individual more than one gene will contribute to genetic risk, and within a population various genes will be identified that contribute to genetic risk. This section focuses on genes other than HLA that are good candidate genes for inflammatory disease based on their roles in immune function. Genes that code for cytokines affect susceptibility to several immune-mediated diseases.

7.3.1 Tumor Necrosis Factor Alpha

Tumor necrosis factor alpha (TNF-α) is known to the clinician through the use of TNF inhibitors like infliximab in the treatment of several inflammatory diseases, including uveitis. The TNF-α gene is localized on the short arm of chro-

mosome 6 between the MHC class II and class I genes. Sometimes this area is also referred to as an MHC class III gene cluster. The close proximity of TNF-α to the HLA genes makes it difficult to determine whether an association with disease is real or due to linkage disequilibrium. Some polymorphisms on the TNF-α promoter region (i.e., position -308A) have been said to increase TNF-α production in vitro [37], whereas others (i.e., at position -863A) have been reported to be associated with reduced TNF-α levels in vitro and in vivo [64]. This last polymorphism has been found with higher frequency in a certain subtype of Crohn's disease [35]. Patients with uveitis have higher concentrations of TNF-α in aqueous humor [61], and this is even more pronounced in HLA-B27 positive patients compared with HLA-B27-negative patients with uveitis [54].

A significant difference in the frequency of the TNF-857T allele was found in patients with AAU in a study of 98 white patients in the United Kingdom [31]. The majority of the patients were also HLA-B27 positive. Linkage disequilibrium between the two genes could not be excluded in this study.

A study of 133 Caucasian Behçet's patients from the United Kingdom found that the -1031C polymorphism was strongly associated with disease (55.6% in Behçet's patients vs. 35.0% in controls) [1]. This was independent of the HLA-B alleles. No disease association with the above-mentioned other TNF promoter polymorphisms could be shown [1]. Further studies will be required to determine the functional effects of this polymorphism.

7.3.2 Interferons

Among the approximately 30 cytokines, interferons (IFNs) have received attention because of their diverse effects, influencing both innate and adaptive immune responses [6]. There have been reports of increased IFN levels in patients with systemic lupus erythematosus correlating with disease activity [73]. Based on their amino acid composition, IFNs have been classified into type I (IFN-α and -β as the most immunologically relevant) and type II (IFN-γ). A new class of IFN, IFN-λ, has been identified recently [29].

The human genome contains only one gene (IFNB) encoding IFN-β, but at least 13 genes (IFNAs) coding for IFN-α. The IFN-α proteins are highly homologous and bind to the same receptor. These genes are localized on the short arm of chromosome 9. IFN-α and -β are expressed by many cell types as well as plasmacytoid dendritic cells in response to infection by diverse viruses. Subsequently, they induce the synthesis of several host cell proteins that contribute to the inhibition of viral replication. As such, IFN-α has been used as an effective therapeutic agent against hepatitis C viral infection. Even though there have been reports concerning the development of autoimmune disease in patients undergoing IFN-α treatment [56], it is also promising as a treatment of another inflammatory disease, Behçet's disease, especially for the associated posterior or panuveitis. Results of an open, uncontrolled, 4-center trial including 50 patients have been published [30].

Linkage analysis of a cohort of patients with multiple sclerosis suggested that IFNA genes, but not the IFNB gene, might play a role in the genetic predisposition to this disease [18, 47]. No data for uveitis and specific IFNA genes exist to date, but a study of a cohort of 102 Japanese patients with sarcoidosis included a significant number of patients with uveitis as they were recruited mainly from the Department of Ophthalmology [2]. Unfortunately for those interested in uveitis, the data are only presented for the whole group of sarcoidosis patients. Still, presumably a large number of patients in this cohort had sarcoidosis-associated uveitis. In this group a variant allele of IFNA (made up of two SNPs, IFNA10, 60A and IFNA17, 551G) was found to be significantly associated with sarcoidosis compared with the healthy control population. This haplotype was also associated with a higher production of IFN-α in vitro. Interestingly, there have been cases reported in which patients undergoing treatment with IFN-α developed sarcoidosis [12, 56].

The IFN-γ gene is localized on the long arm of chromosome 12. IFN-γ is produced by activated T cells, natural killer (NK) cells, macrophages, and dendritic cells. IFN-γ produced through these various cell types and stimuli has been shown to act at various levels of the immune response. Higher levels of production of IFN-γ

in vitro have been observed in patients with a polymorphism in the promoter part of the IFN-γ gene [55]. A recent study of 125 British patients with idiopathic intermediate uveitis found an association with this same polymorphism (A874T) of the IFN-γ gene, but not with two other polymorphisms in the IL-10 gene [66]. However, they did not look at IFNA genes.

7.3.3 CARD15/NOD2

The recent discovery of toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD) proteins has greatly advanced our understanding of host-microbial interaction, and further highlighted the importance of the innate immune reaction in many physiological as well as pathological processes [5]. TLRs are a family of transmembrane proteins primarily responsible for recognizing an array of unique molecules on pathogens that are not found in higher eukaryotes. These foreign molecules include lipopolysaccharide (LPS), a glycolipid endotoxin of Gram-negative bacteria, peptidoglycan (PGN), a component of bacterial wall, and unmethylated CpG dinucleotide in bacterial DNA. A family of proteins that contain NODs may function as intracellular counterparts of TLRs. These related proteins also have the ability to respond directly to bacterial cell wall components and thus have the potential to play a role in the response to invasive or intracellular bacteria.

One member of the NOD protein family, NOD2 (officially called CARD15) was recently determined to be associated with a common inflammatory condition, Crohn's disease [25, 26, 50]. This was followed by the discovery that a source of mutation in a rare, autosomal dominant form of uveitis, Blau syndrome was also the same gene, CARD15 [45]. Blau syndrome (also known as familial juvenile systemic granulomatosis or Jabs disease) is characterized by granulomatous uveitis, joint inflammation, and granulomatous skin inflammation, which presents in childhood [7, 27, 46]. The clinical eye findings include granulomatous panuveitis with vision-threatening multifocal choroiditis [33]. Crohn's disease is characterized by Th1-type recurrent gastrointestinal inflammation. Even though less than 10% of Crohn's disease patients will develop uveitis as an extra-gastrointestinal manifestation, the uveitis phenotype is distinct [38].

CARD15, located on chromosome 16, is polymorphic with over 30 genetic mutations identified, although natural variation in this gene is highly dependent on racial background. To take the emerging understanding of Blau syndrome one step further, patients with a clinical picture virtually indistinguishable from Blau syndrome except with no family history have been found to have de novo mutations in CARD15 [28, 59]. These patients are frequently given a diagnosis of early-onset sarcoidosis because their symptoms include granulomatous inflammation that begins in early childhood [58]. However, unlike adult sarcoidosis, these patients do not have lung involvement.

How might mutations in the same gene cause two very distinct clinical pathologies (i.e., Blau syndrome vs. Crohn's disease)? At least part of the answer is evident in the particular, non-overlapping mutations found in each disease. The mutations associated with Crohn's disease are all in (or near) the functional domain of the protein directly involved in detecting the bacterial cell wall component, muramyl dipeptide (MDP) [19]. These mutations likely impact the ability of the cell to respond to (and possibly clear) intracellular bacteria. In contrast, all of the mutations discovered to cause Blau syndrome are in the NOD functional motif, which is involved in protein–protein interactions necessary for activation of the cell via the signaling pathway dependent on nuclear factor-kappa B (NF-κB) activation. It is postulated that alteration of the tertiary structure of the NOD2 protein caused by these mutations results in increased NF-κB activity, promoting inflammation.

CARD15 is found to be constitutively or inducibly expressed in myeloid cells such as monocytes, macrophages, and dendritic cells. More recently, CARD15 expression has been documented in intestinal epithelial cells, including Paneth cells, and in osteoblasts. With regard to ocular tissue, vascular endothelial cells express CARD15 (Fig. 7.1) [41]. Therefore, NOD2 seems to be more ubiquitously expressed than initially thought. Hence, it is feasible to postulate that CARD15 participates in the host defense of ocu-

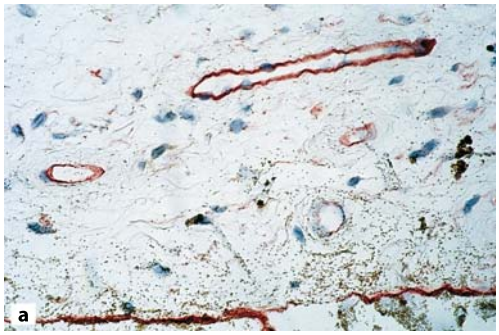

Fig. 7.1 Human iris tissue (paraffin-embedded, 5-μm sections) subjected to immunohistology with polyclonal rabbit-anti-human CARD15 antiserum (Cayman Chemical, Ann Arbor, MI, USA) indicates the presence of CARD15 in vascular endothelium of some iris vessels (**a**, *red staining*). Positive staining was also observed on the posterior lining of the iris. Negative control immunohistology was performed with normal rabbit serum (**b**)

lar tissue against microbial invasion and infection. There are many reports that bacteria such as *Salmonella*, *Shigella*, *Campylobacter*, and *Chlamydia* may trigger HLA-B27-related disease, which includes uveal inflammation.

Given that the mutations in CARD15 cause a form of uveitis (Blau syndrome), that distinct mutations in CARD15 increase risk for Crohn's disease and that a subset of Crohn's disease patients will develop uveitis, it was thought that mutations in this gene could be associated with other forms of uveitis as well. However, in a small study of patients with Crohn's disease comparing patients with uveitis with patients with only intestinal disease, no CARD15 mutations have been found [16]. Neither were CARD15 mutations found to be increased in a cohort of patients with sarcoidosis-associated uveitis [42]. Thus, the role of CARD15 in uveitis has yet to be fully deciphered.

7.4 Concluding Remarks

As an inflammatory "disease," it is no surprise that uveitis bears candidate genes involved in the immune response to environmental stimuli. This is shared among all forms of inflammatory disease. Thus, pivotal immune function genes such as the MHC, TNF-α, IFNs, and CARD15 are found to be associated with the disease. The challenge will be determining which of these genes render an individual susceptible to inflammatory disease in general, and which might be specifically influencing predisposition to uveal inflammation. This will not be an easy task. However, there is no doubt that uveitis exists as a continuum of clinical presentations, with a few well-defined distinct phenotypes identified by the ophthalmologic community. It is these distinct phenotypes of uveal inflammation that will be most useful for future genetic studies.

Association studies can be a powerful means by which to test the genetic risk that particular candidate genes confer on a disease phenotype. This approach dictates that candidate genes be first identified. The utility of the genome-wide scan approach is that genetic regions of linkage can be determined without previous knowledge of candidate genes. The fine mapping of regions identified by the AAU study, and hopefully future genome-wide scans of other uveitis phenotypes, will provide new insights into the genetic determinants that influence uveitis.

Summary for the Clinician

- Despite its current limitations for establishing a diagnosis, genetics offers the potential for insights into disease pathogenesis and ultimately for novel therapies.

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Chapter 8

Cataract Surgery in Patients with Uveitis

8

Jorge L. Alio, Javier A. Montero

Core Messages

- Control of the preoperative inflammatory state is mandatory. A minimum of 3 months of quiescence is necessary before surgery is indicated. Topical, periocular, and systemic steroids and systemic immune suppressants can be used for this purpose. Etiologic diagnostic of the uveitis will be useful if specific treatment can be performed.
- Delicate surgery is the best anti-inflammatory.
- Maximum mydriasis and the use of a dispersive viscoelastic will be of great help in reducing iris trauma.
- A corneal incision of 3 mm is recommended. Careful cortical material removal and posterior capsular polishing are important steps to prevent or delay posterior capsular opacification frequently found in these patients.
- Continuous circular capsulorhexis is less traumatic for the zonule than any other procedure. It should be as regular as possible to overlap the IOL optics; this will decrease posterior capsular opacification as well.
- An anterior vitrectomy can be performed if vitreous opacities are present at the time of surgery.
- Intraocular lenses can be implanted in the majority of the uveitic patients. Until further studies have been performed in this area, implantation of a foldable hydrophobic acrylic lens is the best approach.
- The postoperative control of inflammation is extremely important in these patients, since the associated complications may be severe.
- Chronic juvenile arthritis tends to exacerbate the uveitis after cataract surgery. In these patients, a careful decision regarding intraocular lens implantation must be made.
- In children, the surgery will be of no value unless intensive treatment for amblyopia is performed immediately after the surgery.

8.1 Introduction

Cataract is a common complication among uveitic patients, being more frequent in cases of chronic anterior and intermediate uveitis, reaching 83% among patients with uveitis related to juvenile arthritis (JRA) [49], and up to 50% in patients with pars planitis and Fuchs's heterochromic cyclitis [6, 53]. Cataracts appear not only more frequently among these patients, but also at an earlier age than among the general population. The development of cataracts seems to be associated with the localization, severity, and chronicity of intraocular inflammation as well as with the frequent use of steroid drops.

Chronic inflammation may appear associated with irideal synechiae, iris atrophy, neovascularization, band keratopathy, and secondary glaucoma. Lens defects seem to be most frequently associated with posterior synechiae as localized areas of necrosis in the anterior part of the lens. Severe anterior uveitis may be associated with anterior subcapsular opacities, while chronic cyclitis and the use of topical and systemic steroids may be linked to posterior subcapsular cataracts (Fig. 8.1).

The presence of toxic debris and oxygen free radicals from inflammation, and local ischemia induced by synechiae may damage lens fibers and epithelial cells, leading to the occurrence of opacities. The mechanism of posterior subcapsular cataract induced by steroid use is not well known, although it has been suggested to be related to abnormal cellular metabolism induced by the electrolytic misbalance.

Cataract surgery in uveitic eyes remains a challenge to ophthalmologists with its intraoperative risks and the uncertainty of the postoperative process. Until recently, there was no agreement concerning the timing, preoperative and postoperative care for uveitic eyes undergoing cataract surgery.

There are some facts concerning this surgery that make the surgical approach different from that for other forms of cataracts, such as its presentation in children and young adults and its association with uncontrolled inflammation, hypotony, and phthisis bulbi, among others.

Fig. 8.1 Posterior subcapsular opacification in a patient with chronic topical steroid therapy

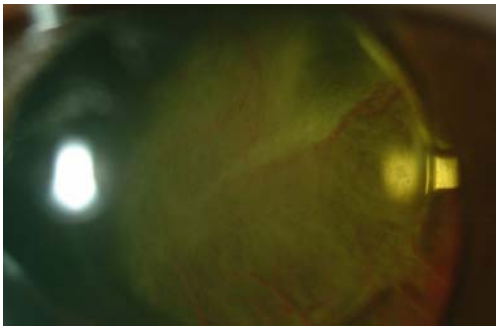

Fig. 8.2 Posterior capsular opacification and neovascularization after chronic inflammation in a uveitic patient with cataract surgery

Several misconceptions have been formed about surgery in uveitic eyes, such as a homogeneously poor outcome of cataract surgery when good visual results have been demonstrated in patients with Fuchs's cyclitis and in patients with good medical control. The appearance of inflammation after surgery can often be controlled with medical therapy, and the incidence of phthisis bulbi and hypotony are more related to the type of uveitis than to the surgical procedure performed. Uveitis is no longer an absolute contraindication for the implantation of intraocular lenses.

However, it is not uncommon for complications to arise during or after surgery, such as the occurrence of band keratopathy, glaucoma, early capsular bag opacification or lens deposits, synechiae, iris atrophy or neovascularization (Fig. 8.2), cystoid macular edema (CME), epiretinal membranes, vitreous hemorrhages, retinal detachment, and even phthisis bulbi.

8.2 Surgical Indication

The main indications for cataract surgery in uveitic patients are: visually significant cataracts with good prospects for improvement of visual acuity; removal of cataracts that impair fundus assessment in patients with suspected fundus pathology or in whom a posterior segment surgical procedure is planned; and removal of a protein leaking lens in an eye with phacogenic uveitis.

Mild cataractous changes in the early course of uveitis have little effect on visual acuity. When

progressing they may reduce visual acuity to the point where cataract extraction is necessary. Cataract is not a reversible disease so we can anticipate a subsequent visual decrease in the following years if no surgery is performed. Potential acuity measurement and laser interferometry are the most reliable procedures in order to estimate postoperative visual acuity in patients in whom standard acuity scales are not sufficient and the health of the macula is unclear. Occasionally, 20/20 visual acuity may be present in patients complaining of blurred vision, generally associated with posterior subcapsular opacities. In these cases, the patient must receive a careful explanation on the nonreversibility of the cataract and the probable increase in symptoms over time, as well as the expected benefits and possible risks of surgery.

8.3 Patient Preparation

A customized approach must be established for each patient, taking into consideration the etiology of the uveitis and the cause of vision loss, as well as the purpose of surgery, whether it is to achieve vision improvement and/or to allow visualization of ocular structures. In order to avoid unreal expectations in the patient, it has to be established whether cataract is the main cause of vision loss, or whether it is caused by optic nerve atrophy, vitreous opacification or retinal damage. Anterior and posterior segment biomicroscopy as well as potential visual acuity measurement have to be performed. Occasionally, B-scan examination and campimetry may be useful [56].

A systematic approach to establishing the diagnosis begins with a comprehensive ocular and systemic history, and an extensive review of medical systems, which gives the possibility of creating a differential diagnosis. An undiagnosed infection by herpes simplex virus in a patient with recurrent anterior uveitis and lack of specific therapy will increase the risk of a poor outcome. Similarly, the surgeon must consider whether associated surgery, such as filtering surgery or vitrectomy, should be performed associated with phacoemulsification.

Summary for the Clinician

- Indications for cataract surgery: visually significant cataracts with good prospects of improvement of visual acuity; cataracts that impair fundus assessment; phacogenic uveitis.
- Preoperative procedure: determine the etiology of the uveitis and the cause of vision loss; establish the goal of the surgery, determine potential visual acuity; perform anterior and posterior segment biomicroscopy; occasionally, B-scan and campimetry.

8.4 Control of Inflammation

It is of utmost importance to achieve proper control of intraocular inflammation before, during, and after surgery. Surgery should be performed when inflammation is as low as possible. Phacogenic uveitis is the only case in which the complete abolition of all inflammatory signs is not necessary before surgery. The definition of "controlled" inflammation is a patient with no cells and up to one (+) flare cell in the anterior chamber; and cell (+/-), no active retinal inflammation and no CME in the posterior segment. It is very important that the surgery is performed in an "undisturbed" eye when the inflammatory reaction has been controlled for at least three months prior to surgery [20].

Topical or periocular steroids can be used to reduce intraocular inflammation for at least 3 months before surgery. The use of systemic steroids is seldom necessary and should be reserved for the severest cases. In severe forms of inflammation, anti-inflammatory drugs may be advisable. Topical nonsteroidal anti-inflammatory drugs (NSAIDs) may be beneficial to reduce the risk of postoperative CME via the inhibition of prostaglandin synthesis. This mechanism may be of importance, especially in eyes where the iris is manipulated, as is often the case in eyes with uveitis developing extensive posterior synechiae. A specific antibiotic therapy should be performed when an infectious etiology such as tuberculosis or syphilis is suspected.

The single most important sign of inflammation is the presence or absence of inflammatory cells in the anterior chamber or vitreous. Aqueous flare in anterior chronic uveitis simply denotes vascular incompetence of the iris and ciliary body, a consequence of vascular damage from recurrent uveitis. Flare should not generally be used as a guidepost for inflammatory quiescence. The activity of inflammation behind the lens may be difficult to assess in patients with dense cataracts. The presence of vitreous cells does not necessarily signify active disease because inflammatory cells clear more slowly than in the anterior chamber. Vitreous inflammation did not appear to be significantly associated with the presence of cataract development in patients with Vogt–Koyanagi–Harada (VKH) syndrome [43].

The purpose of the therapy is to reduce cellular activity in the anterior chamber to less than one (+), with a minimal vitreous infiltration by the time of surgery. Surgery must be delayed when more than 10 cells per high magnification field are detected on the anterior chamber biomicroscopy; however, in some eyes it may be impossible to eliminate the inflammation. In those cases where the degree of inflammation behind the lens cannot be assessed, a prophylactic therapy should be prescribed for a few days before surgery and the patients managed as if an active inflammation was present.

Uveitic patients may be divided into two groups: complicated cases and uncomplicated cases according to the guidelines of the Intraocular Inflammation Society (IOIS). Complicated patients will be those in whom systemic or periocular therapy is necessary to maintain uveitis in a quiescent state or those in whom surgery is expected to be difficult for the surgeon. Uncomplicated patients will be those in whom the uveitis is controlled with topical steroids and an operative routine is anticipated.

Two days to 1 week before surgery the patient should receive a topical steroid (prednisolone acetate 1%), one drop eight times daily, and a topical NSAID such as flurbiprofen four times a day. All patients classified as "complicated" cases also receive 1 mg/kg/day of oral prednisone during the 2 weeks prior to surgery. Systemic steroids in children should not go beyond 3 months due to their possible side effects on growth. The use of systemic NSAIDs is controversial as they may increase the risk of bleeding.

Intraocular inflammation may be associated with high, normal or low intraocular pressure (IOP), depending on the severity of the inflammation and deposits in the trabecular meshwork and the damage to the ciliary body. However, high IOP is often associated with the chronic use of topical steroids.

Uveitic high IOP is usually associated with inflammatory damage to the trabecular meshwork and with peripheral anterior synechiae. Those eyes with posterior synechiae may eventually develop a pupillary seclusion needing a Nd-YAG laser iridotomy before cataract surgery.

Increased IOP may be associated with any kind of intraocular inflammation, and appear as chronic, acute or transient. A specific entity associating high IOP and acute anterior uveitis episodes may appear in the form of Posner-Schlossman iridocyclitis. The term uveitic glaucoma describes high IOP associated with uveitis with optic nerve damage and visual field defects. High IOP during a short period without damage to the optic nerve can be termed uveitis-related hypertension.

The appropriate management of high IOP must include the control of the inflammation as well as the reduction of IOP. Early anti-inflammatory therapy combined with mydriatic and cycloplegic drugs should be used to reduce the appearance of posterior and anterior synechiae, pupillary membranes, pupillary seclusion, and damage to the trabecular meshwork. Proper control of the IOP is recommended 2–3 weeks prior to surgery. However, medical and surgical therapy may be necessary if high IOP cannot be controlled by anti-inflammatory therapy alone. The use of cholinergic drugs should be avoided in these patients as they alter the blood–aqueous barrier (BAB) and tend to increase synechiae formation. The use of prostaglandin analogs has been associated with a higher risk of CME [44].

Low IOP may occasionally appear after severe uveitis episodes. It is generally caused by the formation of cyclitic membranes in the posterior chamber detaching the ciliary body. Occasion-

ally, it may originate from the inflammatory destruction of the ciliary body.

The appearance of a fibrinoid reaction during or immediately after surgery may be dealt with by the injection of 500–700 units of streptokinase or recombinant tissue plasminogen activator (rt-PA), 10–25 μg, in the anterior chamber [18].

8.5 Surgical Procedure

Cataract surgery in uveitic eyes that have been inactive for several months can be performed similarly to that in non-uveitic eyes, through a small corneal incision or via a scleral tunnel and phacoemulsification followed by the implantation of a foldable lens [38]. Phacoemulsification is considered the procedure of choice in uveitic cataract surgery showing better visual outcome and lower incidence of capsular opacification and postoperative inflammation than extracapsular cataract extraction. This has been attributed to smaller incision size, shorter surgical duration, and reduced surgical trauma [50]. The best anti-inflammatory is good, atraumatic surgery. Young patients and patients with high doses of steroids are at an advantage with this technique. Microincision surgery (MICS) is a promising technique for approaching these difficult cases.

The most pro-inflammatory step in extracapsular surgery seems to be nuclear expression causing iris trauma [12]. The size of the incision is significantly related to the degree of inflammation.

Intracapsular surgery is reserved for those cases in which an important phaco-induced inflammatory reaction has appeared after surgery in the fellow eye.

General anesthesia is not necessary (although many patients are young and surgery under general anesthesia is compulsory), and locoregional anesthesia by retrobulbar or peribulbar block is preferred. Topical anesthesia is not contraindicated, but it may be risky and painful in cases in which extensive iris manipulation is expected.

Clear cornea incision has some advantages over scleral tunnels such as the absence of postoperative hyphemas, filtering blebs, and need for cautery, among others. This may be the best approach if no lens or a foldable lens is implanted; where the implantation of a rigid PMMA lens is planned a limbal approach with a short scleral tunnel is performed.

Good pupillary dilation is commonly difficult to achieve in uveitic eyes. Long-standing uveitis is often associated with extensive posterior synechiae and atrophy of the iris sphincter muscle. Viscoelastic substances are routinely used to release adhesions and aid mydriasis. Combinations of hyaluronic acid and chondroitin sulfate (Viscoat) are preferred, and high viscosity materials can be used (Healon GV, Amvisc plus). Stronger posterior irideal synechiae can be eliminated using an iris spatula with the support of a high density viscoelastic material. In more severe cases, further mydriasis can be obtained using four De Juan hooks placed at each quadrant through four small corneal incisions, or by stretching maneuvers with iris hooks under viscoelastic protection of the endothelium. In those cases in which the adherence cannot be removed or extends beyond the iris sphincter, two iridotomies can be performed at the 3 and 9 o'clock meridians. In extreme cases when the previous procedures are not successful, a superior sector iridectomy can be performed. There are some particular situations, in which a prophylactic peripheral iridectomy may be recommended, as in cases of uveitis with a high tendency toward synechiae formation. Patients in whom a vitrectomy has been performed with removal of the posterior capsule do not require an iridectomy.

After dilation a capsulorhexis should be attempted. Continuous circular capsulorhexis (CCC) also seems to be less disruptive to the BAB than can opener capsulotomy, inducing less zonular stress and ensuring in-the-bag implantation isolating the IOL from the uveal vascular tissue. Cases of anterior capsular rim tears have been reported to have a higher inflammatory response in the form of foreign body reaction on the IOL surface. Sometimes it is necessary to use a capsular stain such as trypan blue (Vision Blue) to visualize the anterior capsule. CCC may be difficult to perform due to the presence of secondary fibrosis of the anterior lenticular capsule. In these cases the central part of the capsule may be cut through using intraocular Vannas scissors,

or a circular capsulotomy can be obtained using a vitrectomy probe. Since many uveitic patients develop cataracts at an earlier age the capsule is often more elastic than in older patients. This higher elasticity increases the risk of capsular rim tears. The apparition of hemorrhages during synechiolysis or capsulotomy can be dealt with by the injection of high-density viscoelastic and by raising the IOP.

The phacoemulsification procedure is accomplished by the most suitable technique for each case (Table 8.1), with chop techniques if the nucleus is very hard. The most common type of cataract is nuclear, although the lens nucleus is often relatively soft and the phacoemulsification can be easily performed [1]. Thorough cleaning of the cortex is needed to reduce the risk of postoperative inflammation and posterior capsule opacification. Cortical clean-up and aspiration of the posterior surface of the anterior capsule with low vacuum are mandatory in all cases. Lens epithelial cells contact with IOL will cause the cells to undergo a fibrous metaplasia and disrupt the BAB. Bimanual techniques give excellent results in anterior cortical cleaning.

Table 8.1 Phaco parameters

SOFT CATARACTS	
Power	30% pulsed
Vacuum	400 mmHg
Aspiration	20 cc/min
Burst	30 ms
HARD CATARACTS	
Power	100%
Vacuum	65 mmHg
Aspiration	20 cc/min
Burst	30 ms
FRAGMENT REMOVAL	
Power	65%
Vacuum	400 mmHg
Aspiration	20 cc/min
Burst	30 ms
IRRIGATION/ASPIRATION	
Aspiration	30 cc/min
Vacuum	400 mmHg

Summary for the Clinician

- No inflammation can be allowed in the 3 months prior to surgery: cellular activity in the anterior chamber should be less than one (+) with minimal vitreous infiltration.
- Control of inflammation should be specific if possible, or if nonspecific either topical, periocular and with or without systemic steroids, or with topical nonsteroidal anti-inflammatory drugs.
- Inflammation should be controlled 1 week before surgery.
- In all cases topical prednisolone acetate (1%; one drop eight times daily, or topic NSAID (flurbiprofen) qid should be administered
- In complicated cases: add 1 mg/kg/day of oral prednisone for 2 weeks prior to surgery and immunosuppressive agents may be needed.
- To control high IOP use mydriatics, topical steroids, beta blockers. Avoid prostaglandin analogs and cholinergics.
- In the case of anterior chamber fibrinoid reaction administer 500–700 units of streptokinase, 10–25 μg of recombinant tissue plasminogen activator, or heparin (5,000 IU; 0.2 ml in 500 ml of Ringer lactate infusion

Micro incision surgery allows cataract surgery to be performed through an incision of 1.4 mm or less. A small incision correlates directly with a lower inflammatory rate postoperatively, making this technique less invasive and probably the best way to approach these patients. It seems likely that when the materials and biocompatibility of new IOLs have been improved, this will be the preferred technique. Two side port incisions of 1.2 mm are performed at 90°. After phacoemulsi-

fication a foldable, one piece hydrophobic acrylic IOL is implanted into the capsular bag. Suturing the incision is advocated.

Intraocular dexamethasone phosphate (400 μg) may be instilled into the anterior chamber when the wound is closed [19]. Alternatively, triamcinolone acetate may be injected into the vitreous chamber by the end of combined cataract and posterior segment surgery [3]. Additionally, we use heparin 5,000 IU, 0.2 ml in 500 ml of Ringer lactate infusion to reduce the apparition of fibrin in the anterior chamber (Table 8.1).

Summary for the Clinician

- Use peribulbar or retrobulbar anesthesia.
- Clear cornea phacoemulsification is preferred.
- Use high-density viscoelastics.
- Remove posterior irideal synechiae. De Juan hooks and/or stretching maneuvers may be of help to achieve pupillary dilation.
- Continuous capsulorhexis is less proinflammatory than other capsulotomies.
- Phacoemulsification can be performed as is most suitable for each case: divide and conquer, chopping procedures, etc.
- A thorough cleaning of the cortex is mandatory.
- Dexamethasone phosphate (400 μg) may be instilled into the anterior chamber at the end of surgery.

8.5.1 Choice of Intraocular Lens

The decision whether to implant an IOL in uveitic eyes remains controversial. The issues surrounding intraocular lens (IOL) placement in uveitic eyes after cataract extraction remain a key concern in management of the uveitic patient. Many features unique to a uveitic eye must be considered, such as different types of uveitis, preoperative inflammation and treatment, postoperative inflammation and specific complications. With newer techniques and modern posterior chamber lenses, IOLs are being implanted with fewer complications, especially when the lens is placed in the capsular bag. Many questions remain unanswered regarding the uveitic eye in conjunction with IOL biocompatibility and inflammation. Valuable information can be gained through more experience with IOL use in these eyes.

Presently, it seems that stringent perioperative and postoperative control of inflammation, may help patients with uveitis maintaining high visual acuity over long-term follow-up [52]. There has been concern that inflammation associated with IOL implantation would increase surgical inflammation in compromised eyes. Chronic uveitis has long been considered a relative contraindication for the implantation of IOLs. The major risk factors associated with poor outcome after IOL implantation have been inflammation concentrated in the intermediate zone of the eye, such as pars planitis, and panuveitis. Those patients with a chronic disease that was unlikely to burn out or to be controlled by medical therapy, such as young JRA patients were at highest risk [22].

It has been proven that IOLs trigger a number of reactions (foreign body inflammatory response, and complement and coagulation cascades. The activation of complement system is conducted through classic and alternative pathways. The alternative pathway starts in the presence of IOLs, especially those with prolene haptics, in contact with metabolically active tissues [23]. There are materials such as hydrogel that did not cause any significant activation of this factor.

Many studies have attempted to assess the IOL biocompatibility in risk eyes for intraocular inflammation [2, 21].

Sulcus or anterior chamber implantation have always been contraindicated. Several recent studies have suggested that the implantation of an IOL in the capsular bag does not increase the risk of postoperative inflammation in selected cases provided proper anti-inflammatory treatment is performed. Uveitis is no longer considered an absolute contraindication for the implantation of IOLs. However, cellular and pigment deposits on the IOL surface, or synechiae between the anterior surface of the IOL and the iris may develop (Fig. 8.3). These lesions are often caused by a

Fig. 8.3 Inflammatory precipitates and posterior synechiae after intraocular lens implantation

chronic latent inflammation, which may originate with the uveitis itself or be induced by the lens material.

Pigment dispersion and deposition on the IOL surface seems to be multifactorial, related to surgical trauma, age of patient, and the pre-existing ocular pathology without association with IOL biocompatibility.

Due the heterogeneity and the scarce number of patients, multicentric studies are needed to determine which, if any, IOL material is better tolerated in a uveitic patient by evaluating postoperative responses in the operative eye.

Schauersberger has described the apparition of two inflammatory peaks after cataract surgery [55]. The first one appearing around the 7th day after surgery was attributed to the proliferation of lens cells and the release of PGE2; the second one appeared by the end of the first month and was attributed to a foreign body reaction induced by the IOL and the rebound effect after discontinuation of steroidal therapy.

The selection of the proper type of IOL (material, design, diameter, and configuration) remains a challenge for the surgeon. Few studies have addressed the safety and efficacy of different materials for posterior chamber IOLs, especially foldable lenses, in uveitic eyes.

Polymethylmethacrylate (PMMA) IOLs have been presumed to be relatively inert, but studies have proven their ability to induce foreign body reaction, the postoperative breakdown of the BAB leading to protein and cellular adhesion to the IOL [54].

The use of single-block PMMA lenses may have some advantages in patients with uveitis as they do not activate the complement cascade, a phenomenon associated with polypropylene haptics. They have been the most commonly used IOLs in these cases, and have proved to be inert and stable.

Attempts have been made to either modify the surface of the IOLs rendering them hydrophilic as heparin-modified, or hydrophobic as surface-passivated. Another approach has been to change IOL composition as acrylic lenses.

Surface-modified IOLs such as the heparin-coated models have been introduced and recommended for patients with uveitis as they may decrease the number and severity of deposits on the surface of the IOL. Heparin is electrostatically absorbed onto the surface of PMMA IOLs, converting them into a hydrophilic surface-decreasing electrostatic force. The safety and efficacy of these lenses has been demonstrated in high-risk eyes as well as their higher biocompatibility in terms of lower aqueous cell and protein levels and less cellular deposition in comparison with regular PMMA lenses [40]. Experimental studies with Annexin V-coated IOLs and biodegradable polylactic-glycolic acid disks with sustained release of indomethacin showed a significant decrease in inflammation [13, 47].

Silicone lenses have induced a greater inflammatory reaction in non-uveitic patients compared with other IOL materials (PMMA, hydrogel, heparin-modified), with a higher incidence of early posterior capsule opacification, anterior chamber inflammation, and closure of the capsulorhexis.

Acrylic foldable lenses have demonstrated a statistically significantly lower inflammatory reaction than silicone lenses within the first month of follow-up after surgery. At later visits the differences were no longer significant. Foldable acrylic showed lower inflammation than heparin surface-modified lenses in the first week after surgery, the differences disappearing later. The highest incidence of relapses has been described 1 and 6 months after surgery [1]. Both acrylic and heparin surface-modified IOLs have shown the lowest incidence of relapses, while the highest was for silicon IOLs, although the differences were not significant. Posterior capsular opacifi-

cation also seems to appear earlier with silicone lenses [1]. CME appeared more frequently in eyes implanted with silicone lenses than other materials, although it disappeared with adequate therapy. The apparition of pigment and cellular deposition on the lens, and of pupillary membranes was higher for silicon lens.

A higher incidence of small and giant cell deposition has been found on the surface of silicone IOLs than on PMMA and acrylic lenses. Giant cells are always more significant as they are a sign of a foreign body reaction. Lens epithelial cells originating from the anterior capsule and migrating onto the IOL surface may cause anterior and posterior capsular opacification and phimosis of the capsulorhexis.

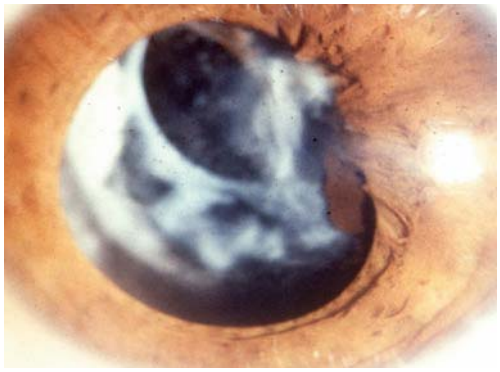

Fig. 8.4 Cellular deposits on the surface of the intraocular lens

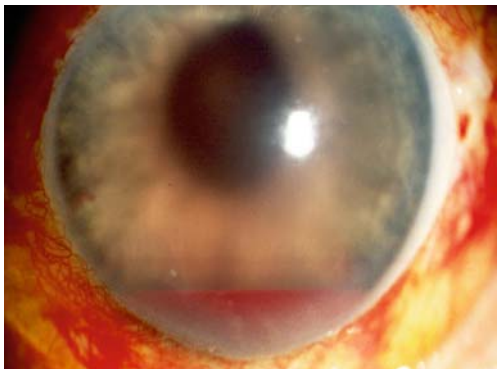

Fig. 8.5 Two types of cells are identified, small fibroblast-like cells that start to disappear by the 3rd month, and large multinucleated giant cells that represent foreign body reaction induced by the intraocular lens

Summary for the Clinician

- Uveitis is no longer considered an absolute contraindication for the implantation of IOLs.
- Poor outcome after IOL in pars planitis, panuveitis, and uveitis, which are unlikely to burn out like JRA.
- Cellular and pigment deposits on the IOL surface and synechiae between the anterior surface of the IOL and the iris may develop originated by the uveitis or induced by the lens material (Figs. 8.4, 8.5).
- Prolene haptics may trigger the alternative pathway in a complementary cascade. Single-block IOLs are preferred
- Sulcus or anterior chamber implantation are contraindicated.
- Surface-modified (hydrophilic heparin-modified, hydrophobic surface-passivated) and acrylic lenses seem to induce less inflammatory response, while silicone lenses induce a greater inflammatory reaction and CME.

8.5.2 Combined Surgery

8.5.2.1 Glaucoma

Glaucoma associated with uveitis is one of the most serious complications of intraocular inflammation. Most patients respond poorly to surgery. It is of primary importance to determine the severity of the inflammation and if possible, the syndrome associated with it. Management includes treatment of the underlying inflammation and of the glaucoma itself.

Special considerations should be given to the management of acute or chronic intraocular inflammation and whether steroids are the cause of the high IOP. Drug therapy (steroids, acetazolamide, beta blockers, cycloplegics, etc.) is the first step in the treatment of uveitic glaucoma.

In general, the outcome of glaucoma surgery in uveitic patients is not as good as it is in patients without uveitis. The following procedures can be performed: laser iridotomy, surgical iri-

dectomy, trabeculodialysis, trabeculectomy, trabeculectomy with wound modulation therapy, ab interno laser sclerostomy, drainage implantation, and cycloablation therapy. Diode cyclophotocoagulation (TSCPC) decreases IOP more effectively than cyclocryocoagulation in children, but complications after TSCPC are more severe than after cyclocryotherapy [36].

Presently mitomycin C is replacing the use of 5-fluorouracil in preventing wound healing after trabeculectomy [11, 62]. The use of antimetabolites in association with trabeculectomy has been used for at least 10 years. Good surgical results have been reported for refractory glaucomas with the use of mitomycin C. However, the higher success rate of filtering surgery with wound healing modulation is associated with an elevated risk of hypotony, bleb leaks, and late bleb-related endophthalmitis [32, 45, 61]. A cellulose sponge soaked in a 0.02 mg/ml concentration of mitomycin C may be placed on the sclera under the scleral flap for 2 min and the remnants left unwashed before suturing.

Drainage implantation seems to be promising when facing progressive secondary glaucoma with uveitis. The most commonly used device is the Molteno implant [42]. What makes this uniquely suited for the benefit of inflammatory glaucoma is the simple fact that the artificial material of the tube is incapable of scarring. The surface area of this plate allows for the formation of a fibrous bleb across from which there is absorption of the accumulated aqueous. These devices are reserved for refractory glaucomatous patients with uveitis who have failed to improve with other medical procedures and when recurrent inflammation is believed to be the reason for the failure of standard filter drainage procedures.

2.2.2.2 Vitrectomy

Cataract surgery can be associated with vitrectomy through a central posterior capsulotomy when marked vitreous organization or membranes are present. The vitrectomy probe can be used to perform a central round capsulectomy. In those cases with extensive vitreous fibrosis and/or exudates, a posterior vitrectomy via pars plana may be necessary. Most surgeons opt for conventional pars plana vitrectomy techniques [24, 27].

Pars plana vitrectomy combined with lensectomy can be the procedure of choice in cases of uveitis with vitreitis refractory to medical treatment. This technique can be useful in patients with JRA or pars planitis. Some disadvantages are associated with this technique including the need for sulcus fixation of the posterior chamber IOL, and the potential difficulties of the aspirating residual cortical debris from the posterior surface of the anterior capsule. In addition, removal of a dense nuclear sclerotic cataract may be difficult to perform.

Combined phacoemulsification and pars plana vitrectomy technique has displayed many advantages over other techniques [4, 35]. Pars plana vitrectomy appears to have beneficial effects on restoring vision, stabilizing vitreous inflammation, and reducing systemic steroid requirements in eyes with thick vitreous opacities associated with sarcoidosis that is resistant to medical treatment [29].

A small phaco-incision guarantees minimal corneal distortion and manipulation, allowing perfect closure during the vitrectomy. Delaying the IOL implantation until completion of vitrectomy if required allows fast visual rehabilitation and functional unaided vision in patients considered poor candidates for aphakic contact lens wear [7].

If a limbar approach to the cataract and posterior pars plana vitrectomy is intended, the scleral incisions for the vitrectomy should be made first, the infusion system sutured, and upper sclerotomies occluded with scleral plugs. A capsulotomy or posterior capsulorhexis must be performed on completion of the vitrectomy due to the fast opacification occurring, and because it allows the access of anti-inflammatory drugs to the vitreous chamber in the postoperative stage.

The presence of vitreous opacities and peripheral retinal neovascularization with a higher risk of vitreous hemorrhage may require cataract surgery to be combined with ablation of the new vessels and vitrectomy.

Summary for the Clinician

- For glaucoma surgery the use of antimetabolites in association with trabeculectomy is recommended.
- Diode cyclophotocoagulation seems more effective than cyclocryocoagulation.
- Drainage implantation seems to be promising.
- For vitrectomy, via the pars plana is preferred to the anterior segment approach.
- This can be combined with lensectomy.

8.6 Postoperative Inflammation

Postoperative inflammation includes the presence of cells, leukocytes, and protein flare in the anterior chamber and engorgement of the iris and conjunctival vessels. The clinical consequence of inflammation is transient or permanent BAB rupture. This phenomenon usually appears associated with postoperative surgical inflammation, is mostly subclinical, and it is very difficult to establish if inflammation has vanished definitively.

The primary clinical variable examined in human clinical studies of postsurgical inflammation is the degree of postoperative intraocular inflammation. Secondary measures of treatment efficacy include measuring the degree of ocular discomfort, bulbar conjunctival redness, ciliary flush, corneal edema, and anterior vitreous reaction. The standard clinical practice includes monitoring the evolution of postoperative inflammation by measuring the presence of cells and flare in the anterior chamber with an inflammation severity score for 4–6 weeks postoperatively. However, subclinical inflammation can only be measured with most modern procedures like fluorophotometry and the Laser Flare Cell Meter. These techniques can detect some clinical events such as late endothelial damage that cannot be detected by other means.

Severe postsurgical exacerbation of pre-existing inflammation should be expected (Fig. 8.6). A strategy for blocking postoperative inflammation is desirable. Topical steroids have become the standard care during the immediate postoperative period to reduce ocular inflammation, prevent structural damage to the eye, and reduce patients' discomfort [41]. The most usual practice in uncomplicated cases is to prescribe prednisolone or dexamethasone four times daily starting immediately after surgery, and then tapering over the following 4–6 weeks. Acetate vehicle is the most adequate due to its superior ocular penetration. Complicated cases may additionally receive systemic steroids started preoperatively and continuing for 2 weeks with gradual tapering over 15 days.

Although topical steroids are currently the most widely used anti-inflammatory agents after cataract extraction, their potential side effects must be considered. This is particularly true for steroid-responsive patients who have a predilection for elevating IOP. Anti-inflammatory agents controlling postoperative inflammation with little effect on IOP would be a useful adjunct to the surgeon. A current strategy underlying the development of new steroidal compounds for ocular use is to identify drugs with marked anti-inflammatory activity with low risk to raise IOP or induce other side effects [63].

No strict guidelines are available for emergency cases. During the postoperative period both topical and systemic steroids may be tapered based on the severity of ocular inflammation. In the most severe cases moderate to high doses of oral prednisone (1–1.5 mg/kg/day), and

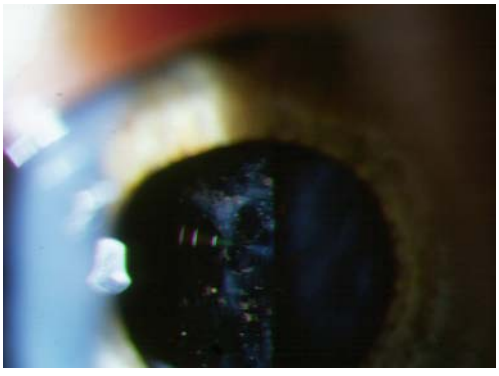

Fig. 8.6 Anterior chamber fibrin formation after phacoemulsification in a patient with chronic anterior uveitis

intensive topical steroid drops should be given and tapered soon afterwards.

Management may be much more difficult in cases of steroid-induced glaucoma, in which temporary immunosuppressive agents may be needed to control inflammation in the very early postoperative period. These drugs have to be administered for at least 2 weeks prior to the surgery because of their latency period.

Several recent studies have demonstrated the effectiveness of NSAIDs in treating ocular inflammation. Most NSAIDs used today are cyclooxygenase inhibitors, thereby decreasing the formation of prostaglandins, which play a major role in ocular inflammation, producing and maintaining the rupture of the BAB. Diclofenac drops were shown to reduce inflammation after cataract surgery [28, 48]. The role of these drugs is controversial in the postoperative control of inflammation even in uncomplicated senile cataracts.

Summary for the Clinician

- A severe postsurgical exacerbation of pre-existing inflammation should be expected.
- Prednisolone acetate (qid) starting immediately after surgery and tapering over the following 4–6 weeks.
- Complicated cases: add systemic steroids started preoperatively and continuing for 2 weeks. Gradual tapering over 15 days.
- In the most severe cases: oral prednisone 1–1.5 mg/kg/day plus intensive topical steroid drops. Gradual tapering over 15 days.
- Topical or systemic NSAIDs may be used.

8.7 Complications

Even low-grade chronic inflammation can result in permanent damage to the optic nerve, retina, anterior chamber angle, and other structures that may preclude our efforts for visual rehabilitation after cataract surgery. Early surgical intervention prior to the development of permanent structural damage from inflammation or steroid therapy may be the option for the future. Strict control of the postoperative inflammation is imperative.

Complications associated with these patients are related to high IOP, corneal edema, endothelial damage, and fibrous membranes described mostly in pars planitis patients [58]. Fibrous membranes may be dense enough to resist rupture by Nd-YAG laser at high levels of energy, and tend to reappear, usually associated with displacements of the lens and retinal detachment.

Decreased visual acuity is mainly caused by CME [17], epiretinal membranes [34], and glaucomatous optic nerve damage [43]. Proper visual acuity has been achieved in the majority of patients in the most important series of patients published [57].

Cystoid macular edema is the most serious postoperative complication in patients with chronic uveitis who undergo cataract extraction. This complication occurs in 19–35% of cases (with angiographic diagnosis) and is present in 80% of eyes with less than 20/40 postoperative visual acuity. CME may be treated with oral acetozolamide, topical NSAIDs, or topical, periocular, intravitreal or systemic steroids [5].

Posterior capsular opacification is a form of postoperative inflammatory reaction. Its apparently higher frequency, up to 56% in the 3rd year seems to be related to the age of the patients [15].

The occurrence of postoperative inflammation relapses is highly related to the behavior and biocompatibility of the IOL. Follow-up shows that the highest incidences of relapses appear at 1 and at 6 months. Both acrylic and surface-modified IOLs show the lowest incidence of relapses and the highest is for the silicone group of lenses.

Summary for the Clinician

- Complications include high IOP, corneal edema, endothelial damage, band keratopathy, fibrous membranes, posterior capsule opacification (Fig. 8.7), CME, epiretinal membranes, glaucomatous optic nerve damage, hemorrhages (Fig. 8.8), chronic low IOP, and phthisis bulbi.

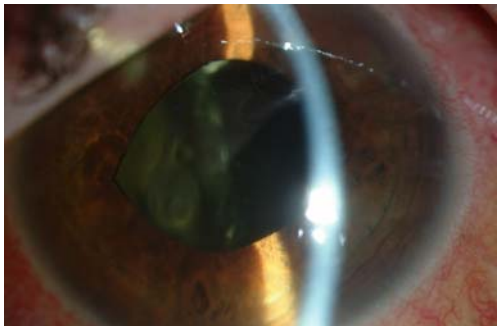

Fig. 8.7 Posterior capsular opacification after cataract surgery in a uveitic patient

Fig. 8.8 Postsurgical hyphema in a uveitic patient after uneventful phacoemulsification

8.8 Cataract Surgery in Specific Cases

8.8.1 Cataract Surgery in Children with Uveitis

By far the most common cause of uveitis in children is the group of joint diseases [33], 30% of patients developing one or more complications during the course of the uveitis. JRA is the most frequent underlying cause of uveitis in children (40% of all uveitis cases and 70% of the anterior uveitis group [59]). Usually, young females affected by monoarticular or pauciarticular JRA with a positive test for antinuclear antibodies and HLA DR5 have the highest rate of complications, with an incidence of cataract of up to 70%, band keratopathy 65%, glaucoma 30%, and chronic hypotony or phthisis 17% [16]. Most of these patients develop these complications in the first decade of life [64]. The primary goal in the management of uveitis in children is early diagnosis and aggressive treatment to prevent ocular complications of the disease.

Surgery in these patients involves serious intraoperative complications, with vitreous loss and retention of cortical material in a good percentage of patients. Patients with JRA frequently have exacerbation of the uveitic process after cataract surgery. Visual prognosis has markedly improved with the introduction of systemic immunosuppressive agents such as methotrexate [3, 51], achieving in some series better than 20/40 visual acuity in all cases [8, 37]. However, its inflammatory component may remain active even in adults, causing vision decrease in 70% of cases after more than 20 years of disease [49]. Uveitis associated with JRA is no longer a contraindication for IOL implantation. With adequate long-term preoperative and postoperative control of intraocular inflammation with systemic immunosuppressive therapy in addition to intensive topical corticosteroid treatment, children with JRA-associated uveitis can demonstrate favorable surgical outcomes after cataract surgery with posterior chamber IOL [37].

2.2.2 Phacoemulsification in Fuchs' Heterochromic Cyclitis

Uveitis associated with Fuchs' heterochromic cyclitis (FHC) tends to be chronic and of low intensity. Posterior synechiae are rarely formed and the patients are usually unaware of the problem until the first complication arises in the form of cataracts or vitreous opacities (Fig. 8.9) [39].

The implantation of IOLs in patients with FHC is generally satisfactory with good visual outcome. Some authors have reported the use of heparin surface-modified lenses for all patients with FHC in whom implantation is indicated [31]. There are reports that suggest that the foldable hydrophobic acrylic IOL is the most suitable one for this type of uveitis [20].

Fuchs' heterochromic cyclitis patients have few postoperative complications, although some isolated cases of vitreitis, hyphema, increased IOP, and cyclitic membrane formation have been reported [46]. Posterior capsular opacification has been described in 8–20% of the patients. The

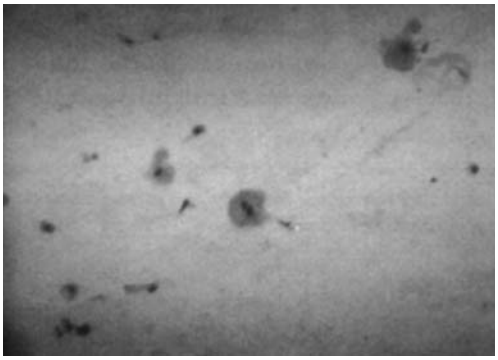

Fig. 8.9 Fuchs's cyclitis. Note keratic precipitates and subcapsular cataract

problem with greatest visual significance is the development of glaucoma, which appears in approximately 10% (range 3–35%) of the patients, and up to 70% of them may require filtration surgery [39]. BAB breakdown following phacoemulsification with posterior chamber lens implantation is relatively mild in eyes with FHC and appears to be fully reestablished to preoperative levels 6 weeks postoperatively, explaining the usually good outcome of cataract surgery with this condition [30, 46].

Some risk factors have been identified in these patients. If glaucoma is present preoperatively, it may worsen postoperatively. In cases of severe iris atrophy, the risk of postoperative uveitis appears to be higher. When rubeosis iridis is present and hemorrhage occurs during surgery, the risk of both postoperative glaucoma and uveitis is higher.

8.8.3 Ocular Toxoplasmosis

Cataract surgery was regularly reported to induce exacerbation in different types of intraocular inflammation; therefore, a group studied and reported an increase in the risk of reactivation of ocular toxoplasmosis following cataract extraction [10]. There was a reactivation rate of 36% for ocular toxoplasmosis within 4 months of cataract surgery and prophylactic treatment with antiparasitic drugs may be considered for patients with ocular toxoplasmosis who are at risk of visual loss.

8.8.4 Pars Planitis, Behcet's Disease, VKH Syndrome, and Multifocal Chorioretinitis

Although macular edema and recurrence of the inflammation may appear in up to 50% of cases of pars planitis after cataract surgery and IOL deposits form in 30% of eyes, adequate control of inflammation, meticulous surgery, in-the-bag IOL implantation, and vigilant postoperative care are keys to achieving a good visual outcome [25].

There are few reports on cataract surgery or phacoemulsification in patients with Behcet's disease, VKH syndrome, and multifocal chorioretinitis.

The incidence of phthisis bulbi and hypotony in Behcet's patients undergoing phacoemulsification has been reported to decrease from 25% to 2% when limited vitrectomy was performed in combination with cataract extraction [33]. It is not clear, however, whether vitrectomy combined with cataract extraction can alter the course of inflammation. Visual prognosis is significantly worse in eyes with Behcet's disease than in other types of uveitis because of the severe posterior segment complications, particularly optic atrophy [9, 14].

Cataract extraction in patients with VKH syndrome can be safely and successfully performed if there is good preoperative and postoperative control of inflammation, careful surgical planning, and a meticulous surgical technique. The final visual outcome depends on the posterior segment complications of the syndrome [26].

Phacoemulsification and vitrectomy in multifocal chorioretinitis with panuveitis has little therapeutic benefit. When an IOL is implanted, a visual improvement of one or two lines can be expected, but visual acuity returns to preoperative values within 6 months. Multifocal chorioretinitis remains poorly understood in terms of its etiology and suitable treatment [60].

Summary for the Clinician

- Uveitis associated with JRA: difficult, but no longer a contraindication for IOL implantation. It is often useful to use immunosuppressive therapy.
- Fuchs' heterochromic cyclitis: generally satisfactory with good visual outcome, although glaucoma may worsen postoperatively.
- Ocular toxoplasmosis: risk of reactivation after surgery.
- Pars planitis: macular edema, lens deposits, recurrences.
- Behcet's disease: worse visual prognosis caused by retinal and optic nerve damage. Vitrectomy may improve visual outcome.

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Chapter 9

Ocular Toxoplasmosis

9

Cristina Muccioli, Rubens Belfort Jr

Core Messages

- Toxoplasmosis is an important cause of ocular disease in both immunosuppressed and immunocompetent individuals with billions of infected people all over the world.
- Cat family members are its definitive hosts.
- Ocular toxoplasmosis is the most common cause of retinitis. It secondarily affects the uvea, triggering uveitis.
- Many if not most of the ocular cases are secondary to postnatal infections. It is estimated that 70–90% of patients with congenital toxoplasmosis and 10–12% of patients with postnatal infection develop the ocular disease.
- The major risk factor for transmission is the ingestion of undercooked meat or food contaminated by cat feces and water.
- The diagnosis of toxoplasmic retinochoroiditis is based on its clinical presentation and the finding of circulating antibodies to toxoplasma.
- Serum IgG titers may be low and IgM may be absent at the time when ocular lesions appear.
- Toxoplasmic retinochoroiditis lesions have the same characteristics, whether they result from congenital or acquired infections.
- They are usually intensely white, focal lesions with overlying vitreous inflammatory haze.
- Active lesions that are accompanied by a severe vitreous inflammatory reaction will have the classic "headlight in the fog" appearance.
- The choroid is secondarily inflamed, but choroidal lesions do not occur in the absence of retinal infection. There can also be an intense, secondary iridocyclitis.
- Some patients with acquired toxoplasmosis could develop atypical ocular lesions (acute, non necrotizing retinochoroiditis) or signs like vasculitis, vitritis, anterior uveitis, without developing a focal necrotizing retinochoroiditis.
- Recurrent lesions tend to occur at the borders of retinochoroidal scars, anywhere in the fundus, which are the remnants of previous disease episodes.
- In most cases *T gondii* infection is self-limiting and asymptomatic.
- The currently available drugs do not eliminate tissue cysts and therefore cannot prevent chronic infection.
- The goal of medical therapy is to prevent damage to the retina and optic nerve, thereby preventing permanent vision loss.
- The association of pyrimethamine, sulfadiazine, and corticosteroids is the most common combination used and is the gold standard.
- Corticosteroids are used to decrease problems associated with secondary inflammation and should not be used without concurrent antimicrobial agents.

Core Messages

- It is probable that long-term use of antitoxoplasmic agents such as Bactrim taken three times a week for months to years decreases the chance of recurrence.
- Toxoplasmosis is believed to be the most common nonviral infection of the brain in patients with AIDS. In HIV-infected patients, ocular toxoplasmosis can occur before the development of AIDS.

9.1 Introduction

Toxoplasmosis is a common disease caused by the obligate intracellular protozoan parasite *Toxoplasma gondii* and can cause severe, life-threatening disease, especially in newborns and immunosuppressed patients and is an important cause of ocular disease in both immunosuppressed and immunocompetent individuals [15].

Toxoplasma gondii is a obligate, intracellular protozoan parasite that belongs to the phylum Apicomplexa, subclass coccidia, which undergoes a complicated life cycle including both sexual and asexual reproduction. Members of the cat family are its definitive hosts, but hundreds of other species, including mammals, birds, and reptiles, may serve as intermediate hosts [18].

T. gondii exists in several forms: the oocyst, the tachyzoite, and the cyst. Oocysts are the products of sexual reproduction and are shed in cat feces. Tachyzoites are the obligate intracellular form of the parasite and are able to invade nearly all host tissues. The tachyzoite form causes a strong inflammatory response and tissue destruction, and, therefore, causes clinical manifestations of disease. Tachyzoites are transformed into bradyzoites under the pressure of the immune response and form cysts [10].

Tissue cysts begin to form as early as 6–8 days after infection and may persist in a viable state in multiple tissues for the life of the host. Bradyzoites, the organisms within these tissue cysts, will continue to replicate slowly by endodyogeny. Tissue cysts contain hundreds of thousands of bradyzoites and form within host cells in brain and skeletal and heart muscles. Bradyzoites can be released from cysts, transform back into tachyzoites and cause recrudescence of infection in immunocompromised patients. Cysts are infective stages for intermediate and definitive hosts [34].

9.2 Epidemiology

Toxoplasma gondii infects up to a third of the world's population and in the United States serologic evidence of *T. gondii* infection ranges from 3 to 70% of the healthy adult population. Prevalence is highest in tropical areas and is relatively low in hot and arid areas, and in cold regions. In some areas of central America, the south Pacific, and western Europe, seropositivity rates exceed 90% by the fourth decade of life [24].

The majority of acquired *T. gondii* infections in immunocompetent children and adults remain asymptomatic. In 10–20% of cases, however, individuals will develop a self-limited lymphadenopathy syndrome [27].

Ocular toxoplasmosis (OT) is the most common cause of posterior uveitis in many countries and represents 85% of the posterior uveitis cases in southern Brazil and 25% of those in the USA. The prevalence of OT in the USA is not well determined, but ranges from 0.6 to 2% according to published reports. In Brazil, according to several published reports, the prevalence ranges from 10 to 17.7% (an area of very high rate of *T. gondii* infection) [4].

Toxoplasmic retinochoroiditis is the most common manifestation in congenital infection and it is believed that the majority of OT results from recurrent infections [1].

Summary for the Clinician

- Patients with toxoplasmosis have to be seen as a movie and not as a picture. The final diagnosis and appropriate management are usually better appreciated after many frames.
- Most knowledge in the field is not based on evidence, but is assumed from induction (such as experience and retrospective work) or seduction (experts, drugs companies, marketing) and not from deduction (randomized clinical trials, etc.)
- As in other chronic diseases with relatively low frequency in a daily practices the referrals of patients for a second opinion should be considered in difficult or unusual patients.

9.3 Infection

Human beings can be infected with *T. gondii* by ingestion or handling of undercooked or raw meet (mainly pork or lamb) containing cysts, or water or food containing oocysts excreted in the feces of infected cats [8].

The major risk factor for transmission of *T. gondii* to adults and children is the ingestion of undercooked meat. Transplacental transmission of *T. gondii* to the fetus occurs when infection is acquired during pregnancy [31]. Most individuals are infected inadvertently; thus, the specific route of transmission cannot usually be established [31]. Several outbreaks of toxoplasmosis in human beings have been linked epidemiologically to the drinking of unfiltered water [9].

In HIV-infected patients, toxoplasmosis is the most common nonviral infection that affects the central nervous system causing toxoplasmic encephalitis. In immunodeficient patients, OT lesions can be more severe and aggressive than those observed in immunocompetent individuals. Ocular toxoplasmosis occurs in 1–2% of patients with AIDS in the USA and in 8% of AIDS patients in Brazil [23].

9.4 Clinical Features

Clinically, infection with *T. gondii* can go unnoticed or could cause signs and symptoms and vary depending on the immune status of the patient.

The most common clinical manifestation of acquired *T.gondii* infection is asymptomatic cervical lymphadenopathy. Other commonly involved lymph nodes are those in the suboccipital, supraclavicular, axillary, and inguinal areas. They will generally be nonsuppurative, discrete, of variable firmness, and nontender [18].

Symptomatic patients may develop sore throat, fever, malaise, night sweats, myalgia, and maculopapular rash. Hepatosplenomegaly may be present, and there may be a few circulating atypical lymphocytes. This disorder resembles infectious mononucleosis, but can be confused with Hodgkin's disease or other lymphomas. Symptoms and lymphadenopathy will usually resolve within a few months [18].

Infection during pregnancy, also usually asymptomatic, can result in a mild systemic illness with lymphadenopathy and skin rash, as in other adults [31].

Summary for the Clinician

- All laboratory/radiology results must be analyzed according to the clinical picture, follow-up, and their predictive values.

9.5 Ocular Disease

The retina is the primary site of *T. gondii* infection in the eye. Toxoplasmic retinochoroiditis lesions have the same characteristics, whether they result from congenital or acquired infections. They are usually intensely white focal lesions with overlying vitreous inflammatory haze (Fig. 9.1). Active lesions that are accompanied by a severe vitreous inflammatory reaction will have the classic "headlight in the fog" appearance (Fig. 9.2). The choroid is secondarily inflamed,

but choroidal lesions do not occur in the absence of retinal infection. There can also be an intense, secondary iridocyclitis (Fig. 9.3) [16].

Recurrent lesions tend to occur at the borders of retinochoroidal scars, which are the remnants of previous disease episodes (Fig. 9.4). Lesions can occur anywhere in the fundus.

The symptoms of recurrent toxoplasmic retinochoroiditis include floaters and blurring of vision. Patients may also develop painful, red eyes from the associated anterior segment inflammatory reaction. Recurrent toxoplasmic retinochoroiditis is not associated with systemic symptoms [19].

9.5.1 Newborns with Congenital Disease

Retinochoroiditis is the most common manifestation of congenital toxoplasmosis, and it will be bilateral in up to 85% of patients (Fig. 9.5) [28].

Retinochoroidal lesions are self-limited and may not develop for months or years after birth, despite well-documented congenital infection.

Less commonly, immunocompetent patients can develop a more severe form of disease, characterized by extensive retinal necrosis and panuveitis.

When *T. gondii* infection involves the optic disc or the retina immediately adjacent to the optic disc, it can result in a papillitis [11].

Patients with macular lesions may also get secondary optic disc swelling.

9.5.2 Signs Associated with Active Disease

Patients with active toxoplasmic retinochoroiditis will occasionally develop inflammatory sheathing of retinal vessels (Fig. 9.6). Vasculitis may develop in response to reactions between circulating antibodies and local *T. gondii* antigens. In general, vascular sheathing disappears rapidly with resolution of other inflammatory signs [36].

9.5.3 Course of Disease

In most cases toxoplasmic retinochoroiditis is a self-limited disease. Untreated lesions generally begin to heal after 1 or 2 months, although the time course is variable, and in some cases active disease may persist for months.

As a lesion heals, its borders become more discrete, and it becomes gray-white and less "fuzzy" in appearance as the inflammatory reaction clears. Over several months the borders of lesions may become hyperpigmented. It may also take weeks to months for all vitreous cells and haze to resolve. Large scars will have an atrophic center that is devoid of all retinal and choroidal elements; the underlying sclera gives the lesion its white center [15].

The frequency of recurrent inflammation attacks varies widely and cannot be predicted.

9.5.4 Immunosuppressed Patients

Toxoplasmic retinochoroiditis is a serious disorder in HIV-infected patients and in other immunosuppressed individuals such as cancer patients or organ transplant recipients. It is generally more severe than in immunocompetent patients, with a broader range of clinical features [2]. In immunosuppressed patients, there can be single lesions, multifocal lesions in one or both eyes, and broad areas of retinal necrosis (Fig. 9.7). Although the majority of reported cases in HIV-infected patients have been unilateral, it is not uncommon to see bilateral cases [14].

In cases with full-thickness necrosis, the retina appears to have a hard appearance, with sharply demarcated borders. There is usually little retinal hemorrhage within the lesion itself. A prominent inflammatory reaction in the vitreous body and anterior chamber has been described in several reports of HIV-associated ocular toxoplasmosis.

Lesions will continue to enlarge without treatment, which probably explains the fact that most patients reported have had extensive areas of retinal necrosis by the time diagnosis is made [7]. In most reported cases of ocular toxoplasmosis in immunosuppressed patients, there have not been pre-existing scars (Fig. 9.8). Central nervous system involvement is seen in 29–50% of HIV-in-

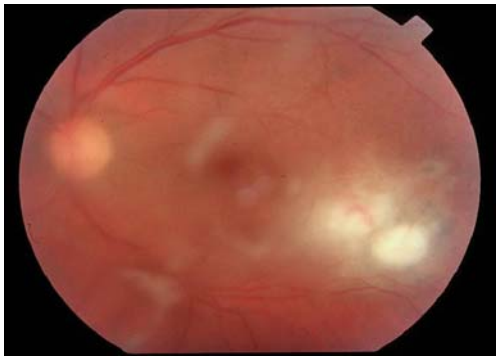

Fig. 9.1 Characteristics of toxoplasmic retinochoroiditis lesions: intensely white, focal lesions with overlying vitreous inflammatory haze

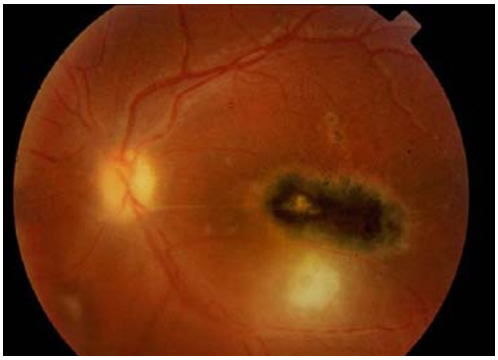

Fig. 9.4 Recurrent lesions at the borders of retinochoroidal scars

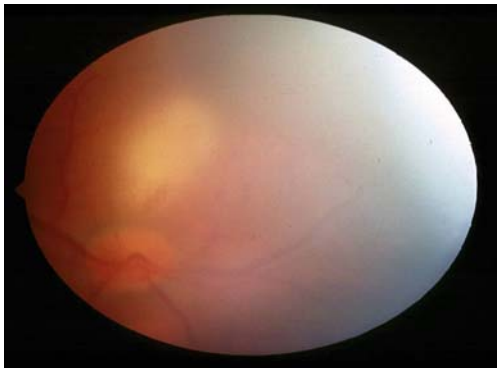

Fig. 9.2 Active lesions: severe vitreous inflammatory reaction ("headlight in the fog" appearance)

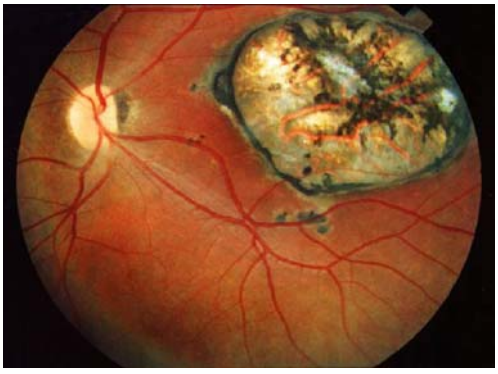

Fig. 9.5 Retinochoroiditis: congenital toxoplasmosis

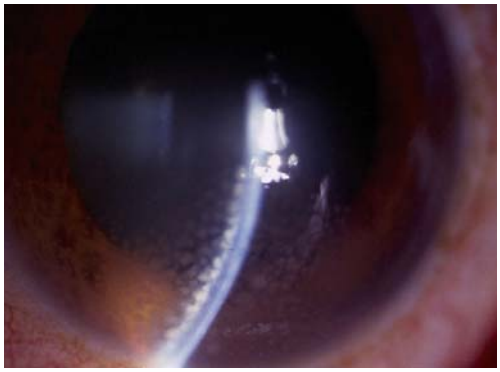

Fig. 9.3 Toxoplasmosis: intense, secondary iridocyclitis

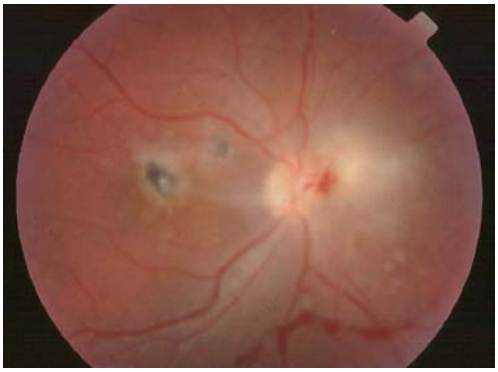

Fig. 9.6 Active toxoplasmic retinochoroiditis: inflammatory sheathing of retinal vessels

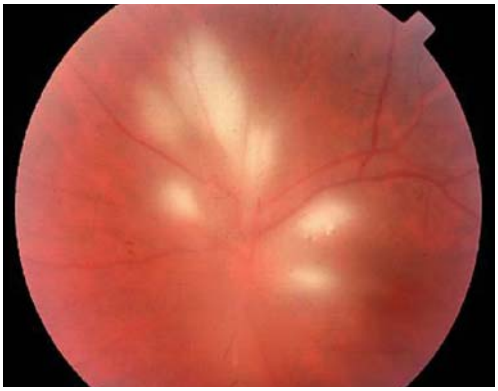

Fig. 9.7 Immunosuppressed patient: multifocal lesions

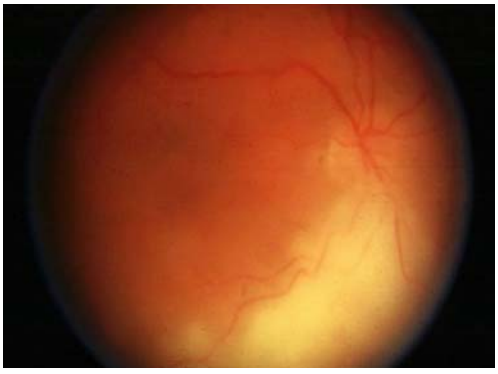

Fig. 9.8 Ocular toxoplasmosis in immunosuppressed patients: no pre-existing scars

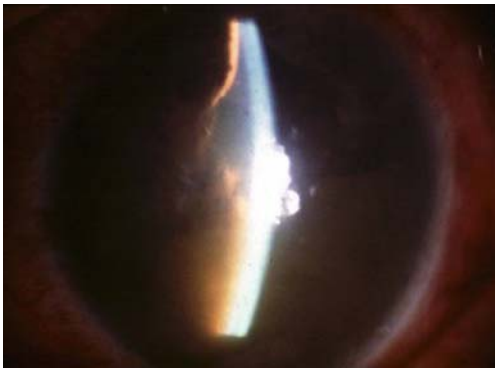

Fig. 9.9 Infection of the iris with *T. gondii* in a patient with AIDS

fected patients with ocular toxoplasmosis. Infection of the iris with *T. gondii* has been reported in a patient with AIDS (Fig. 9.9) [30].

9.6 Clinical Signs and Symptoms

The most common clinical signs of active OT are blurring or loss of vision and floaters. Depending on the location of the lesions, patients can be more or less symptomatic. For example if the macula is involved, patients' complaints could be the presence of a central blind spot. Usually no anterior chamber signs of inflammation (redness, photophobia, ciliary injection) are present. Granulomatous keratic precipitates (KPs) as well as anterior chamber reaction (inflammatory cells and flare) could be present with the active OT lesion [33].

The characteristic sign of OT is the presence of toxoplasmic retinochoroiditis, which is a white or yellow patch of retinochoroiditis with moderate to severe vitreous reaction.

Healed lesions appear as atrophic scars with peripheral hyperpigmentation. Recurrent infections occur at the border of a healed scar as a satellite lesion.

The characteristic sign of ocular toxoplasmosis is necrotizing retinitis with vitreous involvement and anterior chamber reaction, but recent evidence has also confirmed that patients with the acquired infection may present only with vitreitis or even anterior uveitis in the absence of retinochoroiditis. In most cases, the infection is self-limited and asymptomatic [6].

In patients with AIDS ocular involvement is considered to be rare. Ocular lesions may be the first manifestation of intracranial and disseminated disease, but may occur without evidence of intracranial involvement. The clinical picture of ocular toxoplasmosis in AIDS may be different or unusual like the iris infection with *Toxoplasma gondii* causing anterior uveitis [25].

9.7 Serologic Tests

Serologic tests for demonstration of specific antibodies are used commonly to confirm exposure

to *T. gondii* in cases of suspected toxoplasmosis. The presence of anti-*T. gondii* IgG antibodies cannot confirm a diagnosis, however, because such antibodies can persist at high titers for years after an acute infection, and there is a high prevalence of such antibodies in the general population. The presence of antibodies may therefore be unrelated to the clinical disorder being investigated [29].

Several different serologic tests are available to clinicians; they identify antibodies to different *T. gondii* antigens and have unique patterns of change after infection. The most useful tests are the Sabin-Feldman dye test, the enzyme-linked immunosorbent assay (ELISA), the indirect fluorescent antibody (IFA) test, and the modified direct agglutination test [3].

9.8 Imaging and Diagnostic Tests

Toxoplasmic retinochoroiditis is diagnosed clinically. Parasites are found rarely in intraocular fluids, and invasive diagnostic tests such as retinal biopsy are associated with serious risks, which prevents their routine use. Serologic tests should be used only to confirm past exposure to *T. gondii*; it is inappropriate to base a diagnosis of ocular toxoplasmosis on the presence of antibodies alone. Because active retinal lesions are usually foci of recurrent disease, serum IgG titers may be low and IgM may be absent [37].

Toxoplasma gondii DNA has been identified in ocular tissue sections of patients with presumed toxoplasmic retinochoroiditis by polymerase chain reaction techniques, even when typical tissue cysts are not identified on histopathologic examination.

Summary for the Clinician

- Intelligent anamnesis and full bilateral ocular examination in the vast majority of cases are the pillars of the right diagnosis.

9.8.1 Fluorescein Angiography

Edema blocks early fluorescence. The lesion will gradually stain, starting from the borders. The retinitis may be associated with a serous retinal detachment of the overlying retina and, in such cases, the whole area of detached retina stains in late sequences. After the acute stage of toxoplasmosis, the edema gradually regresses. Pigmentary changes become obvious and are initially best seen with fluorescein angiography (FA). After the inflammation resolves, a chorioretinal scar is present. FA may be helpful to diagnose some complications related to the OT itself, like cystoid macular edema (CME), vitreous traction, macular hole, retinal tears, etc. (Fig. 9.10).

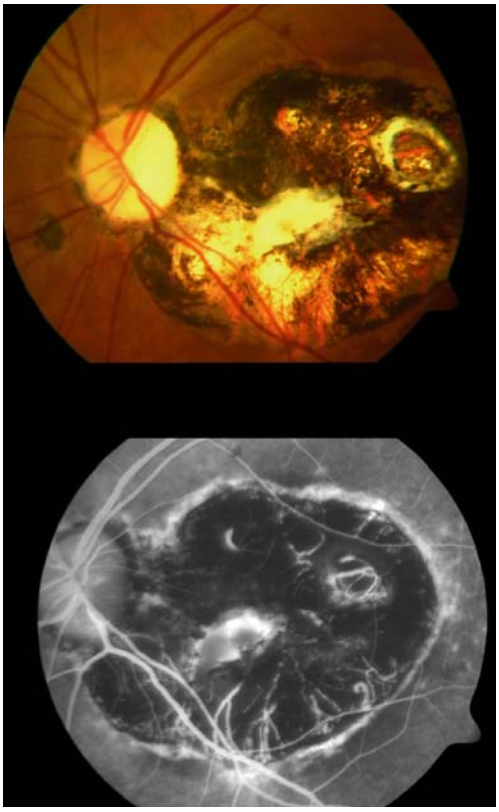

Fig. 9.10 Ocular toxoplasmosis complication (fluorescein angiography)

9.8.2 Indocyanine Green Angiography

Indocyanine green angiography is not useful in the diagnosis or management of OT, but appears to be useful in assessing the extent of choroidal involvement and the evolution of lesions. It might be used as an adjunctive follow-up parameter. Based on reports from the literature, ICG angiography appears to be indicated in the work-up and management of toxoplasmic retinochoroiditis in some special situations (Fig. 9.11).

9.8.3 Optical Coherence Tomography

Optical coherence tomography could be used as a complementary method to FA and be of significant help to the clinician. The OCT enhances the visualization of macular holes, is useful to identify epiretinal membranes as well as cystoid macular edema (Fig. 9.12).

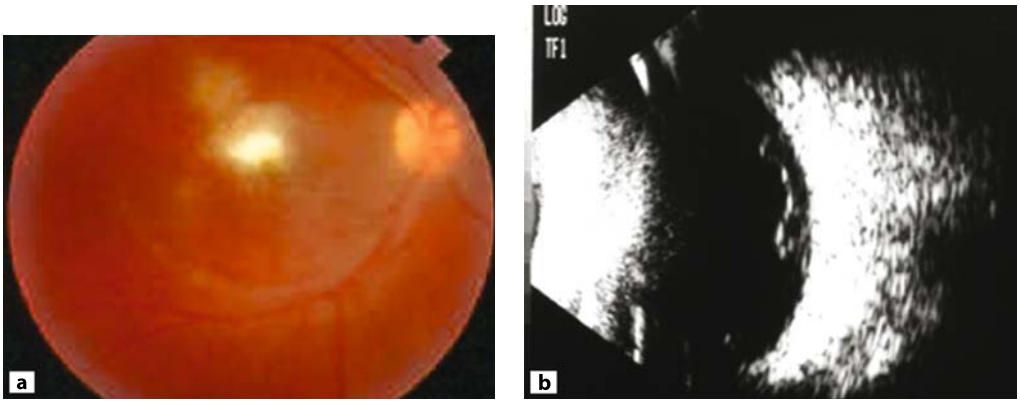

Fig. 9.11 Indocyanine green angiography: assesses the extent of choroidal involvement and the evolution of lesions

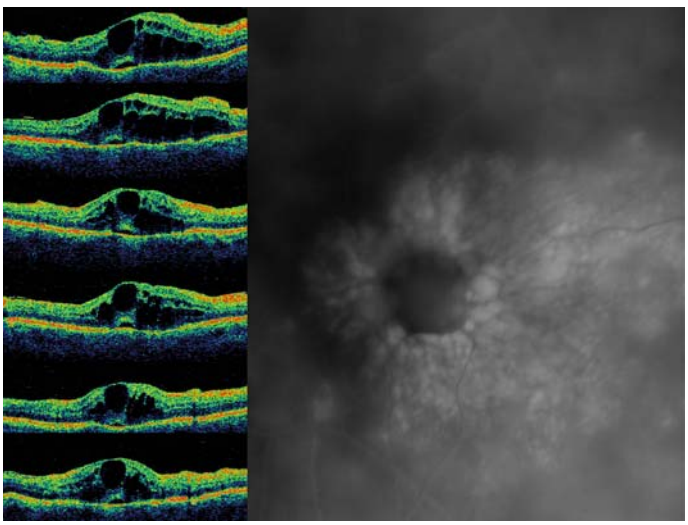

Fig. 9.12 Optical coherence tomography: cystoid macular edema

9.8.4 Ultrasonography

Ultrasound biomicroscopy can be useful for identifying complications related to the anterior part of the eye (ciliary body detachment, intraocular lens placement, angle closure glaucoma, etc). B-scan ultrasound is used for patients in whom fundus examination is difficult or impossible due to the presence of posterior synechiae, cataract or corneal opacification, and to identify complications such as retinal and choroidal detachment, vitreous hemorrhages or opacities and vitreoretinal traction (Fig. 9.13).

Summary for the Clinician

- Cataract surgery in patients with toxoplasmosis also allows the diagnosis and treatment of posterior pole complications. Therefore, patients with cataracts who do not allow examination of the fundus should always be considered for surgery.

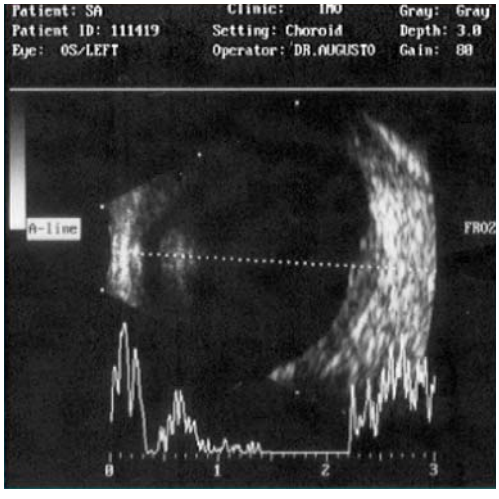

Fig. 9.13 Ultrasonography: opacities and vitreoretinal traction

9.9 Pathology

Ocular toxoplasmosis in immunocompetent patients is characterized histologically by foci of coagulative necrosis of the retina with sharply demarcated borders. Inflammatory changes can be widespread in the eye and involve choroid, iris, and trabecular meshwork. Tissue cysts can be found in the retina with little disruption of the retinal architecture [12].

Scar is characterized by gliosis, obliteration of vessels, and hyperpigmentation at the borders. Calcification can develop.

Eyes from immunosuppressed patients with OT reveals both tachyzoites and tissue cysts in areas of retinal necrosis and within retinal pigment epithelial cells. Parasites can occasionally be found in the choroid, vitreous, and optic nerve [21].

9.10 Treatment

In most cases *T gondii* infection is self-limiting and asymptomatic. Furthermore, currently available drugs do not eliminate tissue cysts and therefore cannot prevent chronic infection. Treatment is therefore not warranted for the majority of *T. gondii* infections. It is also generally accepted that treatment is beneficial for ocular infections [13].

9.10.1 Goal

The goal of medical therapy is to prevent damage to the retina and optic nerve, thereby preventing permanent vision loss.

9.10.2 Antimicrobial Agents

The combination of pyrimethamine, sulfadiazine, and corticosteroids, which is considered "classic" therapy for ocular toxoplasmosis, is the most common drug combination used (Table 9.1).

A number of other drugs have also demonstrated in vitro and in vivo efficacy against *T. gondii* and have been used in the treatment of human ocular toxoplasmosis, but their relative

Table 9.1 Typical therapy for ocular toxoplasmosis

Pyrimethamine	75–100 mg loading dose given over 24 h, followed by 25–50 mg daily for 4–6 weeks depending on clinical response.
Sulfadiazine	2.0–4.0 g loading dose initially, followed by 1.0 g given 4 times daily for 4–6 weeks, depending on clinical response.
Prednisone	40–60 mg daily for 2–6 weeks depending on clinical response; taper off before discontinuing pyrimethamine/sulfadiazine.
Folinic acid	5.0 mg tablet or 3.0 mg intravenous preparation given orally, 2–3 times weekly during pyrimethamine therapy.

efficacies remains uncertain. They are generally used in multiple drug combinations, both with and without oral corticosteroid therapy [20].

The AIDS epidemic has stimulated renewed interest in the medical therapy of toxoplasmosis. A goal of ongoing research is to identify drugs, drug combinations, and treatment regimens that will be cysticidal. Atovaquone is a hydroxynaphthoquinone that interferes with pyrimidine synthesis. The drug is believed to be safe and has been well tolerated by patients; the only reported side effect is a transient maculopapular rash. Treatment does not prevent the recurrence of toxoplasmic retinochoroiditis [32].

Traditional short-term treatments of active toxoplasmic retinochoroiditis lesions do not prevent subsequent recurrences, even with agents such as atovaquone, which have been shown to have cysticidal activity in laboratory investigations.

In a study from Silveira et al., the authors demonstrated that intermittent trimethoprim/sulfamethoxazole treatment can reduce the rate of recurrent toxoplasmic retinochoroiditis during the period of treatment [35]. Treatment also appears to delay the onset of recurrences.

Pyrimethamine plus sulfadiazine is the most commonly used combination for treatment of ocular toxoplasmosis; however, because of the toxicity associated with these drugs, combination of trimethoprim and sulfamethoxazole has been evaluated as a potentially less toxic alternative for the treatment of toxoplasmosis.

The combination of trimethoprim/sulfamethoxazole is generally well tolerated, even when it is administered chronically. The most common side effects are mild gastrointestinal problems and mild skin lesions.

Summary for the Clinician

- There is increasing evidence that long-term treatment with anti-toxoplasmic agents can reduce the risk of recurrence.

9.10.3 Corticosteroids

Corticosteroids are used to decrease problems associated with inflammation, such as macular edema, vitreous inflammatory reaction, and retinal vasculitis. Their use is felt to be especially important for lesions that threaten the macula or optic disc. Because retinal necrosis is believed to be caused by proliferation of *T. gondii*, corticosteroids should not be used without concurrent antimicrobial agents; the suppression of immune defense mechanisms by corticosteroids might lead to fulminant *T. gondii* infection [35].

Topical corticosteroids are used to treat the secondary anterior chamber reaction associated with toxoplasmic retinochoroiditis. The frequency of treatment is based on the severity of reaction and the patient's symptoms of redness and discomfort. Topically applied drug has no effect on the retinal infection [17].

9.10.4 Complications

Toxoplasmic retinochoroiditis can result in permanent loss of vision because of retinal necrosis. Central vision will be lost if lesions affect

the fovea, maculopapillary bundle, or optic disc. Involvement of the optic disc or the peripapillary retina can also result in optic atrophy. Other reported complications include macular edema and the sequelae of anterior segment inflammation (secondary glaucoma, posterior synechiae, secondary cataracts) [5].

Vascular complications include subretinal neovascularization, retinochoroidal vascular anastomoses, and obstruction of branch arterioles or venules that pass through areas of infection.

Neovascularization of the retina and the optic disc can occur, resulting in vitreous hemorrhage. Subretinal neovascular membranes may be a cause of sudden loss of vision.

Rhegmatogenous and tractional retinal detachments may occur. Rhegmatogenous retinal detachment is more common in immunosuppressed patients because they are likely to have extensive areas of retinal necrosis in which retinal holes may develop.

Serous detachment of the macula is an uncommon finding in patients with posterior segment lesions, and these detachments resolve with medical therapy.

Summary for the Clinician

- In many cases loss of vision is not caused by inflammation itself but its complications such as macular edema, retinal detachment and glaucoma.

9.11 Disease in Immunosuppressed Patients

Toxoplasma gondii is a common opportunistic pathogen among immunosuppressed patients and is a particularly severe problem in HIV-infected individuals. The prevalence of anti-*T. gondii* antibodies is 15–40% among HIV-infected individuals in the USA, but is nearly universal where infection rates are very high in the general population. There may also be an increased risk of primary acquired *T. gondii* infections among HIV-infected individuals [22].

Toxoplasmosis is believed to be the most common non-viral infection of the brain in patients with AIDS. Toxoplasmic encephalitis eventually develops in 25–50% of patients with antibodies against *T. gondii.*

9.11.1 Risk Factor

Immunosuppression is associated with an increased risk of life-threatening toxoplasmosis. Immunosuppression also increases the *severity* of ocular toxoplasmosis.

In HIV-infected patients, ocular toxoplasmosis can occur before the development of index diseases diagnostic of AIDS. The risk of life-threatening toxoplasmosis increases when CD4+ T-lymphocyte counts fall below 100/μ1. In one study the median CD4+ T-lymphocyte count of patients with AIDS and toxoplasmic encephalitis was 50/μ1 (range 0–730/μ1). Although the median CD4+ T-lymphocyte count specifically associated with toxoplasmic retinochoroiditis is not known, this infection can occur at higher counts than usually associated with cytomegalovirus (CMV) retinitis [26].

9.11.2 Ocular Disease

In HIV-infected patients, toxoplasmosis is the most common cause of non-viral central nervous system infection leading to toxoplasmic encephalitis. Ocular toxoplasmic lesions can be more severe and aggressive than those observed in immunocompetent individuals. Ocular toxoplasmosis occurs in 1–2% of patients with AIDS in the US and in 8–10% of AIDS patients in Brazil [26].

Toxoplasmic retinochoroiditis is a serious disorder in HIV-infected patients and in other immunosuppressed individuals such as cancer patients or organ transplant recipients. It is generally more severe than in immunocompetent patients, with a broader range of clinical features.

Ocular toxoplasmosis in immunosuppressed patients can be single lesions, multifocal lesions

in one or both eyes, and broad areas of retinal necrosis.

Although the majority of reported cases in HIV-infected patients have been unilateral, it is not uncommon to see bilateral cases.

In cases with full-thickness necrosis, the retina appears to have an indurated appearance, with sharply demarcated borders. There is usually little retinal hemorrhage within the lesion itself.

A prominent inflammatory reaction in the vitreous body and anterior chamber has been described in several reports of HIV-associated ocular toxoplasmosis.

Lesions will continue to enlarge without treatment, which probably explains the fact that most reported patients have had extensive areas of retinal necrosis by the time diagnosis is made.

In most reported cases of ocular toxoplasmosis in immunosuppressed patients, there have not been pre-existing scars.

Infection of the iris with *T. gondii* has been reported in a patient with AIDS.

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Chapter 10

Vogt-Koyanagi-Harada Disease and Sympathetic Ophthalmia

10

P. Kumar Rao, Narsing A. Rao

Core Messages

- Vogt-Koyanagi-Harada disease is bilateral granulomatous uveitis.
- The natural course of the disease includes prodromal stage, uveitic stage, chronic stage, and chronic recurrent stage.
- Extraocular changes such as vitiligo, poliosis, and dysacusis may develop during the chronic or chronic recurrent stage of the disease.
- The acute uveitis stage can be managed with high-dose systemic corticosteroids. The chronic and chronic recurrent stages require immunosuppressive agents to control the ocular inflammation.

10.1 Introduction

Vogt-Koyanagi-Harada (VKH) disease, also known as VKH syndrome, and sympathetic ophthalmia (SO) share virtually all of the same clinical and histopathologic features. Similar to VKH disease, SO can present with bilateral panuveitis associated with retinal detachment and meningismus. However, while a history of penetrating ocular injury is the rule in SO, such an injury is not a feature of VKH disease. Extraocular manifestations such as dysacusis, vitiligo, poliosis, and alopecia can occur in SO, but these are rare. Unlike SO, VKH disease is mainly seen in Japanese, Hispanics, Native Americans, and Asian Indians.

10.2 Vogt-Koyanagi-Harada Disease

Vogt-Koyanagi-Harada disease is a bilateral granulomatous uveitis often associated with exudative retinal detachment, with or without extraocular manifestations. Intraocular manifestations include signs of uveitis associated with multifocal serous retinal detachment and hyperemia of the optic disc. Extraocular changes include meningeal irritation, cutaneous signs, and auditory abnormalities. The meningeal manifestations, which occur early in the disease, consist of headache, meningismus, and rarely, focal neurological signs; CSF analysis may reveal pleocytosis. Auditory disturbances include tinnitus and hearing loss, and, rarely, vertigo. The cutaneous changes that usually develop during the chronic phase of the disease include patchy alopecia, vitiligo, and poliosis of lashes, eyebrows, and scalp hair [20]. The typical clinical course consists of four stages: the prodromal, uveitic, chronic, and chronic recurrent stages. The initial prodromal stage is characterized by the development of a systemic viral-like illness that lasts 3–5 days. This is followed by the uveitic stage, during which patients exhibit the signs and symptoms of acute uveitis; this stage may last for several weeks. In the chronic (convalescent) stage, patients develop integumentary and uveal depigmentation; this stage may last for months to years depending on therapeutic intervention. In the chronic recurrent stage, patients may exhibit resolving chronic uveitis, interrupted by recurrent bouts of anterior uveitis [20].

When a patient presents with the ocular and the extraocular manifestations of VKH disease,

the diagnosis is made with certainty and such cases are considered typical. However, the vast majority of patients with VKH disease may not initially present with *typical* features. Thus, the First International Workshop on VKH disease proposed revised diagnostic criteria to include clinical manifestations at various stages of the disease [29]. These revised diagnostic criteria are summarized in Table 10.1.

10.2.1 Historical Aspects

The association of poliosis with ocular inflammation was reported by Vogt and others [13, 32, 35]. Harada described primary posterior uveitis with exudative retinal detachments and associated cerebrospinal fluid pleocytosis in 1926 [11]. In 1929, Koyanagi reported six patients with bilateral chronic iridocyclitis, patchy depigmentation of the skin, patchy hair loss, and whitening of the hair, especially the eye lashes [16]. Babel in 1932 and Bruno and McPherson in 1949 combined the findings of Vogt, Koyanagi, and Harada and suggested that these processes represent a continuum of the same disease, thereafter recognized as Vogt-Koyanagi-Harada syndrome [2, 4]. The 1st International Workshop on VKH disease, held in 1999, adopted the term Vogt-Koyanagi-Harada disease [29].

10.2.2 Epidemiology

Vogt-Koyanagi-Harada disease appears to be more common in Japan, where it accounts for 6.8% to 9.2% of all uveitis referrals [20]. In the United States, it accounts for 1–4% of all uveitis clinic referrals. Vogt-Koyanagi-Harada disease tends to affect Asians, Hispanics, and Native Americans [20]. In the United States, there appears to be variability in the racial distribution of patients with VKH disease. In northern California, VKH disease was seen mainly in Asians (41%), followed by whites (29%), Hispanics (16%) and blacks (14%) [22]. In contrast, reports from southern California show that 78% of patients with VKH disease were Hispanic, 3% were white, 10% were Asian, and 6% were black [20]. A series reported by the National Institutes of Health (NIH) showed that 50% of patients with VKH were white, 35% were black, and 13% were Hispanic [21]. Most studies report that women tend to be affected more frequently than men; however, Japanese investigators have not found such a female predilection [33]. Most patients are in their second to fifth decades of life, but children may be affected as well [7].

10.2.3 Clinical Features

The clinical manifestations of VKH disease may vary according to the stage of the disease, although extraocular changes have been considered important components of the disease and such changes are not common.

The prodromal stage may last for only a few days and may be limited to headaches, nausea, dizziness, fever, orbital pain, and meningismus. Light sensitivity and tearing may occur 1–2 days after the above symptoms. Neurological signs, such as cranial nerve palsies and optic neuritis, may occur, but these are rare.

The prodromal stage is followed by the development of bilateral posterior uveitis, characterized by thickening of the posterior choroid with elevation of the peripapillary retinochoroidal layer, multiple serous retinal detachments, hyperemia, and edema of the optic nerve head (Fig. 10.1). Fluorescein angiography usually reveals multiple areas of focal leakage at the level of retinal pigment epithelium (RPE) and subretinal fluid accumulation. The inflammation eventually becomes diffuse and extends into the anterior segment. Flare and cells accumulate in the anterior chamber and vitreous. Less commonly, small nodules on the iris surface and papillary margin known as mutton-fat keratic precipitates, may be seen [20]. The inflammatory infiltrate in the ciliary body and peripheral choroid may cause forward displacement of the lens iris diaphragm, leading to acute angle-closure glaucoma or annular choroidal detachment [7].

The chronic or convalescent stage occurs several weeks after the acute uveitic stage. Vitiligo, poliosis, and depigmentation of the choroids occur. Perilimbal vitiligo, also known as Sugiura's sign, may develop at this stage; but this has been described mainly in Japanese

Table 10.1 Modified from the revised diagnostic criteria proposed by the 1st International Workshop on Vogt-Koyanagi-Harada Disease [29]

Complete Vogt-Koyanagi-Harada disease (criteria 1 to 3 must be present)
1. 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 diffuse choroiditis, 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 hyperfluorescence, 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 1a, and either both (2) and (3) below, or multiple signs from (3):
(2) Ocular depigmentation (either of the following manifestations is sufficient):
(a) Sunset-glow fundus, or
(b) Sugiura's sign.
(3) Other ocular signs:
(a) Nummular chorioretinal depigmented scars, or
(b) Retinal pigment epithelium clumping and/or migration, or
(c) Recurrent or chronic anterior uveitis
2. Neurological/auditory findings (may have resolved by time of examination).
a. Meningismus (malaise, fever, headaches, nausea, stiffness of the neck, or a combination of these factors; headaches alone is not sufficient to meet definition of meningismus, however), or
b. Tinnitus, or
c. Cerebrospinal fluid pleocytosis.
3. 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 and either 2 or 3 must be present).
1. Bilateral ocular involvement as defined for complete VKH above.
2. Neurologic/auditory findings; as defined for complete VKH above, or
3. Integumentary findings; as defined for complete VKH above.
Probable Vogt-Koyanagi-Harada disease (isolated ocular disease).
1. Bilateral ocular involvement as defined for complete VKH above.

There should not be a history of penetrating ocular injury or surgery preceding the onset of uveitis.

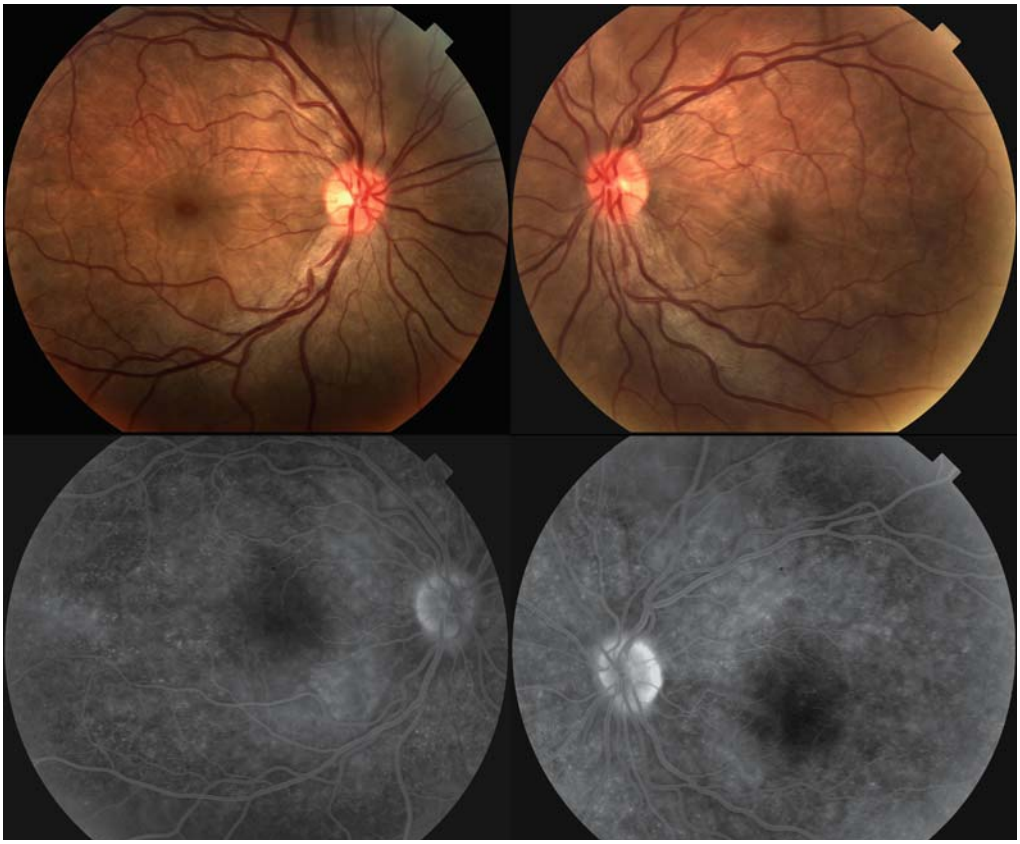

Fig. 10.1 36-year-old Hispanic female presented with bilateral optic disc hyperemia and multiple foci of retinal detachment. Fluorescein angiography reveals multiple pinpoint leaks at the level of the retinal pigment epithelium

patients [20]. Choroidal depigmentation occurs a few months later. This leads to the characteristic bright red-orange choroid known as sunset-glow fundus. The juxtapapillary area may show marked depigmentation. In Hispanics, areas of hypopigmentation and hyperpigmentation may be seen at the level of the retinal pigment epithelium. Yellow, well-circumscribed areas of chorioretinal atrophy may appear in the inferior midperiphery of the fundus. This phase may last for several months.

Smoldering panuveitis, with acute episodic exacerbations of granulomatous anterior uveitis, occurs in the chronic recurrent stage, and posterior uveitis with exudative retinal detachment is uncommon. Iris nodules in the form of round, whitish, well-circumscribed lesions on a background of atrophic iris stroma can be found. Choroidal neovascularization may occur, leading to severe vision loss [18]. Posterior subcapsular cataract, glaucoma (either angle-closure or open-angle), and posterior synechiae have been described [19]. Linear pigmentary changes similar to those seen in ocular histoplasmosis syndrome and arteriovenous anastomosis may be seen in occasional patients [20].

Neurologic, auditory, and integumentary manifestations appear at various stages of the disease. Sensitivity of scalp hair and, neurologic signs and symptoms are most common during the prodromal stage. Findings such as neck stiffness, headache and confusion, focal neurologic

signs, hemiparesis, transverse myelitis, and ciliary ganglionitis may be seen. Cerebrospinal fluid lymphocytic pleocytosis is common and may be found for up to 8 weeks [22].

Dysacusis, tinnitus and vertigo, and cochlear hearing loss develop in the early stage of the disease; this inner ear dysfunction improves in 2–3 months. Vestibular dysfunction is uncommon in VKH disease.

Poliosis of the eyebrows, eyelashes, and scalp hair and vitiligo occur during the convalescent stage and correspond closely with fundus depigmentation, and sunset-glow fundus. The vitiligo is often distributed symmetrically, involving the facial regions, eyelids, and trunk, and the skin over the sacrum [20]. Vitiligo is a variable finding and may be more common in certain races [20]. For example, the occurrence of such cutaneous and extraocular manifestations is relatively low in Hispanics, in spite of their typical ocular and neurologic manifestations [28].

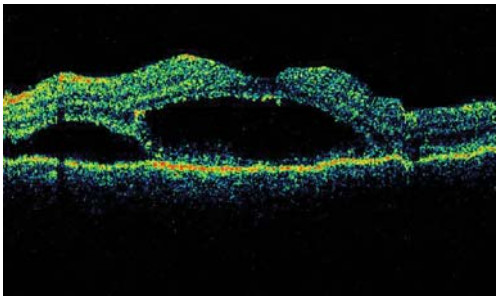

Fig. 10.2 Optical coherence tomography in Vogt-Koyanagi-Harada disease. Note retinal detachment at the posterior pole

10.2.4 Pathogenesis and Pathology

Vogt-Koyanagi-Harada disease is a non-necrotizing, diffuse granulomatous inflammation involving the uvea. Choroidal thickening is prominent in the juxtapapillary area, gradually decreasing toward the anterior part of the uvea. Histopathologic changes vary depending on the stage of the disease [7]. At the uveitic stage, granulomatous inflammation is present throughout the uvea and is characterized by diffuse infiltration of lymphocytes and macrophages, admixed with epithelioid cells and multinucleated giant cells. Occasional eosinophilic leukocytes may be noted in the uveal infiltrate. In the choroid, the infiltrating lymphocytes and macrophages are seen in close proximity to the uveal melanocytes. The epithelioid and giant cells may show engulfed pigment granules. Serous retinal detachments demonstrate the accumulation of proteinaceous fluid between the RPE and neural retina.

Accumulations of epithelioid histiocytes located between Bruch's membrane and the RPE, known as Dalen-Fuchs nodules, may be present. Choroidal infiltrates are predominantly composed of T-lymphocytes. Expression of class II major histocompatibility complex antigen on choroidal melanocytes and on the endothelium of the choriocapillaris has been described [31].

As the inflammatory process reaches the chronic stage, choroidal melanocytes decrease in number and disappear, creating the sunset-glow appearance of the fundus [14]. Histologic evaluation of the numerous, focal, yellowish oval or round lesions seen in the inferior peripheral fundus by ophthalmoscopy reveals focal loss of RPE cells and the formation of chorioretinal adhesions [14]. The choroidal inflammation tends to subside with the disappearance of the choroidal melanocytes; but a mild to moderate degree of lymphocytic infiltration remains in the ciliary body and iris. In the long-standing chronic recurrent stage, the RPE may reveal hyperplasia and fibrous metaplasia, with or without associated subretinal neovascularization [14]. Chorioretinal adhesions with inflammatory cell involvement of the choriocapillaris may be present [14, 23].

Although the exact cause of the inflammation directed at the melanocytes remains unknown, current evidence suggests that it involves an autoimmune process driven by T-lymphocytes. Sensitizations to melanocyte antigenic peptides by cutaneous injury or viral infection have been proposed as possible factors in some cases. The antigenic peptides may include tyrosinase or tyrosinase-related proteins [9, 12]. There is a strong association between the human leukocyte antigen (HLA) DR4, DR1, DRB1*0405, and DRB1*0410 and VKH disease [34].

10.2.5 Laboratory Investigations

When VKH disease presents without extraocular changes, fluorescein angiography, lumbar puncture, and ultrasonography are useful tests to establish the diagnosis. Fluorescein angiography in the acute stage reveals numerous punctuate hyperfluorescent dots at the level of RPE. These dots enlarge and stain the surrounding subretinal fluid. The late phase of the angiogram shows multiple serous retinal detachments with pooling of dye in the subretinal space. Over 70% of patients show disc leakage [20]. Retinal vascular leakage is rarely noted. In the chronic and recurrent stages of VKH disease, the angiogram takes on a *moth-eaten* appearance, with multiple hyperfluorescent RPE window defects without progressive staining.

Ohno et al. found that about 80% of VKH disease patients had cerebrospinal fluid pleocytosis, consisting mostly of lymphocytes [22]. This pleocytosis resolves within 8 weeks, even in those patients who develop recurrent intraocular inflammation.

Ultrasound findings include diffuse, low-to-medium reflective thickening of the posterior choroid, serous retinal detachment located in the posterior pole or inferiorly, and vitreous opacities. The choroidal thickening is most prominent in the peripapillary area, becoming progressively thinner away from the optic nerve. These findings are generally bilateral [6]. Ultrasound biomicroscopic examination during the uveitic stage may reveal a shallow anterior chamber, ciliochoroidal detachment, and a thickened ciliary body.

In the uveitic stage, indocyanine green angiography may reveal multiple hypofluorescent spots throughout the fundus and hyperfluorescent pinpoint changes in areas of exudative retinal detachment. In the chronic recurrent stage of the disease, multiple hypofluorescent spots are present; these spots may persist when the fundus appearance and fluorescein angiogram may not yield diagnostic clues [3]. Recently, optical coherence tomography has been used to monitor serous retinal detachment (Fig 10.2) and cystoid macular edema in patients treated with corticosteroids.

10.2.6 Differential Diagnosis

The differential diagnosis of VKH disease includes sympathetic ophthalmia, uveal effusion syndrome, posterior scleritis, primary intraocular lymphoma, uveal lymphoid infiltration, acute posterior multifocal placoid pigment epitheliopathy, and sarcoidosis [20, 25].

Uveal effusion syndrome may clinically mimic VKH disease. On angiography, the effusion syndrome may reveal numerous fluorescent blotches in the subretinal space. The syndrome can involve both eyes, although usually not simultaneously. The effusion syndrome differs from VKH disease in that there is a lack of intraocular inflammation. Posterior scleritis may present with pain, photophobia, and loss of vision, and the vitreous often reveals cells. Exudative macular detachment and choroidal folds may be noted. Ultrasonography can help differentiate posterior scleritis from VKH disease. In posterior scleritis there is flattening of the posterior aspect of the globe, thickening of the posterior sclera with high internal reflectivity of the thickened sclera, and retrobulbar edema.

Summary for the Clinician

- VKH disease and SO share virtually all of the same clinical and histopathologic features.
- A history of penetrating ocular injury is present in SO but not in VKH disease.

10.2.7 Treatment

High-dose systemic corticosteroids followed by slow tapering over 3–6 months is the treatment of choice for VKH disease [20]. Such treatment may prevent progression of the disease to the chronic recurrent stage and may reduce the incidence and/or severity of extraocular manifestations, including the development of sunset-glow fundus. A relapse of the ocular inflammation after tapering of systemic corticosteroids may reflect a too rapid tapering of

the corticosteroids. Recurrences become increasingly steroid-resistant, and cytotoxic/immunosuppressive agents are usually required to control the inflammation. High-dose oral corticosteroids, 1–2 mg/kg body weight or a 200 mg or higher dose of intravenous methylprednisolone for 3 days, followed by oral administration of corticosteroids (1–2 mg/kg body weight) with a slow taper are the mainstay of therapy for VKH disease [20]. Patients who receive either the intravenous corticosteroid treatment or the high-dose oral corticosteroids require gradual tapering of the corticosteroids over 3–6 months to prevent recurrences.

Although the initial episode of uveitis can be managed successfully in the majority of cases with intravenous and/or oral corticosteroids, recurrences do not respond as well to systemic corticosteroid treatment [20]. Such patients may show some initial response to sub-Tenon's injections of triamcinolone, but they usually require immunosuppressive or cytotoxic agents such as cyclosporine, azathioprine, cyclophosphamide, chlorambucil, mycophenolate mofetil (Cell Cept), and FK506.. Administration of the immunosuppressive and cytotoxic agents requires a pretreatment evaluation and follow-up examinations for any side effects associated with the therapy.

10.2.8 Prognosis and Complications

The complications of chronic recurrent VKH include cataract, glaucoma, choroidal neovascularization, subretinal fibrosis, and optic atrophy. At least 50% of eyes will develop at least one complication [30]. Forty-two percent of eyes develop cataracts, 27% develop glaucoma, 11% develop choroidal neovascularization, and 6% develop subretinal fibrosis. Those patients who developed these complications had a significantly longer median duration of disease and significantly more recurrences than patients who developed no complications. Moreover, eyes with better visual acuity at presentation had better visual acuity at final follow-up, and patients who developed VKH disease at an advanced age had a worse visual acuity [30].

There is general agreement that any cataract surgery that is needed should be delayed until the intraocular inflammation has subsided, at which time safe cataract extraction with posterior chamber intraocular lens implantation can be successfully accomplished. Occasionally, patients with significant vitreous opacities and debris may require a combined procedure of pars plana vitrectomy and lensectomy [19].

Chronic recurrent anterior uveitis and fundus pigmentary disturbances seem to predispose patients to the development of choroidal neovascularization [18]. Indocyanine green angiography is useful for detecting neovascular membranes, and photocoagulation may help in the management of the disease. Photodynamic therapy with verteporfin for subfoveal choroidal neovascularization has been attempted with some success. Although visual prognosis varies depending on the above complications, the majority of patients end up with a visual acuity of 20/40 or better [20].

In summary, VKH disease is a bilateral, granulomatous panuveitis that usually presents with serous retinal detachment and meningeal irritation. The diagnosis of atypical cases may require the use of fluorescein angiography, lumbar puncture, and ultrasonography. Vogt-Koyanagi-Harada disease is treated initially with high-dose systemic corticosteroids, with a gradual tapering over 3–6 months. Complications of this disease include cataract, glaucoma, choroidal neovascularization, and subretinal fibrosis.

10.3 Sympathetic Ophthalmia

Sympathetic ophthalmia (SO) is a rare bilateral, diffuse granulomatous uveitis that occurs a few days to several decades after penetrating trauma to an eye. The injured eye is referred to as the *exciting* eye, and the fellow eye is referred to as the *sympathizing* eye. The clinical signs and symptoms are usually detected in the sympathizing eye within the first 3 months of injury to the fellow eye. The reported incidence of SO varies from 0.19% following penetrating injuries to 0.01% following vitrectomy. Surgical procedures that may lead to sympathetic ophthalmia include cataract extraction, cyclodialysis, iridectomy,

paracentesis, retinal detachment repair, keratectomy, vitrectomy, and laser cyclocoagulation [1, 5, 8, 27]. This intraocular inflammation can occur in all age groups, and there is no sex predilection.

Untreated SO runs a long, variable, and complicated course marked initially by episodes of acute inflammation followed by quiescent periods that can last several months to several years. With time, the disease becomes chronically active, eventually producing irreversible ocular damage and leading to phthisis bulbi.

10.3.1 Clinical Features

The onset of an SO is heralded by the development of inflammation in the sympathizing eye and a worsening of inflammation in the exciting eye. Symptoms in the sympathizing eye include photophobia, blurring of vision, or paresis of accommodation. The exciting eye may have a decrease in vision and an increase in photophobia. Moreover, both eyes may show ciliary injection, and the exciting eye may reveal keratic precipitates.

Posterior segment findings in SO include papillitis, generalized retinal edema, and small yellow-white exudates beneath the retinal pigment epithelium, known as Dalen-Fuchs nodules. The fundus may show exudative retinal detachments and occasionally multiple choroidal granulomas.

The interval between the trauma and the onset of SO has been reported to be as short as 5 days and as long as 66 years after trauma. In general, however, SO rarely occurs less than 2 weeks after trauma, with 80% of cases occurring within 3 months and 90% within 1 year of injury [10, 15, 17].

The diagnosis of SO is a clinical one. Fluorescein angiography may sometimes be quite helpful in establishing the diagnosis. It typically shows persistent multiple fluorescing dots at the level of the RPE in the venous phase. Coalescence of the dye from these foci may occur if there are areas of exudative detachment [8, 17]. Less frequently, there may be early focal obscurations of the background choroidal fluorescence, with later staining similar to the angiographic findings noted in acute posterior multifocal placoid pigment epitheliopathy. Numerous hypofluorescent dark dots may be visible during the intermediate phase of indocyanine green angiography. Some of these dots may become isofluorescent in the late phase.

10.3.2 Pathology and Pathogenesis

The pathologic alterations of SO typically consist of a uveal diffuse granulomatous inflammation made up of lymphocytic infiltration with nests of pigment-containing epithelioid cells and giant cells. In most cases the inflammatory process does not involve the choriocapillaris or the retina. Absence of necrosis is another characteristic feature. The pathologic changes are similar in both the exciting and the sympathizing eye. Eosinophils can also be found and are frequently concentrated in the inner choroids, particularly in heavily pigmented individuals. Nodular clusters of epithelioid cells containing pigment (Dalen-Fuchs nodules) are often seen lying between the RPE and Bruch's membrane; these may appear clinically as drusen-like, yellow-white dots.

Nongranulomatous choroiditis or chorioretinal adhesion, with an inflammatory process involving choriocapillaris, similar to the histologic features of chronic VKH disease, may be seen in chronic cases of SO. Immunohistochemical studies have revealed infiltration of predominantly T-lymphocytes in the uveal tract. Both helper and suppressor/cytotoxic lymphocytes are observed.

The exact cause of SO is unknown. The predominant predisposing factors are accidental or surgical penetrating trauma [24, 26]. A small number of cases are the result of cyclodestructive procedures, contusion injuries with occult scleral rupture and perforating corneal ulcers. The common denominator in the overwhelming majority of cases is the presence of a penetrating injury in which wound healing is complicated by incarceration of uvea. Experimental studies suggest that a breakdown in tolerance to uveal melanin peptide (tyrosinase) might initiate the inflammation. The inflammatory process appears to be directed at the uveal melanocytes. A recent study from Japan reported a significant association between HLA-

DRBI*04 and -DQB1*04 and SO. A similar association was seen in patients with VKH disease.

10.3.3 Differential Diagnosis

Vogt-Koyanagi-Harada disease and phacoanaphylaxis can closely simulate the clinical picture of SO. Although unilaterality may be a clue, phacoanaphylaxis is not invariably unilateral. A careful slit-lamp examination should always be carried out to search for ruptured lens capsule and pieces of lens cortex in the anterior chamber. A history of penetrating trauma is helpful in differentiating SO from VKH disease.

10.3.4 Therapy

The effective management of sympathetic ophthalmia is the prevention of its occurrence, which entails careful microsurgical wound toilet and prompt closure of all penetrating injuries. In eyes with barely discernible or no visual function, or with demonstrable disorganization of the ocular contents, enucleation within 2 weeks of injury has long been advised to prevent the development of SO.

Enucleation of the exciting eye once SO has commenced remains a topic of considerable controversy. Indeed, it is possible that the exciting eye may eventually provide the better visual acuity, and its enucleation would therefore deprive the patient of that visual potential.

Once SO commences, treatment is similar to the management of VKH disease with initial high-dose systemic corticosteroids followed by a gradual tapering of the corticosteroids once the inflammation subsides. Some patients may also require immunosuppressive/cytotoxic agents.

Summary for the Clinician

- Both VKH disease and SO are managed initially with high-dose systemic corticosteroids. The patients with chronic disease require additional immunosuppressive/cytotoxic agents.
- Both conditions require long-term treatment to avoid recurrences and complications.
- Extraocular manifestations are rare during the acute phase of VKH disease except for signs of meningeal irritation.
- Fluorescein angiography of the retina, lumbar puncture for cerebrospinal fluid analysis and ultrasonography of the globe are helpful in confirming the diagnosis of VKH disease during the acute phase.
- Both VKH disease and SO are managed initially with high-dose systemic corticosteroids. The patients with chronic disease require additional immunosuppressive/cytotoxic agents which may include methotrexate, azathioprine, mycophenolate mofetil, and cyclophosphamide.
- In patients with VKH disease, early intervention with systemic corticosteroid administration and slow tapering of corticosteroids over 3–6 months may prevent development of the chronic phase of the disease.
- Both conditions require long-term treatment to avoid recurrences and complications.

10.3.5 Complications and Prognosis

The long-term complications of this ocular inflammation include the development of cataracts, secondary glaucoma, exudative retinal detachments, chorioretinal scarring, and optic atrophy. With prompt and aggressive corticosteroid therapy, and with the use of immunosuppressive

agents in some cases, many eyes with SO retain useful vision of 20/40 or better.

In conclusion, SO is a serious entity, often with many exacerbations and a relentlessly progressive course that frequently results in very poor vision. Long-term follow-up of these patients is essential. It is hoped that with the use of large-dose steroid therapy early in the course of the disease, and supplementation with immunosuppressive agents when indicated, the prognosis for these patients need not be as grim as it has traditionally been.

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Chapter 11

Neoplastic Masquerade Syndromes

11

Sarah E. Coupland

Core Messages

- Neoplastic masquerade syndrome can be defined as neoplastic or proliferative lesions that cause intraocular infiltration of cells, simulating immune-mediated uveitis.
- The most frequent neoplastic "masquerader" is primary intraocular or retinal lymphoma. Less common lymphoid malignancies that can present with signs similar to uveitis include primary choroidal lymphoma, primary iridal lymphoma or secondary intraocular infiltration of a systemic lymphoma.
- Particularly primary retinal lymphoma should be excluded in all elderly patients presenting with a chronic steroid-resistant uveitis.
- Cytological examination (cytomorphology and immunocytology) of rapidly-transported vitreous specimens remains the first line investigation in the exclusion of neoplastic disease in patients with idiopathic chronic uveitis. Although associated with higher morbidity risks, chorioretinal biopsies increase the chances of diagnosing or excluding an intraocular lymphoma. Various techniques, such as IgH-PCR and TCR-γ-PCR, prove to be useful adjuncts to the basic investigations for diagnosing intraocular lymphoma.
- Primary retinal lymphoma is a high-grade lymphoma and demonstrates a very aggressive course, with the development of cerebral manifestation in a large number of patients. In contrast, primary choroidal lymphoma is a low-grade lymphoma and has a good prognosis.
- Other nonlymphoid malignancies that can stimulate immune-mediated uveitis include diffuse amelanotic melanoma, metastatic tumor to the choroid and retina as well as non-neoplastic proliferative diseases of the uvea, such as juvenile xanthogranuloma.

11.1 Introduction

The term "masquerade syndrome" is used in ophthalmology to describe conditions that are characterized by intraocular infiltration of inflammatory cells, simulating immune-mediated uveitis. The "masquerade" syndrome is considered by some to include disease entities such as fungal or chronic bacterial endophthalmitis as well as conditions such as retinitis pigmentosa, ocular ischemic syndrome, and chronic peripheral retinal detachment, which can result in intraocular migration of cells.

The following chapter will concentrate on neoplastic conditions, which cause infiltration of cells into the intraocular space. The neoplastic entities can be divided into lymphoid and nonlymphoid malignancies within either the retina or the uvea. Emphasis will be placed on the former diseases. The lymphoid malignancies occurring in the eye can be further subdivided into:

1. "Primary intraocular (retinal) lymphomas," i.e., those lymphomas predominantly involving the retina and the vitreous;
2 Primary uveal lymphomas;
3. Secondary intraocular (mainly choroidal) involvement of systemic lymphoma, usually leukemia (Table 11.1) [5, 19, 22, 29, 30, 69, 91, 119, 125, 130].

Nonlymphoid malignancies, which can cause a neoplastic masquerade syndrome, include diffuse amelanotic melanoma, metastatic tumor to the choroids and retina, and non-neoplastic proliferative diseases of the uvea, such as juvenile xanthogranuloma.

11.2 Lymphoid Malignancies

11.2.1 Primary Intraocular Lymphoma

Primary intraocular lymphoma (PIOL) – or possibly better termed primary retinal lymphoma – is a high-grade malignant non-Hodgkin's lymphoma (NHL), arising in the retina with involvement of the vitreous and, occasionally, optic nerve [119, 152]. It is the most common "masquerader" according to recent large series investigating the neoplastic masquerade syndromes [126]. PIOL is considered to be a subtype of the primary central nervous system lymphoma (PCNSL), and when occurring simultaneously in patients with PCNSL, the entity is named "oculocerebral lymphoma." Most PIOL are of B cell origin [16, 38, 87, 111], and can be subtyped as diffuse large cell B cell lymphomas (DLBCL), according to the updated World Health Organization (WHO) Lymphoma Classification [82]. Intraocular lymphoma of T cell type is rare, although its existence is becoming increasingly recognized. Most reported cases of intraocular T cell lymphoma, however, represent a secondary manifestation of mycosis fungoides (primary cutaneous T cell lymphoma) or of a systemic T cell lymphoma in conjunction with systemic leukemia (ATL/L) and are associated with human T cell lymphotropic virus type-1 (HTLV1) infection or with acquired immunodeficiency syndrome [8, 16, 21, 28, 38, 49, 55, 60, 66, 67, 80, 86, 89, 90, 94, 97, 99, 102, 122, 123, 129, 137, 139, 155–158]. Very few cases of true primary retinal lymphoma of T cell type, i.e., an intraocular T cell lymphoma whereby neither a cutaneous nor a systemic T-NHL has been diagnosed within 6 months of the intraocular lymphoma manifestation, have been reported in the literature [32, 67, 102].

Table 11.1 Intraocular lymphoma manifestations and relationship to anatomical site. *DLBCL* diffuse large cell B cell lymphoma, *EMZL* extranodal marginal zone B cell lymphoma, *MALT* mucosa-associated lymphoid tissue, *NHL* non-Hodgkin's lymphoma

	Anatomical location	Lymphoma subtype[a]	Immunophenotype
Primary intraocular (retinal) lymphoma	Usually retina in perivascular location or in subretinal space	Usually DLBCL (T cell lymphomas rare)	CD79α+, CD20+, PAX5+, BCL2+/–, BCL6+/– Oct2+, BOB.1+, MUM1+, CD10–/+ MIB1 often >60%
Primary choroid lymphoma	Choroid	EMZL of MALT type	CD79 α+, CD20+, PAX5+, CD43+ mostly, BCL2+, CD10–, BCL6–, cyclin D1–, IgM+, MIB1 5%, maximum 15%
Primary iridal lymphoma	Iris	Usually diffuse large cell lymphoma (T=B)	Dependent upon lymphocyte derivation
Secondary intraocular lymphoma	Usually choroid	Dependent on systemic NHL	Dependent on systemic NHL

[a]According to the new WHO lymphoma classification [82]

11.2.1.1 Epidemiology of PIOL

The exact incidence of PIOL is not known. According to estimates from 1987, it is reported to represent 4-6% of all intracranial tumors, and approximately 1–2% of all extranodal NHL [58]. Although it has been described in some young patients [151, 160], PIOL typically affects those between the fifth and sixth decades [8, 10, 31, 58, 117, 152]. Women are more often affected than men (up to 2:1), and there is no obvious racial predilection [10, 31, 58, 117, 152]. PIOL may be either unilateral or bilateral on initial presentation; however, the vast majority of patients will ultimately develop a bilateral manifestation [15, 17, 58, 65, 117, 152]. Intracranial lymphoma develops in 60-85% of patients with initial ocular disease, usually within the first 2 years of diagnosis [1, 16, 27, 31, 58, 152]. In turn, approximately 15–25% of patients with PCNSL will develop ocular disease [40, 79, 117, 149]. Systemic spread outside the CNS or ocular tissues rarely occurs; it is reported in fewer than 10% of autopsies [77, 110].

An inexplicable increase in the incidence of PCNSL has been reported over the last 15 years in both immunocompetent and immunosuppressed patients, with a clear male predominance [26]. This increase, which has been reported to be up to three-fold, can be only accounted for to a certain extent by the human immunodeficiency virus [45].

Summary for the Clinician

- Primary intraocular (retinal) lymphoma is considered a subset of primary central nervous system lymphoma, with the majority of the lymphomas being high-grade malignant B cell lymphomas.
- Primary intraocular (retinal) lymphoma occurs predominantly in patients between 50 and 60 years of age.
- The vast majority of patients with primary intraocular lymphoma develop central nervous system involvement.

11.2.1.2 Symptoms and Signs of PIOL

When occurring prior to CNS disease, primary intraocular (retinal) lymphoma frequently presents as bilateral idiopathic steroid-resistant chronic uveitis, possibly with accompanying vitritis [1, 8, 9, 31, 58, 64, 65, 117, 121, 126]. Commonly reported symptoms and signs are blurred vision, a painless loss of vision, "floaters," photophobia, and/or red eyes. Less frequent signs include exudative retinal detachment, fundus mass, glaucoma, neovascularization, optic nerve neuropathy, and varying chorioretinal abnormalities [58].

Involvement of the CNS by tumor cells causes nonspecific symptoms and signs with the most frequent single symptom being "behavioral change" [40, 53, 78]. The most common focal neurologic signs include hemiparesis in 40-50% and cerebellar signs (e.g., ataxia) in 15–40% [40, 53, 78]. Seeding of lymphoma cells into the cerebral spinal fluid has been reported in up to 42% of patients with PCNSL [53]. Systemic spread of PIOL/PCNSL is infrequent, although it has been reported to occur [77]. The reason for the PIOL/PCNSL tropism to the central nervous system is unclear, but may be due to a number of certain factors such as the development of a "neurotropic" cellular phenotype, or it may be as a result of the influence of chemokine receptors and their ligands [13, 84].

11.2.1.3 Ophthalmic Findings in PIOL

11.2.1.3.1 Anterior Segment

Anterior segment findings are observed in up to 43% of patients with PIOL [10]. Common findings include corneal precipitates, mild anterior flare, and a pseudohypopyon (Fig. 11.1a) [15, 16, 58, 117, 152]. Most often the posterior segment changes (see Sect. 11.2.1.3.2) precede the anterior segment findings; however, occasionally, anterior segment disease can be the initial presentation of PIOL. Secondary anterior segment changes include neovascularization of the iris and iridocorneal angle with possible glaucoma. In rare

circumstances, PIOL can cause a mass in the iris following secondary infiltration [148, 150].

11.2.1.3.2 Posterior Segment

Vitreous cells and haze ("vitritis") are typical findings, and are present in the majority of cases [15, 17, 58, 117, 152]. The characteristic fundus lesion is a flat creamy orange-yellow subretinal mass. These lesions may be single or multiple, discrete or confluent [15, 17, 58, 63, 85, 117, 152]. The presence of multiple subretinal pigment epithelial masses is considered by some clinicians to be pathognomic of PIOL [39, 63]. Rarely, PIOL presents as a single solitary intraocular mass [57, 100].

11.2.1.4 Diagnostic Techniques

The diagnosis of PIOL can be suspected on fundoscopy when the above-described "classical" retinal or subretinal infiltrates are present. However, additional examinations, such as ultrasonography [144], fluorescein angiography [39, 63, 98, 117, 147], and/or high-resolution neuroimaging of the CNS, are usually performed to support the diagnosis [121].

Ultrasonography enables analysis of the posterior segment of eyes with opaque media; although the findings are not specific, they help to narrow the differential diagnosis. Similarly, fluorescein angiography provides information with regard to the location of the infiltrative process (retina versus choroid), and demonstrates any retinal pigment epithelium (RPE) disturbance in suspected PIOL patients. Due to a blocking effect of sub-RPE tumor masses, hypofluorescent areas may be observed on fluorescein angiography. Alternatively, hyperfluorescent window defects may occur due to atrophy of the RPE. The mixed picture of hyper- and hypofluorescence on fluorescein angiography can result in a "leopard-skin" appearance, considered to be highly indicative of PIOL (Fig. 11.1b).

Neuroimaging studies include computed tomography scans (CT) scans, magnetic resonance imaging (MRI), and positron emission tomography (PET). Current opinion is that MRI is superior to CT scans in detecting lymphoid lesions in the CNS; however, both are limited in the evaluation of any ophthalmic disease [96].

Summary for the Clinician

- Primary intraocular (retinal) lymphoma presents typically as bilateral uveitis that demonstrates no response to steroid therapy.
- Painless loss of vision, blurred vision, and "floaters" are among the most common symptoms.
- Anterior chamber involvement occurs in at least one-third of primary intraocular lymphoma patients.
- Multiple yellow-orange subretinal lesions, with the corresponding "leopard skin" appearance on fluorescein angiography, are considered to be pathognomic for PIOL.

11.2.1.5 Laboratory Studies: Cytological and Histological Diagnosis in PIOL

11.2.1.5.1 Vitreous Biopsy

Cytological studies of vitreous biopsies remain the first line of investigation in the morphological diagnosis of PIOL. Such specimens are obtained by fine needle aspiration [51, 93, 114], vitreal aspiration [102] or via pars plana vitrectomy (PPV) [109, 113].

Vitreous specimens are renowned for containing small numbers of cells with either a reactive cellular infiltrate and/or a necrotic "dirty" background (Fig. 11.1c). Consequently, they can be very demanding and require experience both in their preparation as well as in the cytomorphological interpretation. It is well documented in the literature that "false negative" diagnoses are not uncommon [16, 31, 58, 121, 126, 152], and multiple specimens may be required before a definitive diagnosis of PIOL can be made [31, 121, 126, 152]. Due to the varying and deceptive

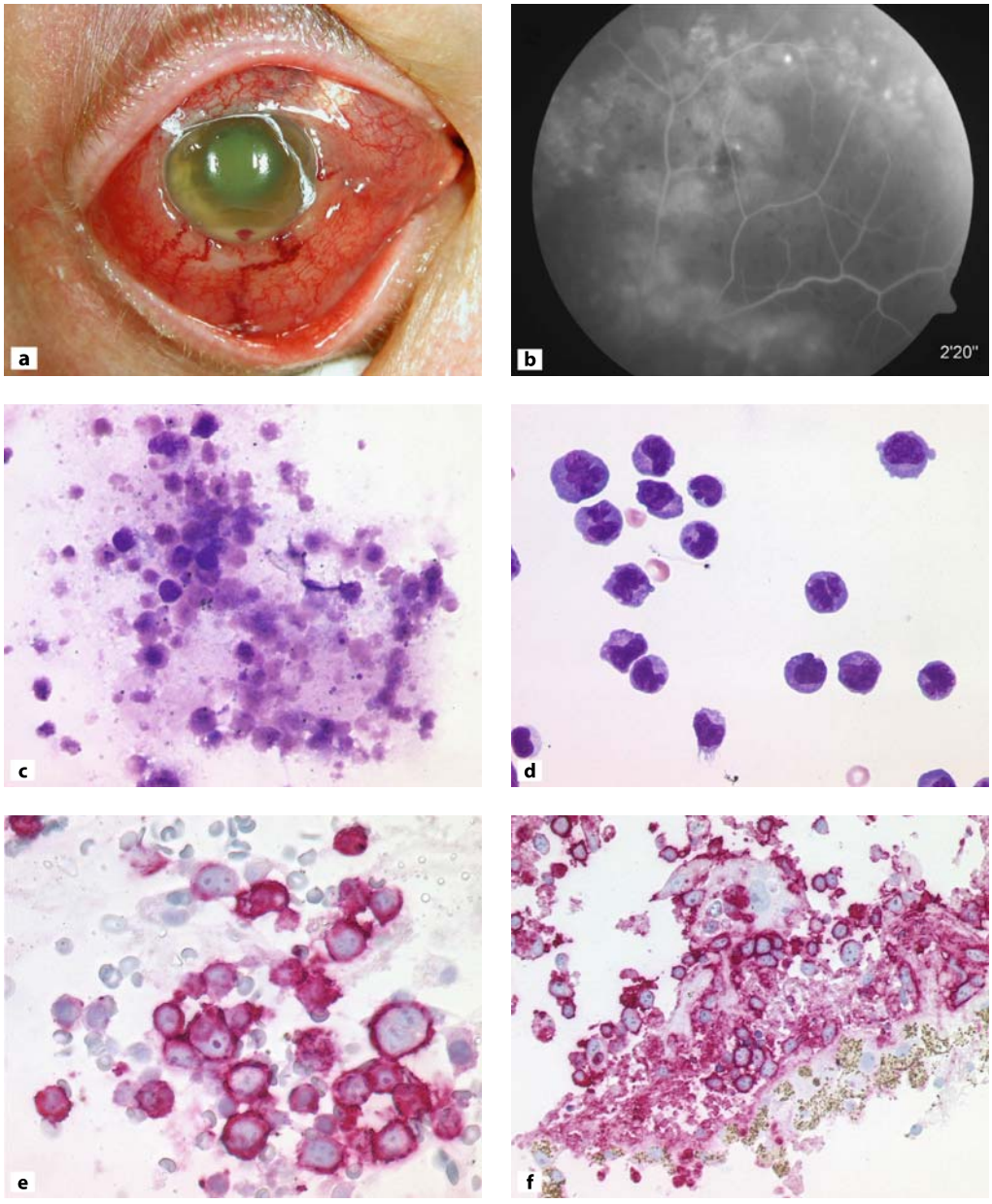


Fig. 11.1 **a** Pseudohypopyon, conjunctival injection and edema as presenting signs of a primary retinal lymphoma in an elderly male patient (courtesy of Prof. N. Bornfeld, University Eye Clinic, Essen, Germany). **b** Characteristic "leopard skin" fluorescein angiographic findings of primary retinal lymphoma. **c** Vitreous aspirate consisting of many lytic cells creating a necrotic "dirty background" with occasional vital pleomorphic tumor cells (May Grunewald Giemsa [MGG] stain, original magnification ×200). **d** Numerous tumor cells in a vitreous aspirate in a patient with primary retinal lymphoma of T cell type (MGG stain, original magnification ×200). **e** Vitreous aspirate of a primary retinal lymphoma of B cell type, with positivity of the tumor cells for CD20 (Alkaline phosphatase anti-alkaline phosphatase [APAAP] staining, original magnification ×400). **f** Corresponding chorioretinal biopsy with extensive infiltration by the neoplastic B cells (APAAP staining, original magnification ×400)

modes of presentation of PIOL as well as to the above-mentioned interpretational difficulties, both clinical and morphological diagnoses are often delayed, according to the literature by between 8 and 21 months [10, 31, 58, 80, 126, 152].

The preparation of vitreous specimens for cytological evaluation varies among laboratories [16, 31, 37, 48, 106, 109, 131]. We are most familiar with the cytospin preparation technique using both unfixed and fixed specimens. The former must be rapidly transported to the cytological laboratory [31]. The latter biopsies, fixed in solutions such as CytoLyt or "H.O.P.E." fixation (Hepes-glutamic acid buffer-mediated organic solvent protectant effect), can be sent via the usual mail, and allow for further examinations such as immunocytology and molecular biological studies [143].

11.2.1.5.2 Cytologic Diagnosis

Vitreous aspirates in PIOL are mildly to moderately cellular, and comprise mature inflammatory cells such as macrophages, small lymphocytes with scattered large atypical lymphocytes and, possibly, fibrinous or necrotic material in the background [114]. The neoplastic cells are usually pleomorphic showing hyperchromatic nuclei with irregular contours and prominent, sometimes multiple, nucleoli (Fig. 11.1d). The cytoplasmic rim is usually narrow or absent. Due to the fragility of neoplastic lymphocytes, a specimen may contain numerous lytic cells. These can cause difficulty in interpreting the immunocytology, as a result of nonspecific background staining [101]. The neoplastic cells are usually positive for B cell antigens, such as CD20, CD79α or PAX-5 (Fig. 11.1e).

11.2.1.5.3 Chorioretinal Biopsy and Enucleation

If vitreous samples fail to demonstrate lymphoma cells, increasingly retinal and chorioretinal biopsies or subretinal aspiration are performed in patients with the subretinal infiltrates considered typical for PIOL [4, 20, 31, 92, 98, 106, 115, 121]. Indeed, in some centers, a chorioretinal biopsy and a vitreous biopsy are performed in the same initial operative procedure. Rarely, enucleation is unavoidable when a blind and painful eye develops due to secondary glaucoma and/or complete retinal detachment, and thus can lead to the diagnosis of malignant lymphoma.

Conventional histology of B-PIOL in the chorioretinal biopsy demonstrates an infiltrate of medium-sized to large atypical lymphocytes in the retina. The neoplastic cells usually have basophilic cytoplasm, reniform-shaped nuclei, and prominent nucleoli. A large number of mitotic figures and apoptotic bodies can be observed. In the enucleated eye, clusters of cells may be seen in the neurosensory retina (often perivascularly), in the subretinal space, and between the RPE and Bruch's membrane. The amount of necrosis within the retina can be extensive. Reactive lymphocytic infiltrates, consisting mainly of T cells, and polyclonal plasma cells may be seen in the adjacent choroid.

The tumor cells are positive for the above-mentioned B cell antigens (Fig. 11.1f), usually demonstrate monotypical expression of an immunoglobulin molecule light and/or heavy chain, and demonstrate a large growth fraction (average 80%) using the MIB1 antibody, directed against the Ki-67 antigen. In addition, B-PIOL cells express immunoglobulin transcription factors such as BCL-2, BCL-6, multiple myeloma protein 1 (MUM1; also known as IRF4), Oct2, and BOB.1 [35]. The differing expression patterns and frequencies of these proteins in PIOL/PCNSL compared with peripheral DLBCL provide further evidence of the notion that "peripherally" and "centrally" located DLBCL differ in clinical, immunophenotypic, and genotypic features, despite their similar morphological characteristics [35].

11.2.1.6 Biochemical and Molecular Analysis of PIOL

In addition to cytomorphology and immunocytology, some centers suggest adjunct investigations such as flow cytometry [38], determination of cytokine concentrations, particularly interleukin-10 (IL-10) [10, 11, 153], polymerase chain reaction (PCR) examining for monoclonal rear-

rangements of immmunoglobulin heavy (IgH) or light (IgL) chains in B cell lymphoma, or T cell receptor genes in T cell lymphoma [31, 88, 132, 154], as well as determination of CDR3 (complementary determining regions) polymorphisms in the variable region of the immunoglobulin gene [68].

Of these, we are most familiar with the assessment of clonality in PIOL using IgH-PCR and TCR-γ-PCR. Demonstration of gene rearrangements, which do not occur at any significant frequency in normal B or T cell differentiation, is strong evidence for the diagnosis of PIOL. These examinations can be performed on unfixed vitreous specimens as well as on fixed and paraffin-embedded tissue biopsies [31, 34]. They should always be performed with a positive and negative control, and preferably repeated for reproducibility. The results of the IgH-PCR and TCR-PCR should be interpreted by someone with experience in the technique, and always in association with the cytomorphology. This is particularly the case with paucicellular vitreous biopsies where "pseudoclones" could be amplified. In enucleated eyes where the tumor cell population may be relatively small compared with the reactive cell population, isolated removal of the tumor cells – either by cutting into the paraffin block ("macrodissection") or from the cut section ("microdissection") [132] – may increase the possibility of obtaining an interpretable DNA amplification product. Newer primers have increased the chances of primer binding in IgH-PCR and TCR-PCR, and, therefore, of detecting neoplastic populations of B and T lymphocytes respectively [146].

Finally, it is possible to perform DNA sequencing of the PCR products to evaluate the somatic mutations in the variable (V) region of the immunoglobulin genes. We showed a high frequency of somatic mutations in the VH genes of PIOL [34]. These data, together with the above-described tumor cell immunophenotype (PAX5/BSAP+, CD20+, MUM1/IRF4+, BCL-6+/-, CD10-/+) [35], would suggest that PIOL of B cell type are derived from mature B cells that have undergone a prolonged interaction in the microenvironment of the germinal center, and are either at the late germinal center stage of differentiation or represent early postgerminal center B cells. Further investigations with a larger number of tissue specimens, however, are required to underline this hypothesis, and to determine which proteins are of diagnostic and prognostic significance.

Summary for the Clinician

- Vitreous biopsy remains the first line of investigation in PIOL diagnosis establishment.
- Both unfixed and fixed vitreous specimens should be rapidly transported to the cytology laboratory, although the latter can be sent using the usual postal system. Close communication between clinician and pathologist is essential to allow for optimal processing of these biopsies.
- Basic evaluations consist of morphological and immunocytological investigations. Additional procedures include PCR for rearrangements of the heavy chain immunoglobulin chain gene, flow cytometry analysis, biochemical analysis determining the cytokine ratios, and determination of CDR3 (complementary determining regions) polymorphisms in the variable region of the immunoglobulin gene.
- Increasingly, chorioretinal biopsies are being performed to achieve an unequivocal diagnosis. These have allowed for more detailed analyses with an attempt to better understand the histogenesis of PIOL.

11.2.1.7 Treatment of PIOL

The treatment recommendations for PIOL with or without CNS disease are constantly in flux and remain controversial. Standard protocols of isolated radiotherapy as well as chemotherapy using traditional regimens are ineffective for the adequate long-term treatment of PCNS/PIOL. In the past, radiotherapy alone to the eyes and CNS was the main form of treatment for PIOL/

PCNSL, due to the sensitivity of lymphoma cells to radiation. Although it gave high rates of initial response, most patients usually succumbed to recurrent disease [105]. Furthermore, ocular radiation was associated with delayed toxicity, including radiation retinopathy, optic neuropathy, dry eye, corneal epithelial defects, loss of limbal stem cells, cataracts, and glaucoma [9]. Significant advances were achieved with the introduction of combined chemoradiation to the CNS and reduced ocular radiation [15]. With multimodality therapy, including a boosted radiation dose (5–10 Gy) to the spinal cord and intrathecal methotrexate, vision could be improved and life prolonged. Recent innovations in treatment include multi-agent primary chemotherapy. This approach was designed to reduce radiation-associated cognitive defects that can occur in up to 40% of patients above 50 years of age [59]. The regimen included methotrexate and procarbazine, and some patients also received vincristine, thiotepa, or both vincristine and cytarabine. Some patients required radiation or further chemotherapy for relapse, but complete remission was seen for as long as 30 months. Others have augmented standard primary treatment by chemoradiation with systemic methotrexate and cytarabine, observing 3 out of 3 cases with a 24-month complete remission [145]. Cytarabine and methotrexate have been used in combination for salvage [118]. Other alternative therapies that require further assessment include intravitreal methotrexate and trofosfamide. Intravitreal methotrexate has been used to reduce the extent of intraocular tumors in patients who have undergone chemotherapy with or without radiation with successful preservation of vision [54, 75]. Similarly, trofosfamide has been administered in patients with recurrent PIOL/PCNSL: it may offer an alternative treatment option for PIOL with a very favorable side effect profile [83].

The management of patients with intraocular lymphoma only is also controversial. Localized radiation to one or both eyes is usually performed. Significant morbidity, however, is associated with irradiation. Promising results for PIOL were obtained with high-dose chemotherapy followed by autologous bone marrow [136].

11.2.1.8 Prognosis of PIOL

Many patients with PIOL succumb to CNS disease within 2 years; however, the median survival of PIOL/PCNSL has increased from 1.0–1.5 to over 3 years with newer therapies [41]. Due to the relative rarity of this disease, the data on prognostic parameters in PIOL/PCNSL is limited to small, usually single-center series. Some authors suggest that tumor cell positivity for BCL-6 protein in PCNSL is a predictor of a poorer prognosis [53]. Furthermore, it has been suggested that PIOL with the translocation t(14;18) might be more aggressive clinically [12]. Larger multicenter studies with clinically well-defined parameters are required to determine if particular proto-oncogenes and chromosomal translocations are of prognostic value. Finally, such studies would also help determine if various subtypes of PIOL/PCNSL actually exist, and whether these should be treated differently.

Summary for the Clinician

- Before commencement of treatment of all PIOL patients, extensive "staging" examinations should be performed to determine the extent of disease (i.e. CNS involvement) and to exclude a secondary ocular involvement of a previously unknown systemic lymphoma.
- Treatment of PIOL remains controversial due to the limited number of patients and, mostly, single-center conducted trials.
- Although the prognosis of patients with PIOL/PCNSL is generally poor, newer therapies provide optimism in prolonging their life expectancy.

11.2.2 Primary Uveal Lymphomas

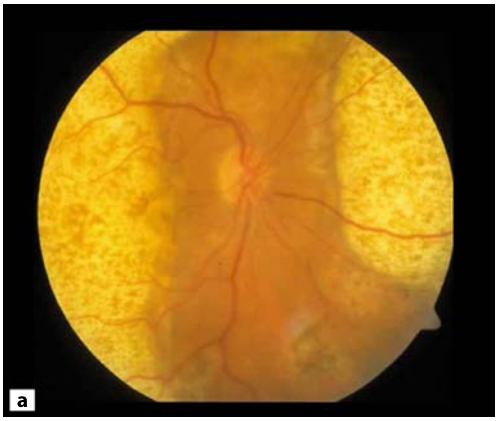
Primary uveal lymphomas are probably the rarest intraocular lymphomas: they can be divided into those arising primarily in the choroid and

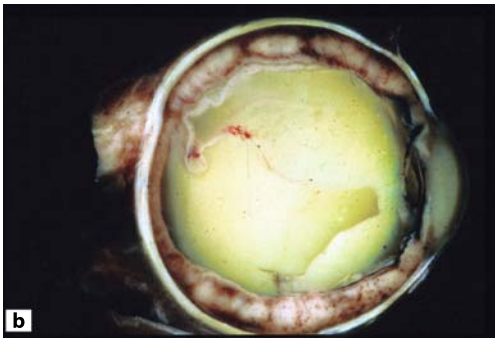
those even more exceptionally rare cases of primary lymphomas with their origin in the iris.

11.2.2.1 Primary Choroidal Lymphoma

Approximately 80 cases of primary choroidal lymphoma have been described since their original description in 1920 [3, 5, 14, 18, 19, 23, 24, 30, 36, 42–44, 50, 62, 69, 81, 85, 108, 127, 128, 134, 135, 142, 161]. They are considered to be tumors with their origin in the choroid, due to the absence of systemic disease at the time of diagnosis, and to their unilaterality in most patients. Most primary choroidal lymphomas are low-grade and are clinically indolent. Consequently, they have been termed "pseudotumors" and "reactive lymphoid hyperplasia" of the uvea in the past. They usually occur unilaterally in men in the fifth decade of life. Typical presenting symptoms include recurrent episodes of blurred vision, painless loss of vision as well as metamorphopsia subsequent to secondary serous detachment of the macula. The key early signs of primary choroidal lymphoma include the creamy choroidal infiltrates on fundus examination with low echogenicity on ophthalmic ultrasound [19, 85]. There may be an initial response to steroid therapy. Ultimately, a diffuse thickening of the uveal tract becomes obvious on fundoscopy (Fig. 11.2a), and, in some patients, subconjunctival or episcleral extension may occur [14, 19, 81, 128].

In the majority of cases of primary choroidal lymphoma reported in the literature, the eyes were ultimately enucleated either due to clinical difficulties in determining the nature of the uveal mass, or due to pain as a result of secondary glaucoma (Fig. 11.2b). In order to establish the diagnosis, other authors performed biopsies of either the episcleral tumor nodules [14, 81] or of the choroidal swellings [18, 33, 70]. On the basis of morphological features and immunophenotype, the primary choroidal lymphoma can be subtyped as "extranodal marginal zone B cell lymphomas" (EMZL) of mucosa-associated lymphoid tissue (MALT) type [30], according to the WHO Classification (Fig. 11.2c).





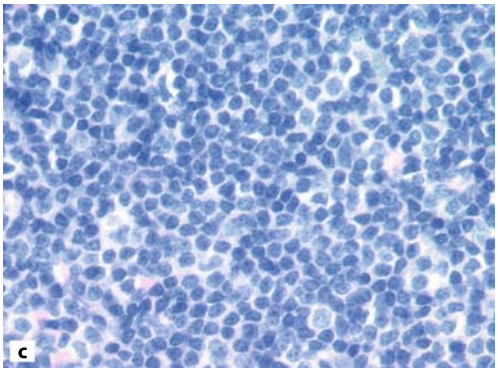


Fig. 11.2 **a** Fundoscopy demonstrating a large choroidal tumor with accompanying retinal detachment (courtesy of Prof. B. Damato, Royal Liverpool Hospital, UK). **b** An enucleated eye of a primary uveal lymphoma, demonstrating the extensive and diffuse infiltration within the choroid without any involvement of the overlying and partially detached retina. **c** The primary uveal lymphomas are low-grade malignant B cell lymphomas consisting of small centrocyte-like cells, monocytoid cells, plasmacytoid cells, and occasional blasts (MGG stain, original magnification ×200)

11.2.2.1.1 Treatment of Primary Choroidal Lymphoma

Prior to any commencement of therapy in patients with primary choroidal lymphoma, a complete lymphoma "staging" investigation, including a complete blood count, serum protein electrophoresis, and abdominal and chest computed tomography is essential [2]. Through such staging investigations, concurrent systemic disease, e.g., primary MALT lymphoma of the lung or stomach, should be excluded. If no systemic disease is found, local treatment is appropriate and can include excisional biopsy of any epibulbar mass, cryotherapy, as well as low-dose irradiation in divided doses [2].

11.2.2.1.2 Prognosis of Primary Choroidal Lymphoma

In distinct contrast to the high-grade malignant B cell lymphoma arising in the retina, the overall survival of patients with primary choroidal B cell lymphoma is very good. Occasional patients with primary choroidal lymphoma have been reported to have developed systemic disease following treatment [30]. Involvement of the central nervous system by primary uveal lymphoma is exceptionally rare.

Summary for the Clinician

- Primary choroidal lymphomas are usually low-grade B cell lymphomas of MALT type.
- A systemic lymphoma (e.g. pulmonary MALT lymphoma) should be excluded prior to therapy commencement.
- If localized, primary choroidal lymphoma can be treated with low-dose irradiation.
- In contrast to primary retinal lymphoma, the prognosis of primary choroidal lymphoma is good. CNS involvement occurs extremely rarely.

11.2.2.2 Primary Iridal Lymphoma

Those lymphomas occurring primarily in the iris are exceptionally rare with 10 cases reported in the literature [25, 28, 67, 86, 102, 120, 148, 158, 159]. The typical presenting symptoms of primary iridal lymphoma include a painful eye, photophobia, and sometimes decreased vision [28, 67, 86, 120, 148, 158, 159]. The clinical signs reported in the literature include uveitis of uncertain nature, nodular or diffuse iridal precipitates, iris discoloration with heterochromia and anisocoria, iridal swelling as well as hyphema or pseudohypopyon. On ultrasound examination, ill-defined tumors of low reflectivity can be observed.

Paracentesis from the anterior chamber and/or iris biopsy with subsequent cytological and histological examinations respectively are the two methods employed, which usually lead to the establishment of a definitive diagnosis. They are usually high-grade malignant lymphomas, similar to those involving the retina. Interestingly, there is an equal incidence of B and T cell lymphomas arising in the iris, in contrast to primary retinal and primary choroidal lymphomas, which are almost exclusively B cell lymphomas.

On establishment of an iridal lymphoma, staging evaluations must be undertaken to exclude either a systemic NHL or a primary central nervous system with secondary iridal involvement. On exclusion of further disease, low-dose irradiation or systemic chemotherapy is the treatment of choice [102].

Summary for the Clinician

- As primary iridal lymphomas are exceptionally rare, a secondary manifestation of a systemic lymphoma and a retinal lymphoma should initially be excluded
- The majority of primary iridal lymphomas are high-grade lymphomas, either of B or of T cell type.
- The prognosis of primary iridal lymphoma is relatively good; although systemic dissemination may occur.

11.2.2.3 Secondary Intraocular Lymphoma or Leukemia

Leukemic involvement of the ocular tissues is the most common form of intraocular lymphomatous proliferation. At least 65% of cases of leukemia were seen to have involvement of the eye at autopsy [91, 125, 130]. Ocular manifestations are rarely the first sign of disease in malignant lymphoma/leukemia, and have been reported in up to 80% of patients at some stage of their disease [2, 25, 29, 43, 91, 112, 150]. They usually occur in the choroid, and less often in the iris. Exceptionally rarely, intravascular lymphoma (also known as neoplastic angioendotheliomatosis) has also been reported to affect the eye [47].

The ultrasonographic appearance of secondary intraocular lymphoma or leukemic infiltration is similar to primary uveal lymphoma with regular low reflectivity. Intraocular biopsy or aspirates have proven useful in determining the nature of indeterminate choroidal tumors in such patients [4, 46].

11.2.2.4 Post-Transplantation Lymphoproliferative Disorder

A well-known complication of solid organ transplantation is the occurrence of lymphoproliferations, such as the post-transplantation lymphoproliferative disorder (PTLD) [71, 72]. This is considered a particular disease entity, most often caused by a chronic Epstein Barr virus (EBV) infection. Some forms of PTLD undergo a malignant transformation with development of a malignant lymphoma [73]. The risk of developing PTLD appears to be dependent upon the duration of immunosuppression. PTLD rarely affects the eye, with fewer than 15 cases being reported in the literature [124]. Interestingly, the predilection site in the eye is the iris [7]. Therapy of PTLD requires a balance between immunosuppression and hindering the EBV infection. In most patients, the dosage of immunosuppression is reduced and a systemic anti-viral treatment is commenced. Where the PTLD is no longer viral driven, and represents a neoplastic population of lymphocytes, chemotherapy is required [141].

11.3 Nonlymphoid Malignancies

11.3.1 Uveal Melanoma

Uveal melanoma is the most frequent primary intraocular tumor in white adults with an incidence of 0.7 per 100,000. These neoplasias, particularly the diffuse form, may present with clinical features suggestive of intraocular or orbital inflammation. Approximately 4.9% of patients with uveal melanoma present with symptoms such as episcleritis, anterior and/or posterior uveitis, endophthalmitis or panendophthalmitis [56]. The use of ocular echography has increased the diagnostic accuracy of uveal melanoma since the above-mentioned study; however, unusual presentations of uveal melanoma may still perplex the clinician, masquerading as other entities, as reported by others [121].

11.3.2 Retinoblastoma

Retinoblastoma is the most common intraocular tumor in childhood occurring in 1 in 17,000 to 24,000 live births and may occur either as a hereditary or as a sporadic tumor. Typically, retinoblastoma presents with leucocoria or strabismus; in very rare cases, it may present as an inflammation [138]. In particular, the rare variant of a diffuse infiltrating retinoblastoma, leading to conjunctival chemosis, pseudohypopyon and/or vitritis, can present with inflammatory signs [107]. Imaging studies – particularly with the presence of dystrophic calcification – are the most reliable in establishing the diagnosis; aqueous or vitreous biopsies are generally not recommended due to the considerable risk of tumor spread.

11.3.3 Juvenile Xanthogranuloma

Juvenile xanthogranuloma is a rare idiopathic cutaneous granulomatous disorder usually occurring in young children [76]. The cutaneous lesions are orange-red papules or macules, predominantly occurring over the face, neck, and upper trunk. Spontaneous resolution of cutaneous lesions occurs between 1 and 5 years.

Histologically, the lesions consist of a collection of foamy epithelioid histiocytes with scattered lymphocytes, eosinophils, and occasional plasma cells [52]. In the mature lesions, the classic Touton giant cell is seen. Ocular involvement usually affects the anterior segment, particularly the iris where it presents as a yellow nodule [162]. Complications of iridal juvenile xanthogranuloma are recurrent hemorrhage, and possibly the development of glaucoma. Occasionally, the posterior segment is involved in juvenile xanthogranuloma, and can be complicated by retinal hemorrhage, detachment, and blindness. Spontaneous resolution has not been observed with ocular lesions. Several treatment modalities have been used for juvenile xanthogranuloma, including corticosteroids, low-dose radiotherapy, and surgical excision [61]. With uveal juvenile xanthogranuloma, however, nonsurgical therapy is recommended due to the risk of severe bleeding.

11.3.4 Metastatic Tumors

11.3.4.1 Uveal Metastases

In most cases of uveal metastases, a previous history of cancer can usually be elicited; on rare occasions, however, the choroidal metastases may be the first manifestation of the malignancy (Fig. 11.3a). The most common metastases to the uvea are bronchial cancer in men (Fig. 11.3b) and breast cancer in women. Metastases are usually painless, are few in number, but lead to a more prominent disturbance of the retinal pigment epithelium [133, 140]. These may present with pale white to yellow lesions on fundoscopy under a serous detachment without involvement of the retina, superficially representing primary uveal lymphoma [95, 133]. Ultrasonography usually demonstrates medium internal reflectivity with no retrobulbar edema; the surface features and size of the tumor can be estimated using echography [6]. Fluorescein angiography reveals irregular widespread leakage in the late phase [74].

11.3.4.2 Retinal Metastases

These are exceptionally rare, with fewer than 30 cases being reported in the literature [104, 116]. Vitreous "floaters" was the most common symptom reported in patients who had most commonly metastatic cutaneous melanomas (known primary), followed by lung carcinoma, gastrointestinal carcinoma, and breast cancer. In patients where no primary malignancy is known, a vitreous aspiration or retinal biopsy may be required to establish the diagnosis.

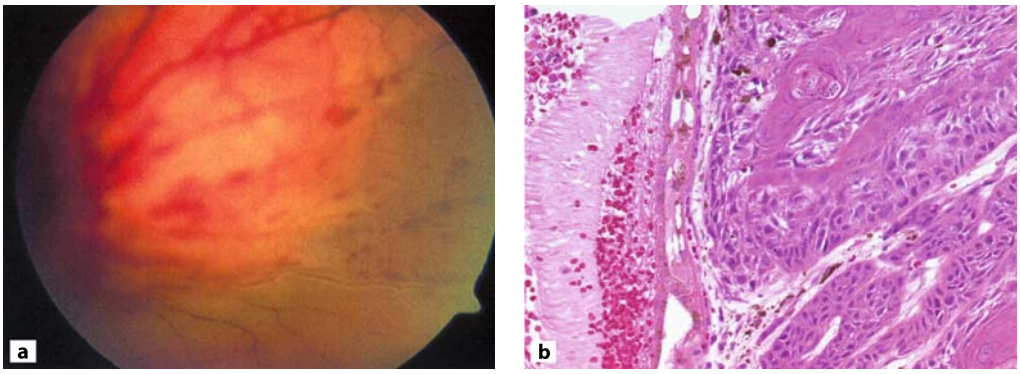


Fig. 11.3 **a** Fundoscopy of a uveal metastasis with accompanying hemorrhage of a previously undiagnosed bronchial carcinoma in an elderly man. **b** High magnification of the metastasis of the well-differentiated squamous cell carcinoma in the choroid (hematoxylin and eosin stain, original magnification ×400)

Summary for the Clinician

Other nonlymphoid malignancies that can stimulate immune-mediated uveitis include:

- Diffuse amelanotic melanoma,
- Metastatic tumor to the choroid and retina,
- Non-neoplastic proliferative diseases of the uvea, such as juvenile xanthogranuloma.

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Chapter 12

Tumor Necrosis Factor Alpha-Targeted Therapies in Uveitis

12

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Core Messages

- Tumor necrosis factor alpha (TNFα) is a key proinflammatory cytokine involved in the pathogenesis of many inflammatory disorders, including non-infectious uveitis.
- TNF α blockade is a highly effective therapy for otherwise treatment-refractory, sight-threatening uveitis.
- There are key differences between available TNFα antagonists in both efficacy and side effects.
- While infliximab is effective for refractory uveitis, the efficacy of etanercept has not been proven, and the role of adalimumab is unknown.
- Serious adverse events are rare, but life-threatening side effects may occur.
- There is a potential role for anti-TNFα therapies earlier in the course of uveitis, and in acute disease relapse.

12.1 Introduction

The clinical impact of neutralizing tumor necrosis factor alpha (TNFα) activity in inflammatory diseases has been likened to that of corticosteroids. This comparison highlights the revolutionary impact anti-TNFα agents have had in the treatment of chronic inflammatory disorders. Randomized controlled trials have proven the efficacy of anti-TNFα agents in the treatment of rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), ankylosing spondylitis (AS), psoriatic arthritis (PsA), and fistulizing Crohn's disease (CD), and the clinical use of TNFα-targeted therapies in these diseases is now widespread [10]. TNFα inhibitors are also being used in many other uveitis-associated inflammatory diseases, such as Behcet's disease (BD) and sarcoidosis.

Considerable published evidence exists, albeit not randomized controlled trials, regarding the efficacy of anti-TNFα therapies in non-infectious intraocular inflammation, including isolated ocular disorders and those associated with systemic disease. Conventional immunosuppression with corticosteroids and adjuvant agents remains the mainstay of treatment for these patients. However, a significant proportion of patients are refractory to these treatments, either due to intolerable side effects or lack of efficacy. TNFα-targeted biologics provide an exciting new therapeutic option for these patients. In the future, the introduction of these agents earlier in the disease process may help prevent the irreversible ocular damage that results from chronic, relapsing inflammation.

While the therapeutic potential of TNFα-targeted biologics is great, caution must be exercised regarding possible adverse effects. However, serious side-effects are rare and must be compared with the considerable risks of conventional treatments. The addition of anti-TNFα agents to the treatment of refractory uveitis offers both increased disease control and the opportunity to taper other medications to less toxic levels. It is likely that in the future anti-TNFα medications will be developed that more exquisitely target the harmful, tissue-damaging effects of TNFα, while leaving its beneficial actions intact.

12.2 Evidence of the Use of Anti-TNFα Agents

12.2.1 What is TNFα?

The pleiotropic cytokine TNFα has a pivotal role in the immune response, as well as in the orchestration of many autoimmune and inflammatory diseases. During inflammation TNFα is predominantly produced by macrophages, as well as activated T cells. It can also be released from a variety of other cell types, including retinal cells such as microglia, Müller cells and retinal pigment epithelium [6]. TNFα is a homotrimeric protein that exists in both a soluble and a transmembrane form. The gene locus for TNFα lies in the MHC class III region. This locus encodes TNFα, lymphotoxin α (LTα; also referred to as TNFβ) and LTβ. TNFα is first produced as a cell-surface transmembrane molecule (memTNFα). Proteolytic cleavage and release by metalloproteinases (MMP) results in the soluble form (solTNFα). Both the membrane and soluble forms of TNFα can interact with two different receptors, designated TNFRp55 and TNFRp75. These receptors are found on the cell surfaces of most somatic cells. Both receptors also exist in soluble forms, which act as natural inhibitors of TNFα. While both forms of TNFα can bind with both receptor types, solTNFα is generally regarded as the ligand for TNFRp55, whereas memTNFα predominantly signals via TNFRp75. The ligand LTα can also bind to both TNFα receptors. Activation of these receptors by TNFα initiates complex intracellular signal transduction cascades leading to diverse effects, depending on multiple factors, including receptor and cell type.

12.2.2 Role in the Immune Response

The proinflammatory activities of TNFα include activation and proliferation of T cells and macrophages, and upregulation of endothelial adhesion molecules and other proinflammatory cytokines. During autoimmune and inflammatory disease, TNFα plays a pivotal role in both the induction and perpetuation of the inflammatory response, which ultimately leads to tissue destruction [6]. However, depending on the context in which TNFα is produced, it can also act as an important mediator of immunoregulation and host defense. TNFα is involved in immune surveillance, mediating apoptosis in tumor cells and leading to downregulation of autoreactive T cell populations. It is a key element in effective granuloma formation and maintenance; thus, in the ability to suppress intracellular organisms such as mycobacteria.

These contrasting proinflammatory and immunoregulatory functions illustrate the complexity of TNFα biology. The conflicting roles of TNFα may be partly explained by the differential activities of solTNFα versus memTNFα, and by the differential functioning of the two receptor types. There is evidence that signaling via TNFRp55 is required for full expression of the inflammatory responses in autoimmunity. Signaling via TNFRp75 is essential for normal lymphoid development, and exerts many of the immunoregulatory functions of TNFα [12]. The level and duration of TNFα exposure is also critical. Acute excessive levels of TNFα are associated with inflammation and tissue damage. If TNFα levels remain chronically elevated there is ongoing inflammation, but also attenuation of T cell responses, resulting in impaired immunoregulation. Chronically subnormal levels of TNFα also lead to impaired immunoregulation [6, 12].

12.2.3 Role in Uveitis – Data in Animals and Man

12.2.3.1 Experimental Autoimmune Uveoretinitis

The role of cytokines in intraocular inflammation has been extensively studied in experimental autoimmune uveoretinitis (EAU), an archetypal animal model of organ-specific autoimmune disease. EAU represents a CD4+ Th1-mediated autoimmune response directed against soluble retinal antigens. It is characterized by infiltration of antigen-specific T cells and activated macrophages, orchestrated by a complex cytokine cascade, and ultimately resulting in targeted and bystander cell death [6].

Studies in EAU provide unequivocal evidence of the pivotal role of TNFα in the inflammatory response and resultant tissue destruction. Increased expression of TNFα has been consistently demonstrated in EAU. Administration of TNFα worsens disease, while TNFα blockade suppresses inflammation [6]. Neutralizing TNFα activity with a TNFRp55 fusion protein suppresses Th1 effector mechanisms, inhibits activation of infiltrating macrophages and prevents tissue damage. In parallel with this improvement in clinical activity, T cell responses within the retina are deviated toward the Th2 type, implying that TNFα blockade may have an immunomodulatory effect in EAU [6].

12.2.3.2 Endotoxin-Induced Uveitis

The pathogenetic role of TNFα is less clear in endotoxin-induced uveitis (EIU), the major animal model of anterior uveitis. In EIU, systemic or intraocular injection of bacterial lipopolysaccharide induces a non-antigen-driven acute ocular inflammation. The predominant infiltrating cells are neutrophils and macrophages, although CD4+ T cells play a role in disease pathogenesis. TNFα is recognized as one of the principal cytokines in EIU, with increased local and systemic production. Intraocular injection of TNFα in animals induces acute uveitis, which closely resembles EIU [2]. However, TNFα activity does not appear essential to the development of EIU. Paradoxically, TNFα blockade has been associated with both suppression and worsening of disease. Gene knockout mice deficient in TNFRp55 and TNFRp75 remain susceptible to EIU, with experiments showing either no difference in inflammation compared with wild-type animals [23] or decreased inflammation [29]. One study found that animals deficient in TNFRp55 alone had decreased inflammation, implying that this receptor subtype is key to the inflammatory effect of TNFα [29]. Another study noted decreased inflammation in mice deficient in both IL-1 receptor type 1 (IL-1R1) and TNFRp55, as well as some animals deficient in IL-1R1 alone [23]. Thus, other cytokines, such as IL-1, may be as important in EIU, and blockade of multiple cytokines may be required to effectively suppress inflammation.

12.2.3.3 Clinical Uveitis

Elevated TNFα levels have been demonstrated in the aqueous humor and serum of uveitis patients with non-infectious posterior segment intraocular inflammation (PSII). This elevation of TNFα both systemically and at the sites of tissue inflammation parallels other inflammatory diseases in which TNFα has been therapeutically targeted. More recently, anti-TNFα agents have been successfully used in various forms of PSII, as described below. TNFα blockade modulates the phenotype of peripheral blood CD4+ T cells in patients with PSII, which correlates with a recovery of visual function [9].

Summary for the Clinician

- TNFα is a key proinflammatory cytokine, but also plays important roles in immunoregulation and host defense.
- SolTNFα is the main ligand for TNFRp55, and signaling via this receptor results in multiple pro-inflammatory activities.
- MemTNFα predominantly signals via TNFRp75, which exerts many of the immunoregulatory functions of TNFα.
- Data from both EAU and human subjects suggests a pivotal role for TNFα in uveitis (PSII).
- However, in EIU other proinflammatory cytokines may be as important as TNFα.

12.3 Anti-TNFα Agents

Medications targeting TNFα act as competitive inhibitors of TNFα, binding to the molecule and thus preventing TNFα–receptor interaction (Fig. 12.1). However, the available drugs demonstrate marked differences in efficacy and safety profiles [10]. This is exemplified by TNFα inhibition in Crohn's disease, in which infliximab has

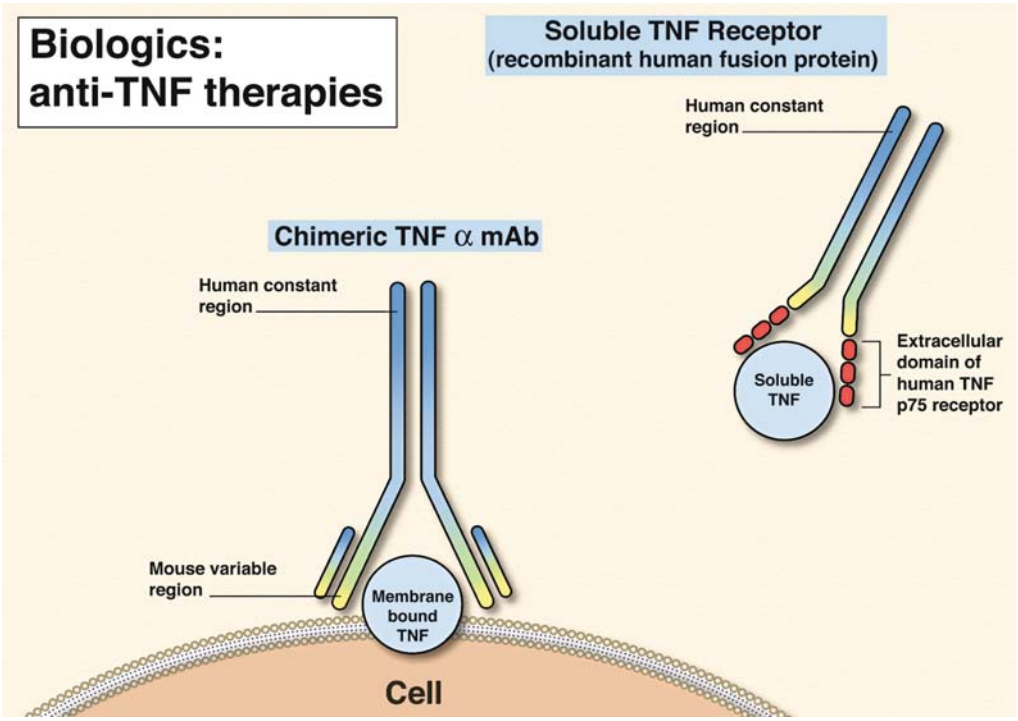

Fig. 12.1 Schematic representation of anti-TNFα neutralizing antibody and TNFα receptor fusion protein. Biological engineering of specific anti-TNFα agents has resulted in the ability to generate a chimeric antibody that is mostly human and has a specific variable region raised in mouse that recognizes TNFα, both membrane-bound and soluble. Also, engineering has generated, by fusing on the tail of human Ig, the TNFα receptor that allows the receptor-fusion protein to bind TNFα. By both mechanisms TNF activity is neutralized to variable extents

been proven to be highly efficacious, while etanercept is no better than a placebo. These disparities are due to fundamental differences in drug structures, pharmacokinetics, and mechanisms of action [24]. The available TNFα inhibitors can be categorized as either recombinant monoclonal antibodies (mAbs) or fusion proteins. The two mAbs in clinical use are infliximab, a mouse–human chimera, and adalimumab, a fully humanized antibody. Fusion proteins consist of the recombinant soluble form of either TNFRp55 or TNFRp75 linked to the Fc component of IgG, and etanercept (TNFRp75-Ig) is the only form clinically available.

12.3.1 Neutralizing Antibodies

12.3.1.1 Infliximab

Infliximab, the most commonly used mAb, is a chimeric monoclonal antibody composed of the variable regions of a mouse antibody joined to the constant region of human IgG1. Infliximab binds with high affinity to both soluble and transmembrane forms of TNFα [24]. TNFα is bound rapidly and irreversibly, and when infliximab is present in excess it can block all three receptor binding sites on TNFα. Once bound, infliximab maintains a stable complex with both solTNFα and memTNFα, preventing the interaction of either molecule with either of its receptor subtypes. The result is complete and sustained neutraliza-

tion of the bioactivities of TNFα. Infliximab is not known to bind any other ligand.

The method of administration is summarized in Table 12.1. The long-term infusion schedule depends on indication and response. Table 12.2 shows the drug doses used in published uveitis studies. The bolus dose method of administration of infliximab results in great variability in serum drug concentrations over time, with high peaks separated by low troughs [18].

12.3.1.2 Adalimumab

Adalimumab is a fully humanized recombinant IgG1 monoclonal antibody specific for TNFα. While less information is available regarding the pharmacokinetics of this agent, it appears to result in a sustained neutralization of TNFα in a manner similar to that of infliximab. However, the subcutaneous delivery method results in smooth and uniform concentration–time pro-

Table 12.1 Key characteristics of anti-tumor necrosis factor α (TNFα) therapies. *MemTNFα* cell-surface transmembrane molecule, *PSII* other idiopathic posterior segment intraocular inflammation, *LTα* lymphotoxin α, *RA* rheumatoid arthritis, *AS* ankylosing spondylitis, *JIA* juvenile idiopathic arthritis

Characteristic	Infliximab	Adalimumab	Etanercept
Type of drug	mAb – chimeric	mAb – humanized	Fusion protein
TNFα interaction			
– TNFα-drug binding	High affinity, stable	High affinity, stable	Unstable
– MemTNFα blockade	Good	Good	Poor
– Other ligands	Nil	Nil	LTα
Administration			
– Route	Intravenous	Subcutaneous	Subcutaneous
– Periodicity	0, 2, 6 then 8-weekly	Weekly to 2-weekly	Twice a week to weekly
– Dose	3, 5 or 10 mg/kg	20 mg/week, 40 mg/2-weekly	25 mg/biweekly, 50 mg/week
Concentration-time profiles (steady state)	Non-uniform (high peaks, low troughs)	Smooth, uniform	Smooth, uniform
Half-life (approximate)	10 days	14 days	4 days
Efficacy profile:			
– Inflammatory arthritis	RA, AS, JIA	RA, AS, JIA	RA, AS, JIA
– Crohn's disease	Effective	Effective	No effect
– PSII	Effective	Unknown	Unknown
– Anterior uveitis	Effective	Unknown	Controversial/unclear
Side-effect profile:			
– Tuberculosis risk	High	High	Low
– Lymphoma incidence	Increased	Increased	Increased
– Demyelination	Reported	Reported	Reported
– Autoantibodies	Common	Common	Common
– Anti-drug antibodies	Common	Less than infliximab	Rare

Table 12.2 Studies of infliximab in PSII – reports of more than two patients. *n* number of patients studied, *PNICS* prospective, noncomparative interventional case series, *RNICS* retrospective, noncomparative interventional case series, *BD* Behcet's disease, *MFC* idiopathic multifocal choroiditis, *Birdshot* birdshot retinochoroiditis, *IU* idiopathic intermediate uveitis, *B27+ AU* HLA B27 positive anterior uveitis, *PU* idiopathic panuveitis, *RV/PU* idiopathic retinal vasculitis and panuveitis, *PSII* other idiopathic posterior segment intraocular inflammation, *Ps* psoriasis, *JIA AU* chronic anterior uveitis associated with juvenile idiopathic arthritis, *Scl/PUK* scleritis and peripheral ulcerative keratitis, *Tb* tuberculosis, *Infl* ocular inflammatory parameters, *VA* visual acuity, *CME* cystoid macular edema

Reference/ study type	*n*	Type of uveitis	Response to treatment	Dose	Side effects	Follow-up (months)	Treatment indication
[3] PNICS	7	5 BD 1 Sarcoid 1 MFC	Infl – 6/7 improved VA – 1 lost 1 line, 3 improved, 3 stable	5 mg/kg	0	≥36	Refractory uveitis
[4] RNICS	12	5 BD 2 PU 2 B27+ PU 2 Ps PU 1 B27- PU	Infl – 12/12 improved VA – 10/12 improved ≥3 lines	5 mg/kg	0	Mean 17.4 (8–30)	Refractory uveitis
[11] PNICS	5	3 BD 2 PSII	Infl – 4/5 improved VA – 4/5 improved	5 mg/kg	1 Tb	6	Refractory uveitis
[14] PNICS	13	6 BD 5 PSII 1 Sarcoid 1 Birdshot	Infl – 13/13 improved VA – 5/6 BD and 6/8 other patients stable or improved[a]	200 mg (approx 3 mg/kg)	2 skin rashes – drug stopped	≥24	Refractory uveitis
[15] PNICS	10	6 IU 2 BD 1 B27+ AU 1 Other	Macular thickness – 10/10 decreased thickness VA – 10/10 improved	5 mg/kg	0	6	Refractory, chronic CME[b]

[a]Most eyes in this study were noted to have pre-existing irreversible ocular damage causing permanently decreased visual acuity

[b]Uveitis patients without clinically evident ocular inflammation but with impaired visual acuity due to chronic cystoid macular edema (duration of at least 6 months prior to infliximab treatment – mean duration 14 months)

Table 12.2 continued

Reference/ study type	*n*	Type of uveitis	Response to treatment	Dose	Side effects	Follow-up (months)	Treatment indication
[16] RNICS	7	1 IU 1 RV / PU 1 JIA AU 3 Scleritis 1 Scl/PUK	Infl – 6/7 improved (other patient had partial response, but infusion reaction precluded further treatment)	200 mg (approx 3 mg/kg)	1 infusion reaction – drug stopped	Mean 12 (4–22)	Refractory uveitis
[19] PNICS	13	13 BD	Mean number of uveitis attacks/14 weeks – reduced significantly (nil in 10/13) VA – 9/13 improved	5 mg/kg or 10 mg/kg	1 Tb	14 weeks on treatment, 26 weeks of safety data	Refractory uveitis
[26] PNICS	25	25 BD	Infl – 25/25 improved VA – 25/25 improved Mean number of uveitis relapses per 32 weeks (15 patients) – significantly reduced	5 mg/kg	Nil	32 weeks	Acute relapse and 32-week protocol[c]
[31] PNICS	23	4 BD 3 Sarcoid 3 Birdshot 8 IU 3 PU 1 Crohn's 1 MFC	1. 10-week protocol – Success[d] in 18/23 2. 52-week protocol (14 patients) – Success[d] in 7/7 who completed the 52 weeks; however, in 5/14 treatment stopped due to serious adverse events	3 mg/kg (*n*=20) or 5 mg/kg (*n*=3)	2 pulmonary embolus 1 congestive heart failure 2 lupus-like reaction 1 myocardial infarct 1 endometrial cancer 2 vitreous hemorrhage	10-week protocol: 23 52 week protocol: 14	Refractory uveitis

[c]All patients were treated with a single infusion at immediate onset of uveitis relapse. In addition, 15 of these patients were enrolled in a 32-week protocol of ongoing infliximab infusions, and frequency of relapses and final visual acuities were compared during the 32-week periods pre- and post-treatment

[d]Success was judged by a composite clinical end point composed of VA, control of inflammation, tapering of concomitant medications, and reduction of CME

Table 12.2 continued

Reference/ study type	*n*	Type of uveitis	Response to treatment	Dose	Side effects	Follow-up (months)	Treatment indication
[32] PNICS	13	13 BD (all male)	Mean number of attacks/mean VA – lower/better while on treatment than pre- or post-treatment Primary outcomes[e]: – remission in 4/13 – sustained remission in 1/13	5 mg/kg	0	54 weeks: treated weeks 0–22, observed weeks 23–54	Refractory uveitis (≥2 uveitis attacks in 6 months despite maximal treatment)
[34] RNICS	4	4 BD	Infl – 4/4 improved VA – 4/4 improved	5 mg/kg	Nil	7–22 months	Refractory uveitis

[e]Remission or sustained remission were defined as the absence of uveitis attacks during the infusion period (weeks 0–22) or entire study period (infusion plus observation period) respectively. Nine patients had 13 uveitis attacks while on treatment, and 10 of these attacks occurred at the end of the 8-week period after the last infliximab infusion.

files at steady state [18]. Whether this has clinical implications regarding efficacy or side effects is as yet unclear.

12.3.2 Fusion Proteins

12.3.2.1 Etanercept

Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of TNFRp75 linked to the Fc domain of human IgG1. In contrast to infliximab, etanercept forms unstable complexes with TNFα, binding quickly but dissociating rapidly. The TNFα that dissociates is bioactive; thus, etanercept may only transiently neutralize the activity of an individual TNFα molecule. Even in the presence of excess etanercept, one TNFα receptor binding site remains open on each TNFα trimer. The binding of etanercept to memTNFα is particularly unstable, and thus this drug may have little effect on signaling via TNFRp75. Etanercept is not specific to TNFα, also binding to LTα.

12.3.2.2 Other Fusion Proteins

Fusion proteins incorporating the extracellular domain of TNFRp55 (TNFRp55-Ig) exist, but are not commercially available. The incorporation of TNFRp55 instead of TNFRp75 confers a much greater affinity for solTNFα, which should result in more complete neutralization of solTNFα bioactivity. Intravenously administered TNFRp55-Ig has demonstrated efficacy in EAU and PSII, as discussed below.

Summary for the Clinician

- There are fundamental pharmacokinetic differences between anti-TNFα medications, and this translates clinically into different efficacy and side effect profiles.
- The key features of the clinically available agents are summarized in Table 12.1.

12.4 Studies in Uveitis

12.4.1 Posterior Segment Intraocular Inflammation

Published data suggest that infliximab is highly efficacious in sight-threatening PSII that has proven refractory to conventional treatment with maximal tolerated doses of corticosteroids and adjuvant immunosuppressive agents (Table 12.2). Studies have targeted the most severely affected patients, who often have pre-existing ocular damage from previous inflammation. The consistent demonstration of clinical improvement in this subgroup of patients is compelling evidence of the efficacy of infliximab in PSII, despite the absence of any randomized controlled data. Infliximab appears to have a rapid effect in suppressing ocular inflammation, and in some patients the disease-suppressive effect lasts longer than would be predicted by the pharmacokinetics of the drug. However, long-term remission of PSII is not induced by anti-TNFα therapy, and periodic infusion is necessary to prevent disease relapse. Thus, the induction of immune tolerance with anti-TNFα treatments is generally not achieved.

12.4.1.1 Behcet's Disease

Behcet's disease (BD) is a logical target for anti-TNFα therapies, due to the established link between elevated TNFα levels and the ocular and systemic manifestations of the disease. Treatment with infliximab has been shown to rapidly and dramatically suppress ocular inflammation, decrease frequency of relapse, reduce chronic cystoid macular edema, and improve visual acuity (Table 12.2). Infliximab is also beneficial for the non-ocular manifestations of BD. The spectacularly rapid action of infliximab in BD-related acute ocular inflammation suggests a potential role for this agent not only in the chronic suppression of disease, but also as rescue treatment in acute relapse. A rapid therapeutic effect is vital in BD, in which episodes of severe acute inflammation often leave permanent structural damage.

Most studies in ocular BD have used an infliximab dose of 5 mg/kg body weight, administered at weeks 0, 2, 6, and then every 8 weeks thereafter. The dose periodicity may then be extended to 10–12 weeks; however, intervals longer than 6–8 weeks may result in more frequent disease relapse [4, 32]. In patients with chronic inflammation, remission may not be achieved until the patient has received up to three infusions. Corticosteroids and other immunosuppressive agents are gradually tapered to the lowest level that maintains disease control and prevents a human anti-mouse antibodies (HAMA) response (discussed in Sect. 12.5.5). Many patients need ongoing adjuvant immunosuppression, albeit at a lower level than pre-treatment. Further studies are necessary to determine optimal drug dosage and administration schedule.

12.4.1.2 Other Posterior Segment Intraocular Inflammation

A similar treatment benefit of infliximab exists in other forms of PSII (Table 12.2). A phase 1/2 study also demonstrated the efficacy of a TNFRp55-Ig fusion protein, administered by intravenous infusion, in 15 out of 17 patients with refractory PSII [17]. Disease diagnoses included BD (2 patients), tubulo-interstitial nephritis-uveitis (TINU) syndrome (1), sympathetic ophthalmia (1), and idiopathic PSII (13). Within 1 month of therapy, 53% of patients achieved an improvement in visual acuity of at least 2 lines, 57% had a reduction in BIO score to 0, 56% showed resolution of macular edema, and 71% achieved complete remission. Concomitant immunosuppression could be reduced in 11 patients (65%), although all but one continued to require adjuvant therapy. Measures of quality of life also showed significant improvement. The median duration of response was 3 months, and all but 3 patients relapsed during the follow-up period.

Most published reports include patients with a heterogeneous group of PSII. It is likely that this disease spectrum includes some disorders in which cytokines other than TNFα play a pivotal role. In rheumatological disease, it is well recognized that different patterns of disease may involve differing cytokine responses. For example, while TNFα inhibition suppresses disease in most JIA patients, the 10% with systemic onset JIA (SoJIA) respond poorly to anti-TNFα treatment. A recent study demonstrated increased IL-1β expression in these patients, and administration of an IL-1 receptor antagonist suppressed their disease [20]. At present, there is no clinically available method to determine which uveitis patients would benefit most from TNFα-targeted therapies. However, the impressively high response rates to infliximab suggest that TNFα plays a key pathogenic role in the majority of PSII patients.

In most reports of TNFα inhibition in uveitis, therapy was tolerated well. However, a recent phase 2 study demonstrating the efficacy of infliximab in refractory PSII also documented a high rate of serious adverse events [31]. While causality was unclear in many cases, other side effects were clearly drug-related.

There are no published reports regarding the use of adalimumab in PSII. Etanercept therapy has not been widely used in PSII, due to concerns regarding the potential for exacerbation of uveitis, as discussed below.

12.4.2 Anterior Uveitis – Juvenile Idiopathic Arthritis and Ankylosing Spondylitis

There is considerable controversy regarding the effect of etanercept therapy on anterior uveitis. Etanercept is widely used in uveitis-associated rheumatic diseases such as juvenile idiopathic arthritis (JIA) and AS, and there is randomized controlled trial evidence of the beneficial effect of etanercept on the joint manifestations of these disorders. However, etanercept appears to have a differential effect on joint versus ocular disease, with a clear benefit in joint disease, but there is conflicting evidence regarding its effect on ocular inflammation. Case reports have implicated etanercept in the development or worsening of anterior uveitis in patients whose rheumatic disease was successfully treated [22, 30], raising

concerns as to whether etanercept can in fact trigger uveitis in susceptible individuals.

In a retrospective study of 16 patients who received anti-TNFα treatment for ocular inflammation associated with a variety of rheumatological diseases, only 4 out of 14 patients treated with etanercept obtained improvement in ocular inflammation, despite the universal benefit for joint disease [30]. Five patients developed uveitis for the first time whilst on etanercept. A retrospective survey of the use of etanercept in JIA also suggested a lack of treatment effect on the frequency and severity of uveitis episodes [25]. In a randomized, placebo-controlled, double-masked clinical trial of 12 patients with JIA-associated uveitis and stable joint disease, no difference in ocular inflammation between etanercept and placebo was demonstrated [28]. Another randomized controlled trial of 20 patients with various forms of uveitis who were in clinical remission and undergoing tapering of methotrexate found no significant difference in the rate of relapse or final visual acuity between treated patients and controls [8].

These findings are in contrast to an open, prospective study of 10 children treated with etanercept for treatment-resistant JIA-related chronic uveitis, which suggested a treatment benefit [21]. Three randomized, controlled trials demonstrating the efficacy of etanercept in AS-related joint disease found no statistically significant difference in iritis frequency; however, a trend toward treatment benefit was documented in all studies [22]. A recent meta-analysis of etanercept and infliximab therapy in AS suggested that treatment with either agent was associated with a statistically significant decrease in the number of anterior uveitis flares [5].

Infliximab appears to be effective in anterior uveitis, and for associated joint disease. A prospective series of 5 patients evaluated the use of infliximab in HLA B27-associated acute anterior uveitis [7]. A single 10 mg/kg infliximab infusion given at the onset of the attack achieved total resolution of uveitis in 4 patients, with the fifth requiring additional topical prednisolone. The therapeutic effect was rapid, with a median flare duration of 8 days.

12.4.3 Other Ocular Inflammation

Patients with scleritis, with or without associated systemic disease, and scleritis-associated peripheral ulcerative keratitis (PUK) have been successfully treated with infliximab [16, 30]. One patient with chronic cystoid macular edema associated with adult-type vascular pseudotumor also responded to infliximab treatment [15]. In theory, other clinical forms of ocular inflammation that share similar pathogenetic features with PSII may also benefit from TNFα-targeted treatment.

Summary for the Clinician

- Infliximab therapy is effective in sight-threatening, treatment-refractory non-infectious PSII, including Behcet's disease.
- It appears to be effective for both joint and ocular manifestations of uveitis-associated rheumatological disorders.
- It has been successfully used in patients with other forms of ocular inflammation, including scleritis and peripheral ulcerative keratitis.
- Etanercept therapy may not be effective in treating or preventing anterior uveitis, and has been associated with worsening or triggering of uveitis.
- It may have a differential effect on joint versus ocular inflammation in uveitis-associated rheumatological disorders.
- Adalimumab therapy has not been reported in uveitis patients.
- Long-term disease remission is not achieved with anti-TNFα agents, and periodic infusion is required to prevent disease relapse.
- Most patients require ongoing adjuvant immunosuppressive therapy, both to prevent disease relapse and to decrease the incidence of anti-drug antibodies.

12.5 Caveats

Experience with infliximab and etanercept in rheumatic disorders now extends over a decade, allowing the accumulation of considerable safety data. There is a lack of long-term follow-up in uveitis patients, and inherent differences in disease-related immune dysregulation could potentially alter the side effect profile. However, available data suggest that adverse events are likely to be similar to those reported in other inflammatory disorders.

Minor, short-term side effects of TNFα inhibition are common with all agents. The most frequently reported adverse effects of infliximab include headache, nausea, upper respiratory tract infections, dizziness, fatigue, and infusion-related reactions such as fever. Serious adverse events are rare; however, potentially fatal side effects include opportunistic infections, lymphoma, and cytopenias. TNFα inhibition has also been proven to exacerbate congestive heart failure. Important differences in side effect profiles exist between the available TNFα inhibitors.

12.5.1 Inhibition of Macrophage Function – Tuberculosis

Blockade of TNFα has been associated with the development of granulomatous infectious disease, in particular tuberculosis. Histoplasmosis and other opportunistic infections have also been reported. Many tuberculosis cases have involved patients with no past history of tuberculosis who were from non-endemic areas, and fatalities have occurred due to disseminated infection.

Containment of mycobacterial infection relies on the unimpaired formation, activity, and maintenance of granulomas by T cells and macrophages. TNFα is intimately involved in this process, promoting inflammatory cell migration to the site of infection and inducing antimycobacterial macrophage activities. The absence of TNFα activity in gene knockout models, or by administration of TNFα neutralizing agents, results in impaired granuloma formation, disintegration of established granulomas, and dissemination of mycobacterial infection [33].

Infliximab and adalimumab convey a substantially greater risk of granulomatous infectious disease than does etanercept. In the largest study of granulomatous infections associated with infliximab and etanercept, Wallis et al. reviewed all US Food and Drug Administration Adverse Event Reporting System reports from 1998 to the third quarter of 2002 [33]. Over this time period the overall risk of granulomatous infection was 129 per 100,000 infliximab-treated patients compared with 60 per 100,000 for etanercept ($p<0.001$).

This marked difference in infection risk is consistent with the differential therapeutic efficacy of TNFα inhibitors in granulomatous inflammatory conditions such as Crohn's disease (CD). These differences may be due to the more complete neutralization of TNFα bioactivity with infliximab. The differing effect of the agents on signaling via TNFRp75 may be important, as this pathway appears key to the antimycobacterial effects of TNFα. In addition, infliximab has been shown to induce apoptosis in monocytes from CD patients, and in granulomatous lesions in M. tuberculosis-infected mice, and this could result in elimination of T cells with specific reactivity against mycobacterial antigens [33].

It is mandatory that patients have thorough pre-treatment screening for tuberculosis. A chest X-ray should be supplemented with Mantoux testing or other investigations as indicated, and screening guidelines have been developed in many countries. The possibility of opportunistic infection must be considered if ocular or systemic disease worsens after anti-TNFα treatment. Joseph et al. [11] described a patient who developed disseminated tuberculosis with worsening ocular inflammation after infliximab treatment for presumed PSII, despite receiving extensive pre-treatment work-up to exclude tuberculosis.

12.5.2 Lymphoma Development

Treatment with infliximab, etanercept or adalimumab in rheumatoid arthritis increases the standardized incidence ratio for lymphoma (SIR; the ratio of observed lymphoma cases divided by the number of expected lymphomas in normals). Re-

ported cases have predominantly been B cell non-Hodgkin's lymphomas. In a review of database information on 18,572 RA patients collected over a 4-year period, the SIRs for lymphoma were 3.8 (95% confidence interval [95% CI] 1.9–7.5) for etanercept, 2.6 (95% CI 1.4–4.5) for infliximab, 1.7 (95% CI 0.9–2.5) for methotrexate alone, and 1 (95% CI 0.4–2.5) in the absence of methotrexate or biologicals [35].

Patients with RA are recognized to have a disease-related, treatment-independent lymphoma risk of approximately twofold compared with the general population [35]. This risk is strongly related to disease severity and activity. Past or concurrent use of methotrexate or other immunosuppressives may also increase lymphoma risk. It is unclear what proportion of the increased lymphoma risk associated with anti-TNFα therapy should in fact be attributed to these confounding factors. A causal association has been suggested in some patients by lymphoma regression after discontinuation of anti-TNFα treatment.

It is not known whether patients with isolated ocular inflammation have a disease-related, treatment-independent risk of lymphoma, and lymphoproliferative malignancy has not yet been reported in a uveitis patient treated with anti-TNFα. Thus, as with conventional immunosuppressive agents, it is difficult to accurately predict future lymphoma risk. While there is no evidence that TNFα inhibition increases the risk of other tumors, it is unknown whether the risk of tumor recurrence is increased in patients with a past history of malignancy. Rapid and fatal lymphoma recurrence has occurred in patients whose pre-existing lymphoma was in remission at the time of initiation of etanercept. Caution is advised in any patient with a past tumor history or who is at high risk of tumor development.

12.5.3 Multiple Sclerosis and TNFα Blockade

Despite considerable data suggesting a key pathogenic role for TNFα in multiple sclerosis (MS), there is randomized controlled trial evidence that anti-TNFα therapy exacerbates MS [13]. In patients receiving treatment for other inflammatory disorders, demyelinating syndromes, including optic neuritis, have been reported [27]. It is unclear whether this represents unmasking of latent disease or the development of new disease.

Subsequent studies point to contrasting actions of TNFα in demyelination. MS is considered to result from abnormal T cell autoreactivity to myelin antigens, and elevated TNFα levels worsen the inflammatory response. However in animal models, the absence of TNFα is associated with the persistence of autoreactive T-cells directed against myelin antigens [12]. Thus a chronic, low level of TNFα may be required to inactivate the aberrant T cell response, and there is evidence that this protective action is signaled via TNFRp75 [12].

Patients with ocular inflammation must be adequately screened for demyelinating disease prior to anti-TNFα therapy. This is particularly pertinent in intermediate uveitis, which may be associated with MS. If visual deterioration occurs post-treatment, the possibility of demyelination must be considered.

12.5.4 Other Autoimmune Disease and Autoantibody Production

All of the clinically available TNFα inhibitors have been associated with autoantibody induction, particularly anti-nuclear and anti-double-stranded DNA antibodies (ANA and anti-dsDNA respectively), but also anticardiolipin antibodies. However, despite a high frequency of ANA induction, drug-induced autoimmune disease is rare. In all reported cases of secondary disease, serology and symptoms have reversed upon cessation of anti-TNFα therapy. Autoantibody development has not been linked to decreased drug efficacy, development of anti-drug antibodies or other adverse events.

In a prospective study of infliximab therapy in uveitis, positive ANA titers were documented in 15 out of 20 patients completing three drug infusions, of whom only 1 had a detectable titer prior to treatment [31]. Seven patients reached a titer of 1:160, and 5 of these were positive for anti-ds-

DNA. Rheumatological symptoms, temporally related to the development of ANA positivity and resolving on cessation of infliximab, developed in 2 patients, both of whom had elevated anti-streptolysin O titers at the time of onset of symptoms. In another study of 13 infliximab-treated BD patients, only 2 became ANA positive, with no related symptoms [19].

The mechanisms responsible for autoantibody induction require further clarification. Inhibition of the cytotoxic T cell response, which normally suppresses autoreactive B cells, is likely to be involved. Drug-induced autoimmune disease must be considered if a suggestive clinical syndrome develops during anti-TNFα treatment.

12.5.5 Anti-Drug Antibodies

Anti-drug antibodies may develop during treatment with any of the clinically available anti-TNFα agents. Patients treated with mouse–human chimeras such as infliximab can develop HAMA. A fully humanized molecule like adalimumab will not induce a HAMA response, but human anti-human antibodies (HAHA) may develop, which bind to the unique antigen-binding site of the molecule to which the immune system has not been tolerized [1]. Treatment with fusion proteins can induce antibodies directed at the TNFR–IgG junction.

The clinical relevance of this response relates to the potential for infusion reactions, and a shortened duration of drug response [1]. Adalimumab is less immunogenic than infliximab, and anti-etanercept antibodies are the least common. Higher medication doses and increased frequency of administration are associated with a lower frequency of anti-drug antibodies. There may be an increased antibody incidence in CD, as bacterial triggers may play an important role. In RA patients, there is proof that concomitant therapy with low-dose methotrexate reduces the incidence of anti-infliximab antibodies, and this is now part of routine clinical practice. As anti-drug antibodies are unique to each medication, patients who develop decreased efficacy or antibody-related infusion reactions with one agent may be trialed on an alternative anti-TNFα drug.

Summary for the Clinician

- Minor side effects are common with anti-TNFα therapy, and serious adverse events rarely occur.
- Patients must receive pre-treatment screening for tuberculosis, and the risk of granulomatous infectious disease is higher with infliximab and adalimumab than etanercept.
- The risk of lymphoma may be increased by anti-TNFα therapy.
- Demyelinating disease must be excluded prior to treatment with anti-TNFα agents, as TNFα blockade has been proven to worsen MS.
- ANA induction is common with anti-TNFα treatments, although secondary lupus-like syndromes are rare.
- Low-dose adjuvant immunosuppression has been proven to reduce the incidence of anti-drug antibody formation.

12.6 Future Directions

The impressive efficacy of TNFα-targeted therapies in sight-threatening, otherwise treatment-refractory uveitis is an argument in favor of expanding the role of these agents in non-infectious ocular inflammation. Introduction of these agents earlier in the course of the disease may prevent the structural damage that results in permanent visual impairment. The rapid action of anti-TNFα agents could also be utilized as a rescue therapy in acute severe relapse, particularly in patients who have contraindications to high-dose corticosteroids.

Although infliximab has been effective in most treated uveitis patients, more information is required regarding which subgroups of patients are most likely to respond. The development of clinically available serological tests to aid in patient selection would represent a major advance, while the ability to determine a patient's individual cytokine response would allow truly directed biological therapy. In the meantime, treatment decisions must be based on clinical experience in treating similar patients.

While it appears that infliximab is beneficial in uveitis, the efficacy of etanercept in ocular inflammation is unclear, and little is known about the role of adalimumab. The subcutaneous method of administration is a potential advantage of this agent. This highlights the urgent need for more controlled clinical trials in uveitis patients, in order to determine the optimal agent and treatment regimen, as well as to obtain additional long-term efficacy and safety data.

Clinical experience with TNFα-targeted therapies raises intriguing scientific questions regarding TNFα biology. Further study is required to explain the differences between agents, and the differential efficacy of etanercept in joint versus eye disease. Recent studies highlighting the role of TNFRp55 signaling in disease pathogenesis suggest that targeting this receptor may achieve a more directed therapeutic action. Alternative future directions include methods to prevent TNFα production, or to increase the soluble release of receptors by MMP. While targeting TNFα may not have achieved the ultimate therapeutic aim of immunomodulation, these agents do represent a revolution in the management of inflammatory disease.

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Chapter 13

Immunotherapy of Uveitis: is Gene Therapy in our Future?

13

Rachel R. Caspi

Core Messages

- Idiopathic uveitis in humans is often considered to be of autoimmune origin, resulting from deficient self tolerance to retinal antigens.
- Animal models of autoimmune and inflammatory disease in the eye allow the scientist and clinician to study the basic mechanisms of disease, and serve as templates for the development of therapeutic approaches. Experimental autoimmune uveitis (EAU), induced by immunization with purified retinal antigens, is an autoimmune model. Endotoxin-induced uveitis (EIU) is an inflammatory model.
- Therapies can be classified as systemic vs. local, or antigen-specific vs. nonspecific. Each has specific advantages and limitations that can make it suitable for a particular situation.
- Gene therapy holds the promise of permanent cure through long- term expression of gene(s) designed to correct an underlying disease process. However, its success is highly dependent on the gene delivery system and in some cases on the ability to control gene expression.
- Antigen-specific therapies applied systemically have the potential to "reprogram" the immune system for tolerance to the target antigen(s). Nevertheless, these approaches require that the antigen(s) involved to be known, and caution must be used when considering the introduction of a disease-related antigen into a primed host.
- Antigen-nonspecific therapies, that affect common activation functions of lymphocytes, do not require knowledge of the inciting Ags(s), but may also inhibit immune responses to infection. Antigen-nonspecific therapies delivered locally into the eye have the lowest potential for undesirable side effects, but will leave the underlying disease process unaffected.

13.1 Human Inflammatory Uveitis of Putative Autoimmune Origin

Idiopathic uveitis in humans, where an infectious origin cannot be documented, is often considered to have an autoimmune origin [15, 29]. The prototypic ocular autoimmune disease is sympathetic ophthalmia (SO), where an exposure to antigens from the retina due to a trauma to one eye, may result after weeks or months in a destructive inflammation in the uninjured "sympa-

thizing" eye. Other inflammatory uveitic diseases may arise without a defined triggering event, and their causes are unknown, for example, birdshot retinochoroidopathy (BSRC). Other ocular inflammations considered to be autoimmune may be part of a broader syndrome, where other organs are also affected, such as Vogt-Koyanagi Harada (VKH) disease, Adamantiades-Behcet's disease (ABD) or systemic sarcoidosis.

Uveitic diseases are often chronic and if untreated can be sight-threatening. In a large population-based study uveitis has recently been reported to have an incidence of 52.4/100,000 person-years and a prevalence of 115.3/100,000 persons [14]. In the USA, inflammatory uveitis is estimated to account for about 10% of severe visual handicaps; thus, this group of diseases is neither rare nor trivial.

What these diseases have in common is strong HLA associations that are typical for each disease entity, which, however, may differ among ethnic groups [29, 32]. The patients often exhibit cellular and humoral responses to retinal antigens such as retinal arrestin (retinal soluble antigen, S-Ag) or interphotoreceptor retinoid binding protein (IRBP), which were observed in patients with SO, BSRC and ABD. Responses to tyrosinase-related proteins (TRP1 and TRP2) are often observed in VKH patients. Antibodies to recoverin are typically observed in cancer-associated retinopathy (CAR) and are thought to participate in disease pathogenesis. These retinal antigens, when injected into experimental animals, induce severe uveitis that is autoimmune in nature and is used as a model for these diseases ([15] and see Sect. 13.2).

T lymphocytes specific to these retinal antigens are felt to play a central role in driving the pathogenesis of uveitis, a notion that is supported by the improvement often seen with T cell targeting agents such as cyclosporin A, tacrolimus, rapamycin, and more recently with the promising experimental therapy with Daclizumab (=Zenapax), a humanized anti-interleukin-2 receptor antibody that targets activated T cells, which typically express this receptor [19, 30].

Current therapies in use for uveitis depend chiefly on treatments with broad spectrum immunosuppressives. In addition to steroids, macrolides such as cyclosporine and rapamycin are used, and in some cases cytotoxic agents such as cyclophosphamide and chlorambucil, and antimetabolites such as azathioprine, methotrexate, and lefunomide [29]. While often effective, these drugs have potentially serious side effects and they compromise protective immunity to pathogens. A more specific approach to therapy, that will target primarily the cells involved in pathogenesis and leave the rest of the immune system alone, is urgently needed.

Summary for the Clinician

- Idiopathic uveitis in humans is usually considered to be of autoimmune origin, with a central role for retinal antigen-specific T cells.
- These notions are supported by a lack of a demonstrable infectious etiology, strong HLA associations, frequent responses of the patients to retinal antigens, and an improvement seen with T cell-targeting agents.

13.2 Experimental Autoimmune Uveoretinitis – a Model for Human Autoimmune Uveitis

Experimental autoimmune uveitis (uveoretinitis; EAU) has been developed as an animal model that represents human autoimmune uveitis. EAU is induced in susceptible animals, rodents as well as primates, by immunization with the same retinal antigens that often are recognized immunologically by lymphocytes of human uveitis patients [15]. The animals are typically immunized with one or more injections of the retinal antigen in complete Freund's adjuvant (CFA), which contains mycobacteria. In some cases an additional adjuvant in the form of pertussis toxin is also required. EAU in rodents develops 9–12 days later, and the inflammation reaches a peak within a week (Fig. 13.1). In mice, the antigen of choice for eliciting EAU is IRBP, whereas in rats either

IRBP or S-Ag may be used. The mycobacteria and pertussis toxin serve as an immunological adjuvant, without which EAU will not develop. Our immune system has evolved to deal swiftly and effectively with bacterial pathogens. Innate recognition of bacterial components drives a strong inflammatory immune response accompanied by a massive production of the cytokines IFN-γ and TNF, which deals effectively with the "pathogen", but at the same time also drives the immune response to the co-administered retinal antigen toward an inflammatory, tissue-destructive phenotype. The specific fragments (epitopes) of S-Ag and IRBP that elicit uveitis in Lewis rats as well as for some strains of mice (B10.RIII, C57BL/6 and B10.A) have been defined, and can be synthesized as short peptides, so that it is no longer necessary to use the whole protein to elicit disease [1].

EAU is wholly T cell dependent: it cannot be induced in T cell-deficient animals even with repeated immunizations, and can be transferred from immunized donors to genetically compatible unimmunized recipients by T cells, but not by serum. Finally, T cell lines specific to retinal antigen can be cultured in vitro for long periods of time and can transfer disease to unimmunized recipients without formation of detectable serum antibodies. In fact, this method of EAU induction, with T cells from immunized donors that are activated in culture before transfer with the immunizing retinal antigen, or with cells from a T cell line, serves as an alternative method of inducing EAU, bypassing the priming stage and obviating the need to use bacterial adjuvants (Fig. 13.1). Although antibodies by themselves cannot elicit EAU, possibly due to their inability to penetrate an intact blood–retinal barrier, they can modify the course of disease if the barrier has already been breached by infiltrating T cells [33]. The ability of antibodies to exacerbate EAU may in part be connected to complement activation, which is emerging as a significant factor in the pathogenesis of EAU [36]. The pathogenic T cell in EAU has a defined phenotype known as Th1. These effector cells, when triggered with antigen,

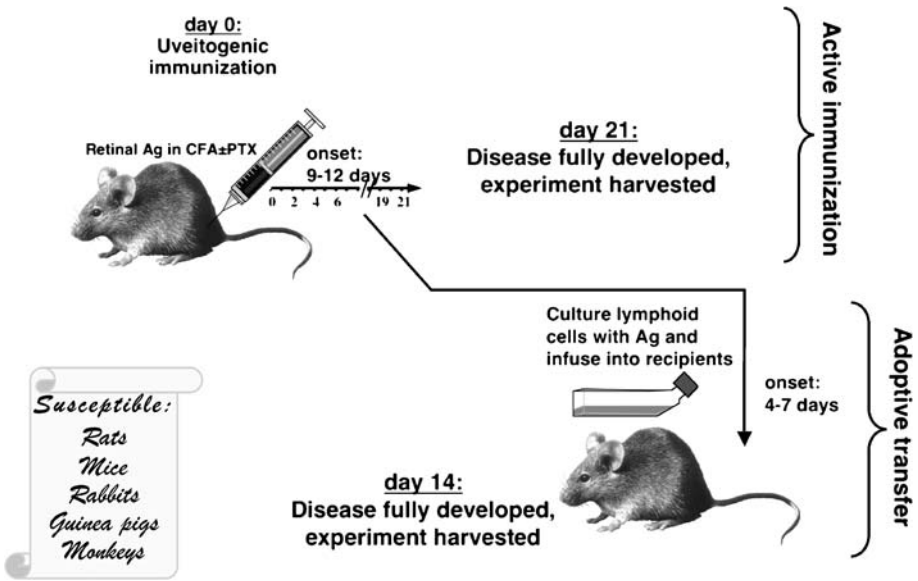

Fig. 13.1 Experimental autoimmune uveitis (EAU) induction in rats and mice. EAU is induced by active immunization with a retinal antigen in complete Freund's adjuvant with or without pertussis toxin (*PTX*) as additional adjuvant, or by adoptive transfer of cells from immunized donors. The cells are stimulated in culture with the specific antigen before being re-infused into mice. *Ag* antigen, *CFA* complete Freund's adjuvant

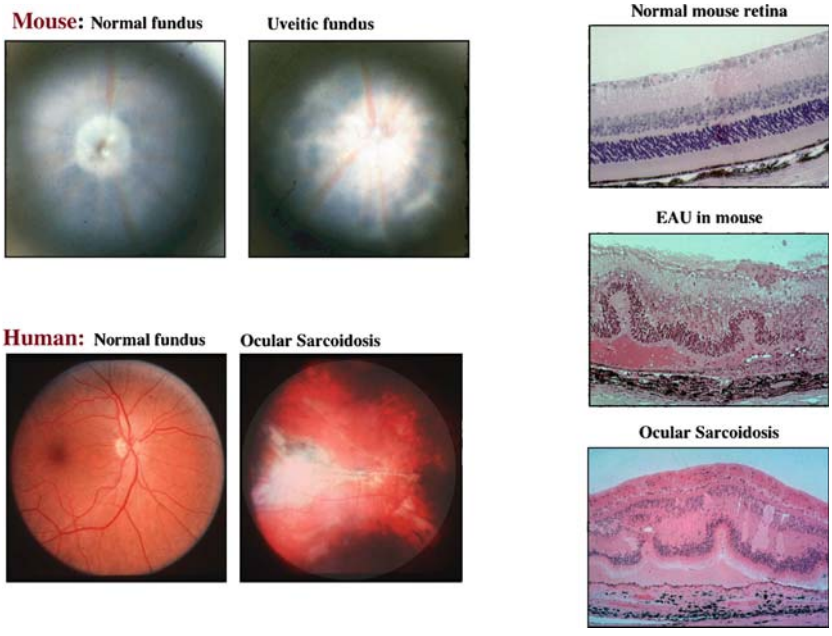

Fig. 13.2 Experimental autoimmune uveitis (EAU) vs. human uveitis (ocular sarcoidosis). Fundus photographs and retinal histology of normal and uveitic eyes are compared. Histology is on hematoxylin and eosin-stained sections embedded in methacrylate. Original magnification: mouse ×100, human ×40. The fundus and histology photographs of human sarcoidosis were kindly contributed by Dr. Chi-Chao Chan, NEI, NIH. The case has been reported [8]

produce high amounts of IFN-γ but not IL-4 or IL-5. Genetic predisposition to mounting dominant Th1 immune responses to an antigenic challenge tends to be associated with EAU susceptibility in rodents [7]. More recently, another type of inflammatory pathway, based on induction of T cells producing IL-17 has also been implicated in tissue-destructive autoimmune responses [18] and appears to also contribute to EAU (Luger, Cua, and Caspi, submitted for publication).

Although no animal model fully reproduces the broad spectrum of human uveitic disease, the EAU model appears to share many essential features and mechanisms with human uveitis (Fig. 13.2, Table 13.1). Importantly, the EAU model has allowed the cellular mechanisms involved in the pathogenesis of autoimmune ocular disease to be studied at a level that would not be possible in human patients. This knowledge is critical for devising novel approaches to therapy, based on specifically targeting the processes and cells that participate in initiating and orchestrating the disease process.

Studies in the EAU model have allowed critical checkpoints in the disease process to be defined. A fundamental function of the immune system is self–nonself discrimination, which is programmed into the immune repertoire during thymic selection of the maturing T cells. It is now known that retinal antigens that induce EAU are present in the thymus, and directly affect selection of the immune repertoire [4, 12]. As an example, IRBP-specific T cells that are potential EAU-inducing effector cells are shown to be eliminated as a result of the presence of IRBP in the thymus [4]. However, thymic elimination of self-reactive T cells is never complete. Normally, self-reactive T cells that escaped elimination in the thymus get a second chance to be tolerized in the periphery, as they recirculate through the body and encounter tissue antigens under non-inflammatory conditions. Those T cells are then turned off, a

Table 13.1 Experimental autoimmune uveitis (EAU) vs. human uveitis: similarities and differences. *Ag* antigen, *S-Ag* retinal soluble antigen, *IRBP* interphotoreceptor retinoid binding protein, *MHC* major histocompatibility complex

	EAU vs human uveitis: similarities and differences	
	EAU	**Uveitis**
Triggering event	induced	"spontaneous"
Reactivity to retinal Ag	immunizing Ag	S-Ag, IRBP, recoverin, others
Clinical course	acute or chronic	usually chronic
Pathology		
chororoiditis	yes	yes
retinitis	yes	yes
subretinal neovasc	some	some
iridocyclitis	yes	yes
Genetric control	MHC & background	MHC (background?)
MHC genes involved	class II	class I and class II
Central role for T cells	yes (lines, clones)	yes (efficacy of T cell targeting treatments)
Role of antibodies	Modifying	Suspected

process aptly known as peripheral tolerance, so that they are now less able to become activated when they subsequently encounter self antigens in the context of bacterial or endogenous "danger" signals. We believe that for retinal antigens peripheral tolerance does not operate efficiently due to the relative inaccessibility of retinal antigens, which are sequestered away from the immune system as part of the immune privilege of the eye. In this scenario, T cells specific to retinal antigens that have exited the thymus cannot be tolerized peripherally, and remain available to be activated as a result of exposure to a retinal or a cross-reactive antigen. If activated in the presence of (in)appropriate "danger" signals they may differentiate into pathogenic effector cells of the Th1 or Th-IL-17 phenotype. Activated T cells express adhesion molecules and matrix-degrading enzymes, and are able to extravasate and penetrate the blood–retinal barrier much like a metastatic tumor cell. Data suggest that the first activated uveitogenic T cells may find their way into the eye (which is intact at that point) at random [34]. Once in the eye, it is thought that these T cells must recognize their specific retinal antigen in order to initiate an inflammatory cascade, involving massive production of cytokines and chemokines as well as of adhesion molecules on the retinal and uveal microvasculature, that culminates in a massive recruitment of blood-borne leukocytes into the eye and a destructive retinal inflammation [9, 34, 49].

Another model of uveitis should be briefly mentioned. Endotoxin-induced uveitis (EIU) is an acute anterior uveitis of short duration that is not autoimmune (reviewed in [42]). EIU is induced in rats and mice by systemic injection of endotoxin from Gram-negative bacteria (lipopolysaccharide = LPS). This is followed by a rapid infiltration of inflammatory leukocytes into the anterior chamber within 24 h. Involvement of proinflammatory cytokines of the innate immune system, including IL-1, IL-6, and TNFα, has been documented [42]. Although it is not clear whether there is an equivalent disease entity in humans, this model has been extremely useful in studying various aspects of ocular inflammation and in evaluating therapeutic interventions.

Summary for the Clinician

- Experimental autoimmune uveitis (EAU) is an animal model that represents human uveitis.
- It is induced in experimental animals by immunization with retinal antigens or their fragments, or by infusion of T cells specific to these antigens.
- Experimental autoimmune uveitis has allowed the basic mechanisms involved in autoimmune or immune-mediated ocular disease to be studied and the critical checkpoints in the disease process that can be targeted therapeutically to be defined.
- Endotoxin-induced uveitis (EIU) is a useful model for studying local aspects of inflammation.

13.3 Immunotherapeutic Paradigms: Antigen-Specific vs. Non-Antigen-Specific; Systemic vs. Local

Based on an understanding of the critical checkpoints in the disease process, two major approaches to immunomodulation have emerged which seek to target the autopathogenic T cells that orchestrate the disease process: antigen-specific, vs. non-antigen specific. The antigen-specific approach targets the disease-related T cells based on their receptor for antigen (T cell receptor, TCR). For this approach to be successful, the antigen(s) that drive the disease must be known. Antigens that may be involved in ocular autoimmune diseases in humans are increasingly being identified. The best known is retinal S-Ag, to which many uveitis patients exhibit lymphocyte activation responses. Importantly, in a "humanized" uveitis model in HLA-transgenic mice this antigen was shown to elicit typical uveitis, supporting the notion that it is causally involved in human disease [33]. Another example are antigens related to melanin and its metabolism, such as GP100, MART1, TRP1, and TRP2, which elicit responses in lymphocytes of VKH patients [16, 47, 54]. Notably, some patients treated for melanoma by immunotherapeutic approaches targeting melanin antigens have resulted in fundus depigmentation, and in some cases even in overt uveitis [38].

The clear advantage of the antigen-specific approach is that it targets only the cells specific to disease, and (at least in theory) leaves the rest of the immune system unaffected. However, in many autoimmune entities the target antigens are not known, or the process of epitope spreading and antigen spreading may have occurred, and more than one antigen becomes involved. The alternative approach, still more specific than a general immunosuppressant, is to target activated lymphocytes based on common markers or processes involved in immune activation and/or function. One example of this would be therapy directed at activation receptors such as the IL-2 receptor, based on the premise that they will be expressed by the pathogenic effector T cells driving the disease [30]. Although – at least in this particular case – the mechanisms involved appear to be much more complex than was initially assumed [19], the immunotherapeutic paradigm of targeting activated T cells irrespective of their antigenic specificity appears to hold promise in terms of its effectiveness and relative paucity of side effects.

Although the manifestations of uveitis are local, the immune response fueling the disease process is systemic. It is generally believed that antigen-specific effector T cells are primed in the peripheral lymph nodes, not in the target tissue where they express their function. This is likely to be particularly true for the eye, which as part of its immune privileged status constitutes a profoundly immunosuppressive environment. The immune privilege of the eye is manifested both as a paucity of major histocompatibility complex (MHC) class II-expressing cells that could serve as productive antigen-presenting cells, and as a presence of multiple cell-bound and soluble factors that inhibit immune and inflammatory responses [46]. There is no experimental evidence that naive uveitogenic T cells can be primed and undergo clonal expansion within the eye (although previously activated T cells can recognize their antigen in the eye, as discussed in Sect. 13.2). Therefore, to intervene in the disease-perpetuating processes that take place in the periphery,

i.e., priming of new T cells and their entry into the effector pool, one has to employ systemic approaches. On the other hand, intervention at the systemic level may be too toxic or too drastic in a given situation. Fortunately, the eye is particularly well suited to local therapy, which obviates the undesirable and often serious side effects of systemic intervention. However, it must be kept in mind that a local therapy can by its nature only control symptoms, and will not affect the underlying (systemic) disease process.

Summary for the Clinician

- Systemically applied antigen-specific therapies uniquely target the cells involved in the pathogenic process, but in many cases the antigens involved are unknown.
- Therapies targeting surface molecules, receptors, or functions common to all activated T cells are a close second, but may affect desired immune responses having to do with host defense.
- Local therapies have the lowest potential for side effects, but will not affect the underlying systemic disease process.

13.4 Gene Therapy as an Approach to Immunotherapy

Unlike traditional treatment approaches, which must be applied again and again, gene therapy, applied at the DNA level, holds promise of becoming self-sustaining. The introduced DNA integrates, permanently or transiently, into the genetic material of the host, and – at least in theory – becomes expressed along with the host's own genes. The success of gene therapy depends on the gene delivery system, which must be capable of efficient transfer of genetic material into a variety of tissues, and have minimal pathogenic effects on the tissue as well as minimal or no immunogenicity. For some genes, the ability to control their expression, e.g., turn them on or off at will by means of inducible promoters, will be important. The current principles of gene therapy, including vector development and the advantages and limitations of various delivery systems, have recently been discussed in depth by Verma and Weitzman [50].

In this part of the review, we will concentrate specifically on gene therapy as it has been applied experimentally to the eye. Although gene therapy of retinal degeneration caused by single-gene defects has been under investigation for some time, gene therapy applied to uveitis is still a new concept and, to date, only a few gene therapy studies in uveitis models have been attempted. However, the results have been promising. I shall discuss here two systemic approaches to antigen-specific gene therapy, whose goal is essentially to reprogram the immune system for tolerance to retinal antigen (Figs. 13.3, 13.4), and a local approach based on expressing the immunoregulatory cytokine IL-10 within the eye (Fig. 13.5).

Summary for the Clinician

- Gene therapy holds the promise of permanent cure through long-term expression of gene(s) designed to correct an underlying disease process.
- However, its success is highly dependent on the gene delivery system and in some cases on the ability to control gene expression.

13.4.1 Gene Therapy of EAU by Peripheral Expression of a Uveitogenic Retinal Antigen

As discussed in Sect. 13.2, one of the contributing factors in susceptibility to ocular autoimmune disease may be deficient peripheral tolerance, due to the relative sequestration of retinal antigens within the eye. This concept is supported by the finding that widespread expression of IRBP as a transgene, under control of the MHC class II promoter, dramatically enhances resistance to EAU and inhibits associated immunological responses [52]. Further support for this notion was provided by the observation that infusion

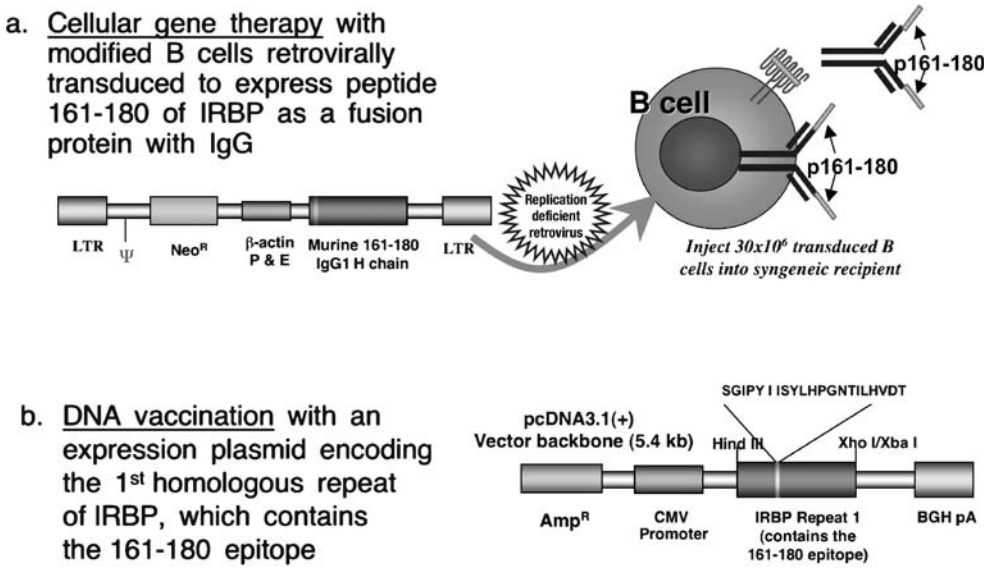

Fig. 13.3 Approaches to peripheral expression of interphotoreceptor retinoid binding protein (IRBP) in mice by gene transfer. **a** Cellular therapy with modified B cells. Schematic of the construct and its expression is shown. **b** Naked DNA vaccination. DNA construct is shown. *LTR* long terminal repeat, *P&E* promoter and enhancer, *CMV* cytomegalovirus, *BGHpA* Bovine growth hormone Poly A

13

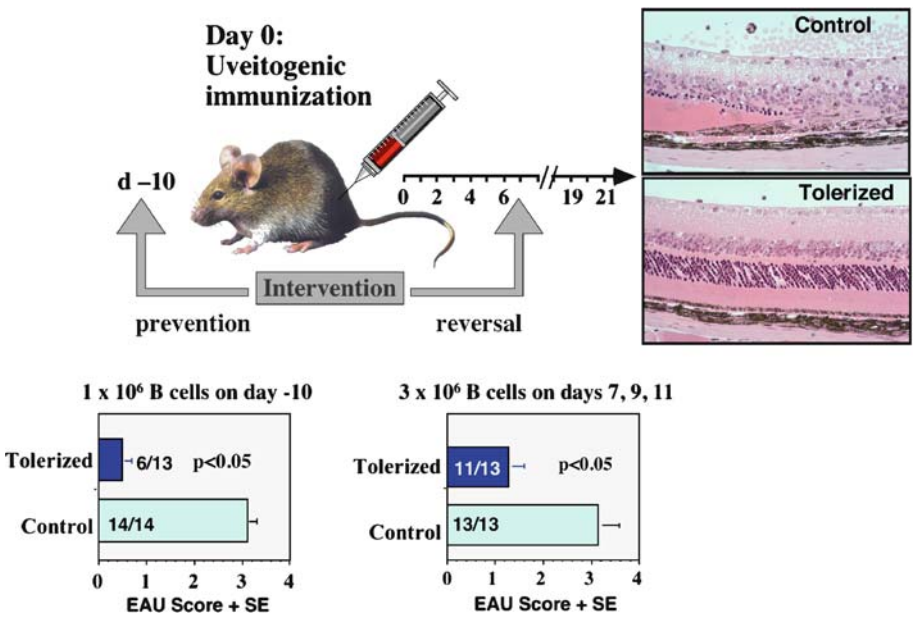

Fig. 13.4 Therapy with IRBP p161-180 transduced B cells. Mice are infused with 30×10^6 transduced B cells either 7 days before, or 7, 9, and 11 days after uveitogenic immunization with p161-180. EAU scores are graded on a scale of 0 (no disease) to 4 (severe disease). Histology illustrates representative scores (H&E/methacrylate, original magnification ×100). *SE* standard error

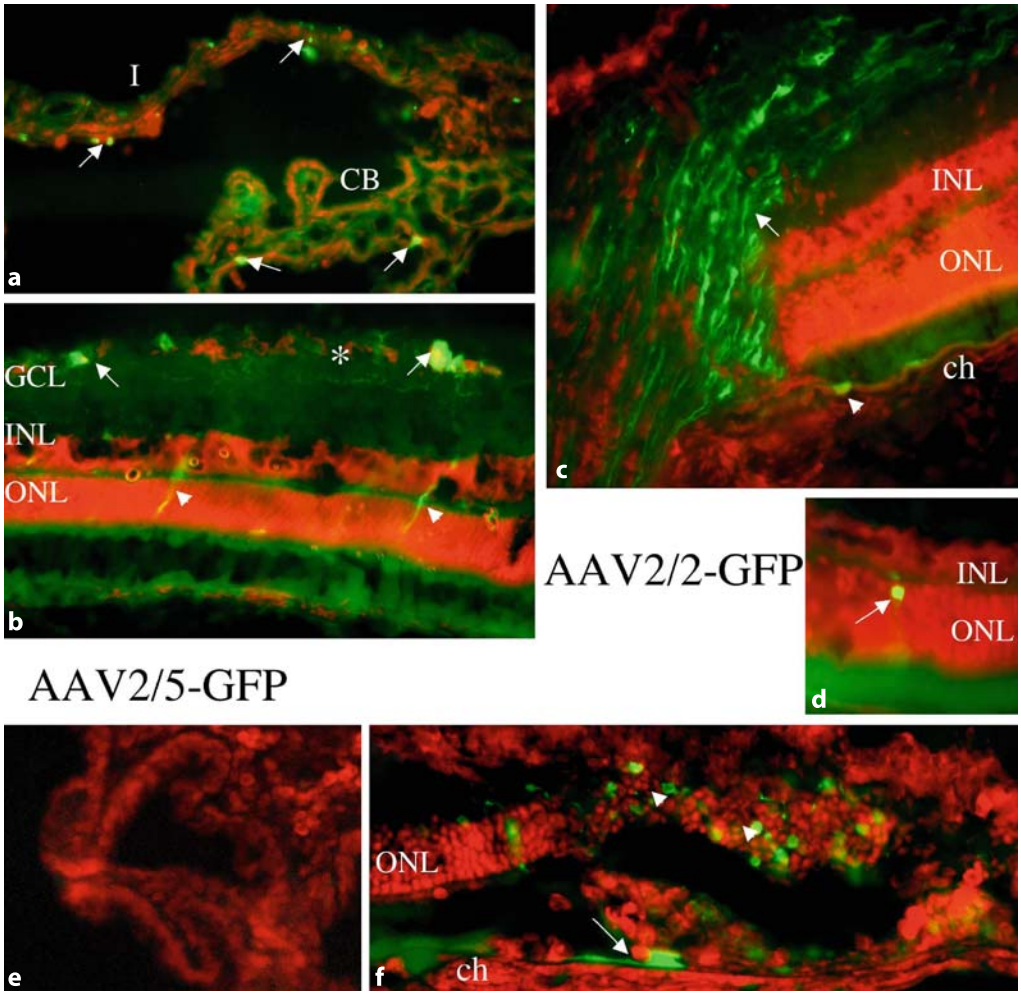

Fig. 13.5 Green fluorescent protein expression in ocular tissues of AAV2/2- and AAV2/5-injected eyes in Lewis rats. **a** Intravitreal injection of AAV2/2-GFP-induced GFP expression in the epithelium of the iris (*I*) and ciliary body (*CB*; *arrows*). In the retina, different cell types were shown to express GFP: **b** Müller glial cells (*arrowheads*), ganglion cells in the ganglion cell layer (*GCL*; *arrows*), nerve fibers (*asterisk*), **c** retinal pigment epithelium (*arrow*), and **d** photoreceptors in the outer nuclear layer (*ONL*). Since numerous ganglion cells were transduced, GFP was detected in numerous nerve fiber axons at the optic nerve head level (*arrow*; **c**). **f** Intravitreal injection of AAV2/5-GFP induced GFP expression in the photoreceptor cells (*arrowheads*) and in the retinal pigment epithelium (*arrow*), **e** but no expression was found in the iris or ciliary body. The photographs were kindly contributed by Dr. Yvonne de Kozak, Inserm, Paris, France. *INL* inner nuclear layer, *Ch* choroid

of naïve (MHC class II-expressing) spleen cells from these IRBP-transgenic mice (which co-express IRBP) into EAU-susceptible wild-type recipients, render them also resistant to EAU (Avichezer and Caspi, unpublished).

The first successful prevention of EAU by gene transfer was reported by McPherson et al. [23], who showed in Lewis rats that transducing the gene encoding S-Ag into bone marrow cells, which are then infused into lethally irradiated Lewis recipients, protects them from develop-

ing EAU upon subsequent immunization with S-Ag. These findings demonstrated that revoking the immune privileged status of a retinal antigen by expressing it peripherally can be exploited to enhance peripheral tolerance and increase resistance to EAU. However, as a potential therapy this paradigm poses a difficulty when considered clinically, because of the need to myeloablate the recipient. In order to circumvent this problem, our group has used two gene transfer methods to achieve expression of IRBP in the periphery of the adult animal that do not require myeloablation: cellular therapy with autologous B cells retrovirally transduced to express a uveitogenic epitope of IRBP, and naked DNA vaccination with a plasmid expressing a uveitogenic portion of IRBP (Fig. 13.3).

13.4.1.1 Cellular Therapy with Autologous B Cells Expressing a Uveitogenic Epitope

One interpretation of the resistance to EAU of wild-type mice infused with splenocytes from IRBP-transgenic donors (mentioned above) is that donor MHC class II-positive cells expressing the endogenously produced transgenic IRBP act as tolerogenic antigen-presenting cells (APC) to peripherally tolerize the recipient. Based on this notion, we adapted a tolerance paradigm employing antigen-transduced B cells, which was originally developed by Zambidis et al. [55] in a model system of phage lambda repressor protein, to systemic gene therapy of EAU. In this highly tolerogenic paradigm, peripheral B cells are transduced with a genetically engineered retroviral construct encoding the antigen fused in-frame to the IgG1 heavy chain. The B cells must be stimulated with lipopolysaccharide (LPS) from gram-negative bacteria to activate them and cause them to proliferate, as retrovirus can only infect actively cycling cells. The activated B cell provides the light chain gene, which – together with the engineered heavy chain that has been introduced – results in a secretable IgG1 molecule carrying the fused antigenic fragment. The B cells also express the antigen on their surface in the context of MHC class II. This last property turns out to be critical for induction of tolerance, as similarly transduced B cells that are MHC class II-deficient are unable to tolerize (Fig. 13.3a) [13].

To adapt this tolerance induction method to the EAU model, an oligonucleotide encoding residues 161–180 of IRBP, a major pathogenic epitope that elicits EAU in the highly susceptible B10.RIII mouse strain, was fused to the murine IgG1 heavy chain gene as part of a retroviral fusion construct. The recombinant replication-deficient retrovirus was used to transduce purified B cells collected from B10.RIII mice. The modified B cells were subsequently infused into genetically identical recipients, simulating an auto-transplant situation. The recipients were challenged for EAU by standard uveitogenic immunization with peptide 161–180 in CFA. Compared with control mice that received B cells transduced with the original Zambidis phage lambda repressor construct, which were not protected from EAU, the recipients of B cells transduced with p161–180 construct were highly protected from disease. Importantly, protection could also be achieved in animals that had been given the uveitogenic immunization a week before the tolerizing treatment, although in this situation three infusions of the tolerogenic B cells were required to achieve a good protective effect (Fig. 13.4). It should be pointed out that interrupting the disease process 7 days after active immunization is extremely difficult in this model, possibly due to the presence of CFA and the very strong innate stimulation that it causes. An alternative "reversal" model that is not complicated by the presence of CFA is infusion of a p161–180-specific T cell line, representing a mature uveitogenic effector population. In this adoptive transfer EAU model, complete protection from disease could be achieved with a single infusion of the p161–180-transduced B cells (Agarwal et al., unpublished). Importantly, this gene therapy approach can be generalized for a variety of antigens and autoimmune diseases, and is not limited to inducing tolerance to single epitopes: Larger antigenic peptides as well as whole proteins can be engineered as an Ig fusion product, and efficacy has been shown in other autoimmune disease models [24].

The mechanism involved in protection is not completely clear, and may involve tolerization of the high-affinity antigen-specific effector T cells [2] as well as induction of regulatory cells [44].

The mechanism that predominates may be determined by the antigen being used as well as by the genetic background of the animal.

When considered clinically, this gene therapy paradigm presents a number of advantages. Large numbers of peripheral B cells can easily be obtained. There is no need to myeloablate the host, as must be done with bone-marrow based methods to permit engraftment of the transduced stem cells. Also important is the fact that transduction of the B cells is done ex vivo, obviating the need to inject large amounts of antigenic virus into the host. In this way, generation of a strong immune response to the retroviral vector would be avoided, conceivably permitting repeated infusions of such transduced B cells if needed. Tolerance induced by these B cells is extremely long-lasting: mice challenged for EAU as long as 10 months after a single infusion of tolerogenic B cells (almost half of a mouse's lifespan) are still protected from disease (Agarwal and Caspi, unpublished). Finally, the ability of this gene therapy paradigm to interrupt an existing disease process, bodes well for its clinical efficacy. This paradigm has been validated in preclinical studies using whole human S-Ag as a tolerogen in Lewis rats and in HLA-DR3 transgenic mice [56], and is currently in the process of being adapted for a clinical trial in uveitis patients.

13.4.1.2 DNA Vaccination for Tolerance

An alternative method of gene delivery into cells, that circumvents the use of viral vectors entirely, is transduction with naked plasmid DNA encoding the gene of interest. There are a number of approaches to delivering naked DNA into cells, including gene gun, intramuscular injection, and systemic delivery. To achieve widespread peripheral expression of antigen, as is our goal here, it would seem that systemic delivery is the most promising. However, DNA injected intravenously is rapidly degraded by DNases in the blood. To circumvent this, we have used a method of hydrodynamic DNA delivery, described by Song et al. [45]. The method consists of a rapid infusion of a large volume into the tail vein, which temporarily overcomes cardiac output, forcing DNA into internal organs through the inferior vena cava. Most of the injected DNA becomes expressed in the liver, with marginal levels of expression observed in spleen, kidney, and lung [45].

To adapt this to the IRBP-induced uveitis model, we constructed a mammalian expression plasmid encoding the first homologous repeat of IRBP, which contains the pathogenic 161-180 epitope (Fig. 13.3b). After hydrodynamic injection of as little as 10–20 μg of plasmid DNA, IRBP is expressed in the liver and can easily be detected in tissue extracts by Western blotting. The "vaccinated" mice are highly protected from development of disease when subsequently challenged for EAU using a uveitogenic regimen of p161-180, with protection lasting for at least 3 months [3, 41]. Liver is known to constitute a tolerogenic environment [10], but the cells presenting the transduced antigen have yet to be identified. Unlike with the B cell protocol, only partial protection was apparent in DNA-vaccinated mice in a disease reversal regimen, as represented by infusion of a uveitogenic T cell line, suggesting that this type of tolerance mostly affects naïve T cells that are being exposed to the antigen for the first time. As a therapeutic protocol, this type of tolerance is still of value, as it can prevent the priming of new uveitogenic T cells and their entry into the effector pool, a process that is thought to fuel chronic autoimmune disease.

In comparison to vaccination by the more conventional intramuscular injection route, the hydrodynamic vaccination was 700 times more efficient: achieving the level of protection obtained after a single hydrodynamic infusion of 10 μg of DNA required seven intramuscular injections of 100 μg of DNA. Clinically, hydrodynamic injection can be considered analogous to portal vein infusion. While clearly not a trivial procedure, in severe disease portal vein infusion is being used to deliver various therapeutic modalities, including chemotherapeutic substances, gene constructs, and transplanted islets of Langerhans. Conventional intravenous administration of naked DNA is not practical, because most of the DNA would be degraded in the general circulation before it reaches the tissues where it is to be expressed. However, novel DNA encapsulation and stabilization technologies may well make intravenous delivery of DNA a practical solution in the not-too-distant future. It is tempting

to speculate that when the APCs that are important for tolerogenesis in the liver are identified, it will be possible to specifically target the DNA to these cells using antibodies to surface molecules or receptor targeting technologies, thus maximizing transduction efficiency.

A word of caution is in order concerning tolerance-inducing protocols such as the ones described above that involve the introduction of the disease-related antigen into a primed host. While neither we with EAU, nor others in different autoimmune disease models, have ever seen adverse effects with the B cell protocol in experimental systems, with the DNA vaccination method we did observe a minimal and transient cellular infiltration into the eyes of mice vaccinated with a high dose of IRBP plasmid DNA, as a consequence of vaccination alone [3]. This did not occur with a lower dose of DNA vaccination. Although the low-grade infiltration resolved spontaneously, and the animals were protected from an acute EAU challenge, it underscores that antigen-specific tolerance regimens must be carefully controlled by adjusting the dose and treatment regimen to minimize potential side effects and maximize the benefit.

Summary for the Clinician

- Antigen-specific therapies of various flavors have the potential to "reprogram" the immune system for tolerance.
- The modes of delivery can be diverse, and will affect the location, the efficiency of transduction, and the duration of expression.
- Gene therapy based on inducing expression of a normally sequestered retinal antigen in the periphery is effective in preventing, and (depending on the particular paradigm) potentially also in reversing, the disease process.
- Nevertheless, despite the highly tolerogenic nature of these protocols, caution must be used when considering the introduction of a disease-related antigen into a primed host.

13.4.2 Local Transfer into the Eye of Genes Encoding Immunoinhibitory Molecules

An approach that has been explored by a number of groups is the introduction of genes producing the immunoinhibitory molecules directly into the eye, using viral and nonviral delivery systems. Verwaerde et al. were the first to report that local delivery of IL-10 or CTLA-4-Ig into the eye of rats using either intravitreal injection of recombinant adenovirus (AV) encoding the gene, or cultured retinal glial Müller cells transduced with the gene ex vivo, had a protective effect against EAU [51]. This paradigm is an example of an antigen-nonspecific therapy directed against common activation and function requirement of T cells, so that the antigen(s) involved need not be known. Thus, CTLA-4-Ig blocks the CD28 molecule on T cells, thus preventing a costimulatory signal, which is necessary for productive activation of T cells that have recognized their specific Ag, whereas IL-10 is a regulatory cytokine produced by a variety of cells, and controls the function of T as well as non-T cells at a number of levels [26]. Although neither of these delivery systems has direct clinical applicability (adenoviral vectors are highly immunogenic, and metabolically active injected glial cells could proliferate within the eye) this result represented proof of concept, which was then further developed by this group and by others using the less immunogenic adeno-associated virus (AAV) as vector, or using electroporation to transduce naked DNA directly into ocular tissue [5, 6, 43].

Systemic treatment with IL-10 has been used to treat some experimental as well as human autoimmune disorders, in some cases with considerable success [17, 26]. However, in addition to its regulatory effects on APC and effector T cells, IL-10 has stimulatory effects on B cells and even on some T lymphocyte subsets [26]. These drawbacks can be partly overcome by using the viral instead of the cellular form of IL-10, which has been shown to exhibit mainly the immunosuppressive properties of the cytokine [26]. Nevertheless, used systemically, over the long term this kind of approach might have side effects in terms

of inhibiting host antimicrobial responses and eventually eliciting some unwanted responses, but when applied locally, this problem is circumvented. We have previously demonstrated that IL-10 plays a protective role in EAU [37, 48]. Furthermore, it is able to inhibit mature uveitogenic effector cells, as represented by a highly uveitogenic T cell line, which even TGF-β cannot do [37, 53]. This makes IL-10 a good candidate for local therapy, where inhibition of the function of already primed effector cells, incoming from the periphery, is required. Furthermore, cells other than the antigen-specific T lymphocytes, including local APC and recruited inflammatory cells from the circulation, may also be inhibited.

Adenovirus and adeno-associated virus are both able to deliver genes to nonreplicating cells [50] and therefore can both be used to deliver genes into ocular tissues; however, AAV is less immunogenic than AV; thus, it is less likely to induce ocular inflammation as a result of an immune response to the vector itself. While the IL-10 produced from the construct that has been introduced would tend to counteract inflammation in response to the vector, with AAV inflammation was not observed even in eyes treated with control vector. It might perhaps be proposed that the immune privileged status of the eye contributes to reducing immunogenicity of vectors, as the eye not only constitutes an immunosuppressive environment, but also elicits anterior chamber-associated immune deviation (ACAID) [46]. However, immunogenic components introduced into the eye as an AAV construct can elicit uveitis, as in the case of the RPE65-deficient dog [28] that is used as a model for Leber's congenital amaurosis, so any protection that might be afforded by immune privilege in this case seems partial at best.

Adeno-associated virus is available in a number of serotypes, with differing tropism to particular cell types. The choice of serotype will therefore be influenced by the precise anatomical location and type of expression desired. The shell and DNA of different serotypes may be mixed to produce a cross-packaged virus with improved transduction efficiency in the desired location within the eye, or other modifications may be made (reviewed in [21, 35]).

Smith et al. [43] explored intravitreal injection of an AAV2-based construct encoding viral IL-10 (AAV2/2-vIL-10) controlled by a tetracycline-inducible promoter, as well as the same AAV2-based construct cross-packaged in an AAV5 shell (AAV2/5-vIL-10) to modulate S-Ag-EAU in Lewis rats. Expression of the construct can be triggered by including tetracycline in the drinking water, which allows the promoter to be turned on or off at will. The use of inducible promoters addresses the issue, inherent in introducing genes encoding biologically active molecules, of controlling their expression. Interestingly, the two serotypes gave very different results in terms of the ocular tissues they tended to transduce, as evidenced by a test construct in which green fluorescent protein (GFP) was inserted in place of the vIL-10 gene, and in terms of the protection they afforded from EAU. While intravitreally injected AAV2/2-GFP was expressed in the iris, ciliary body, and retina, AAV2/5-GFP was only expressed in the retina, but not in the anterior uvea (Fig. 13.5). Furthermore, only the AAV2/2-vIL-10 construct achieved detectable levels of vIL-10 mRNA and protein within the eye and was effective in protecting against uveitis pathology, whereas the AAV2/5-vIL-10 construct did not achieve high levels of vIL-10 in ocular fluids, and was unable to protect. Curiously, in another study [35] it was AAV2/2 that was found to be inferior to the 2/5 combination for ocular expression. This underscores the many unknowns still associated with the use of these constructs, where the specific insert itself may affect expression; thus, it is difficult to predict the final outcome. A recently published study by Broderick et al. in the mouse EAU model, induced with the uveitogenic peptide 1-20 of IRBP, confirms and extends these results by demonstrating protection using a subretinal administration of AAV2 vector encoding murine rather than viral IL-10 [6].

It is important to emphasize that, in both studies, the protected animals had undiminished systemic immune responses to the uveitis-inducing antigen. This confirms the strictly local nature of this type of intervention, which has the potential to avoid unwanted systemic effects. However, at the same time, this treatment paradigm would leave unaffected the circulating pool

of uveitogenic effectors, likely necessitating continued local expression of IL-10 to keep disease under control. The duration and the stability of expression over the long term of these constructs, as well as the possible consequences of re-injection of the AAV vector encoding the construct into the eye of a host that may have become primed to the vector, still remain to be examined.

The problems inherent in the use of viral vectors may potentially be circumvented by a recently developed nonviral delivery system based on electroporation. Electroporation (also known as electropermeabilization or electrotransfer) dramatically enhances plasmid gene transfer in vivo, and has successfully provided plasmid gene transfer into a variety of ocular tissues, such as corneal cells, adult ganglion cells, and neonatal photoreceptors [22, 25, 31, 39], but has not previously been used as gene therapy for uveitis.

Bloquel et al. [5] adapted this technique for the in vivo transfection of ocular ciliary muscles, to create a reservoir for prolonged intraocular expression and secretion of therapeutic molecules. The model that was used is that of endotoxin-induced uveitis (EIU) in the rat, an acute anterior chamber inflammation where cytokines such as TNFα play a central role. Using specially designed electrodes (a needle cathode inserted through a corneal tunnel, and a contact anode overlying the ciliary body region), naked DNA plasmids were delivered directly into the ciliary body muscles and their expression was verified by using plasmids encoding GFP or luciferase. The therapeutic potential of this technique to inhibit EIU was examined using a gene encoding human TNFα receptor I in soluble form (hTNFR-Is). Soluble TNF-α receptors are widely used clinically as systemic treatment for various types of arthritis (Enbrel/Remicade). Anti-TNFα treatment was reported to inhibit EAU in rodents, and in a nonrandomized, open-label, clinical pilot study infusion of soluble TNFα receptor was reported to lead to remission of posterior uveitis in human patients [11, 27], although most required continued low-dose adjunct immunosuppressive therapy. The hTNFR-Is construct electroporated into ciliary muscles was expressed at levels sufficient to result in a significant decrease in the aqueous levels of TNFα (note that human and rat TNF-α are homologous; thus, the human receptor binds the rat TNFα molecule). In parallel, inflammatory clinical and histological signs of EIU were ameliorated [5].

Summary for the Clinician

- Local delivery of genes encoding anti-inflammatory cytokines, such as IL-10, can be effective in reducing inflammation.
- Efficiency of transduction is vector dependent and determines whether the gene product will reach therapeutic levels within the eye.
- Local expression of therapeutic genes may be less likely to produce undesirable side effects, especially if gene expression can be controlled by an inducible promoter engineered into the construct.
- Viral delivery systems may be superseded by the development of approaches, such as electroporation of naked DNA directly into ocular tissues, that could offer better clinical applicability.
- However, it must be kept in mind that local intervention can by its nature only treat the symptoms, and will leave the underlying disease process unaffected.

13.4.3 RNA Interference as a Future Prospect

It is of interest to mention RNA interference (RNAi), a relatively new technology for gene silencing that has been gaining momentum and is rapidly replacing the antisense DNA approach [20, 40]. RNAi may find application in ocular therapeutics to inhibit expression of transcription factors or of proinflammatory cytokines instead of the currently used toxic pharmacological agents. Small inhibitory RNA (siRNA) is a highly conserved mechanism believed to have evolved as protection that eukaryotic cells use to inhibit expression of unwanted RNA species, such as viruses and transposons. Due to its complementary nature, it is highly specific to its target sequence, although as therapy one must

take into account the possibility of cross-reactive, off-target and nonspecific effects. In order to achieve long-term expression, cells are transduced with a DNA construct under control of the specialized Pol III promoter that drives its transcription into short hairpin RNA (shRNA); shRNA is recognized by an intracellular enzyme known as Dicer and is cleaved into siRNAs, the final inhibitory effector sequences. Regulatory and inducible elements can be built into the construct. Although complete inhibition of the target gene is almost never achieved, RNAi can provide an efficient and long-term gene knockdown, and a heritable expression that can be exploited for the production of gene knockdown mice. However, the method of introducing the siRNA-generating DNA fragments into cells rely on the same viral and nonviral methods as "traditional" gene therapy, so in that regard similar caveats will apply.

13.5 Conclusions

Gene therapy is a very attractive therapeutic option, as it carries the promise of more or less permanently curing a clinical condition. As our understanding of the critical checkpoints in the pathogenesis of autoimmune ocular disease improves, more and more potential intervention points and candidate therapeutic targets are identified. New technologies emerge, promising more specific and more easily applied therapies. Nevertheless, serious concerns about vector development and delivery methods remain to be addressed, including such issues as vector immunogenicity, method of administration, efficiency of transduction, duration of gene expression as well as the ability to turn expression off when deemed necessary. None of the therapy paradigms offers a perfect solution. In the case of antigen-specific therapies, arguably the most desirable approach, choice of antigen(s) is a central issue, as in many cases the participating antigen is uncertain and multiple specificities may be involved. Not to be ignored is also the potential for eliciting unwanted immune responses by the introduction of an autoantigen into an already primed host. Paradigms targeting common lymphocyte activation functions have the potential of inhibiting desired immune responses as well, whereas strictly local therapies, while having the potential for the fewest side effects, leave the underlying autoimmune process unaffected.

Acknowledgements

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Chapter 14

Optic Neuritis from the Perspective of an Ophthalmologist

14

Klaus Ruether

Core Messages

- In principle, typical (demyelinating) optic neuritis (ON) occurs in patients between 20 and 50 years of age.
- The diagnosis of ON is ensured by history and clinical examination (onset within days, pain with eye movement, afferent pupillary defect, monocular decrease in visual acuity, visual field defects, optic disc unremarkable or swollen).
- After a first isolated episode of optic neuritis the probability of developing MS over about 10 years is 22% in cases with no white matter lesions in the MRI and 56% in those patients with one or more significant lesions (overall risk of 38%, results of the "Optic Neuritis Treatment Trial" [ONTT]).
- If the patient is willing to learn more about her/his disease a brain MRI should be performed.
- Therapeutic options in the case of acute ON are intravenous methylprednisolone (1,000 mg) per day or no drugs.
- Application of intravenous methylprednisolone accelerates the disappearance of pain and the resolution of vision, but does not reduce the risk of developing MS in the long run.
- Prednisone at dosages of 1–1.5 mg/kg body weight is not indicated.
- As immunomodulatory agents (IMAs) possibly reduce risk of developing MS in patients who have isolated optic neuritis and significant white matter lesions on MRI, the ophthalmologist is basically obliged to inform the patient about the association between ON and MS.
- The care for patients suffering from ON should be performed in cooperation with neurologists, in particular in cases of recurrent disease.
- Atypical optic neuritis should be considered in cases of bilateral involvement, lack of pain, patients outside the age range of 20 to 50 years, marked optic disc swelling and/or retinal hemorrhage and/or retinal exudates, and no improvement in vision after 6 weeks.

14.1 Introduction

As a rule, the first episode of optic neuritis leads a patient to consult an ophthalmologist and eventually a neuro-ophthalmologist. For the latter, optic neuritis is one of the most prevalent diseases. However, there is a clear overlap with other medical disciplines, primarily with neurology and, where appropriate, with pediatrics. In the case of recurrent episodes, the neurologist

becomes the primary contact person, especially if optic neuritis is part of a chronic inflammatory disorder of the central nervous system. In general, demyelinating optic neuritis is a rare disease. The incidence is about 3 in 100,000 of the population per year in the USA [19] and this may be similar for Europe. About 75% of ON patients are female.

The most important task of the ophthalmologist in the management of optic neuritis is to find out whether the patient has typical optic neuritis (ON) or something else. "Typical" means monocular demyelinating optic neuritis, no known systemic disease besides multiple sclerosis, and characterized by the symptoms listed in Sect. 14.3.1. Optic neuropathy emerging in patients suffering from multiple sclerosis is looked upon as typical optic neuritis. The most important differential diagnosis of typical optic neuritis, the subject of this chapter, is atypical optic neuritis in which a defined cause can be detected. In addition, anterior ischemic optic neuropathy, toxic, hereditary, and compressive optic neuropathy, as well as retinal disorders, have to be excluded.

The Optic Neuritis Treatment Trial (ONTT) [5] and the subsequent investigations based on the original cohort [4, 6–10, 24, 25] plays the continuing role of defining diagnostic procedures and therapeutic options in optic neuritis patients. In addition, counseling and the use of cerebral MRI have been influenced by the CHAMPS (Controlled High-Risk Subjects Avonex Multiple Sclerosis Prevention Study) [12, 18]. However, although the ONTT in particular and its related investigations have made available a vast amount of information about the natural course of optic neuritis, there are still open questions regarding adequate diagnostic procedures as well as the optimal treatment regimen.

Summary for the Clinician

- Optic neuritis (ON) is a rare disease (3 per 100,000 of the population per year), and females are more often affected (75%).
- The most important task for the ophthalmologist with regard to the management of ON is to rule out other diseases such as atypical ON, compressive optic neuropathies, or retinal disorders.
- For the management of ON the results of the Optic Neuritis Treatment Trial (ONTT) and the CHAMPS Study (Controlled High-Risk Subjects Avonex Multiple Sclerosis Prevention Study) are of great relevance.

14.2 Pathogenesis

14.2.1 Pathology and Immunology

Studies concerning the pathogenesis of ON are quite rare and most knowledge has been drawn from patients with definite multiple sclerosis (MS). MS is a disease of the white matter and demyelination is the most important pathologic event; however, axonal damage can also be seen. Data from MS patients indicate that there are probably heterogeneous pathogenetic mechanisms. Local perivascular mononuclear cell infiltration of the myelin can be observed. It has been shown that some patients reveal T cell- or T cell plus antibody-mediated myelitis indicating an autoimmune process, while others show an oligodendrocyte dystrophy reminiscent of virus- or toxin-related demyelination [22]. Other studies imply apoptotic cell death of oligodendrocytes and microglia activation in myelinated tissue [3], findings that might have therapeutical implications in the future. Astrocytic proliferation leads to gliotic lesions explaining the term "sclerosis." While there seems to be less inflammation in cortical lesions compared with white matter lesions, cortical demyelination finally leads to axonal transection, dendritic transection, and neuronal death by apoptosis [26].

In many patients with ON an abnormal B cell response can be detected. Typically, it can be shown by IgG oligoclonal bands in the cerebrospinal fluid (CSF). Antibodies for myelin basic protein (MBP), phospholipid proteins, and myelin oligodendrocyte glycoprotein (MOG) could be detected in the serum [10, 28]. The aim of studies of this kind is to be able to detect those patients with an episode of ON who are at risk of the early development of MS using a simple blood test. However, these results are still controversial [21]. Recently, promising work concerning other biomarkers has been published (summary in [11]). All these studies are directed at optimizing the time of onset and the mode and the extent of therapeutic interventions.

14.2.2 Relationship Between Optic Neuritis and Multiple Sclerosis

It has been long established that there is a relationship between ON and the occurrence of MS (e.g., [30]). Moreover, it has been hypothesized that if ON were the first symptom of MS this might be indicative of a relatively benign course of MS [13, 29]. In previous times there was no reason to inform an ON patient about the risk of developing MS and respective probabilities because there was no therapy for MS available. Today, although there is still no fully satisfactory treatment, reports on some beneficial effects of early therapy in those patients at a high risk of developing MS basically alter the situation. For this reason, ophthalmologists should know how many patients experiencing the first event of ON will develop MS. However, respective data previously published showed an unacceptably high variability ranging from 11.5 to 85% [1]. The ONTT gives valuable information on this issue. Throughout this study the risk of developing MS after isolated ON is 30% after 5 years [24] and 38% after 10 years [9]. This number is refined by taking into account the results of the brain MRI. The number of lesions (white matter lesions with a diameter of equal to or more than 3 mm on T2-weighted MRI) is correlated to the risk of developing MS. Patients with no white matter lesions were at a 22% risk of MS, while the risk of those with one or more significant lesions was 56% after 10 years [8]. In those patients with no lesions, male gender, and signs of atypical ON such as missing pain, no light perception, pronounced disc swelling, peripapillary hemorrhage or retinal exudates the probability of getting MS after 10 years decreased further. As there are comparable data in more recent studies these numbers seem to be reliable and help the ophthalmologist taking care of patients with a first episode of ON.

Summary for the Clinician

- In ON due to MS demyelination and axonal damage can be observed.
- There are hints at an autoimmune as well as at a viral or toxic origin of the disease.
- Astrocytic proliferation leads to gliotic lesions explaining the term "sclerosis."
- In patients with ON an abnormal B cell response directed at different proteins, such as myelin basic protein or myelin oligodendrocyte glycoprotein, can be detected.
- The ONTT renders estimates for the development of MS after an episode of ON: 22% of patients with no lesions and 56% of patients with one or more significant lesions on MRI developed MS within 10 years.
- The patient with ON should be carefully informed about the possible association with MS because he/she should be able to decide on possible early treatment options.

14.3 Diagnosis

14.3.1 Ophthalmologic

Before talking to the patient and before any kind of examination, the age of the patient is an important issue rendering the diagnosis of optic

neuritis more or less probable. Typically, the age of the patient is between 20 and 50 years. Accordingly, in the ONTT the eligibility criterion concerning age was 18 to 46 years [5]. The mean age of ON patients seen in our institution is 35 years (SD 8 years). The present history of the patient gives important clues to a correct diagnosis. Typically, symptoms arise within days. If there is a more sudden onset vascular diseases have to be excluded, in particular anterior ischemic optic neuropathy in older patients. In contrast, a history of long-standing visual loss over months or even years favors compressive or hereditary optic neuropathy. Sometimes, patients report worsening of vision on elevating body temperature, e.g., in the bathtub or with physical exercise (Uthoff symptom).

The typical ophthalmological symptoms of ON are:

1. Pain with eye movement;
2. Monocular decrease in visual acuity;
3. Relative afferent pupillary defect (RAPD);
4. Visual field defects;
5. Impairment of colour vision;
6. Optic disc unremarkable or swollen;
7. Prolonged peak-latency of the P100 of the pattern reversal visually evoked potential (VEP).

A case of ON is illustrated in Fig. 14.1. The pain is located behind the eye or periorbitally. Although it is a frequent and important sign there are definitely cases of ON in which there is no pain. However, lack of pain should be a reason to give more attention to clues indicating atypical optic neuritis. The decrease in visual acuity may be mild or even lacking, but can decline to "no light perception." The RAPD must be present, but is sometimes subtle so it might escape detection. This is a particular problem in patients who had previously suffered an occasional episode of ON in the fellow eye without knowing it.

As a basic visual field examination a 30° threshold perimetry should be performed. In addition to the typical visual field defect, the central or centrocecal scotoma, a wide variety of patterns of visual field loss may occur. Visual field defects respecting the vertical or horizontal midline are of special interest as they may give a hint as to whether there is a central (if vertical) or a vascular (if horizontal) disorder. No pain on eye movements and swelling of the optic disc do not exclude typical optic neuritis, but atypical forms should be included in the differential diagnosis with more emphasis than in cases with pain and unremarkable optic disc (for example see Fig. 14.2).

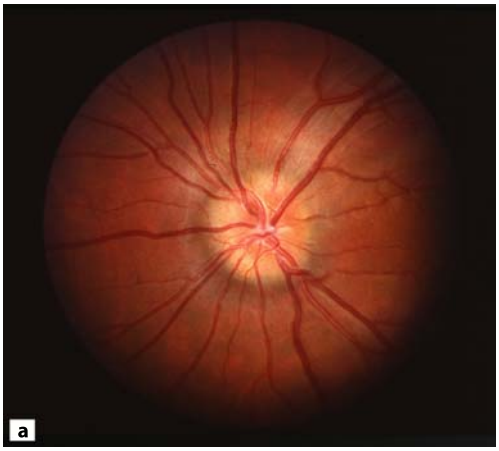
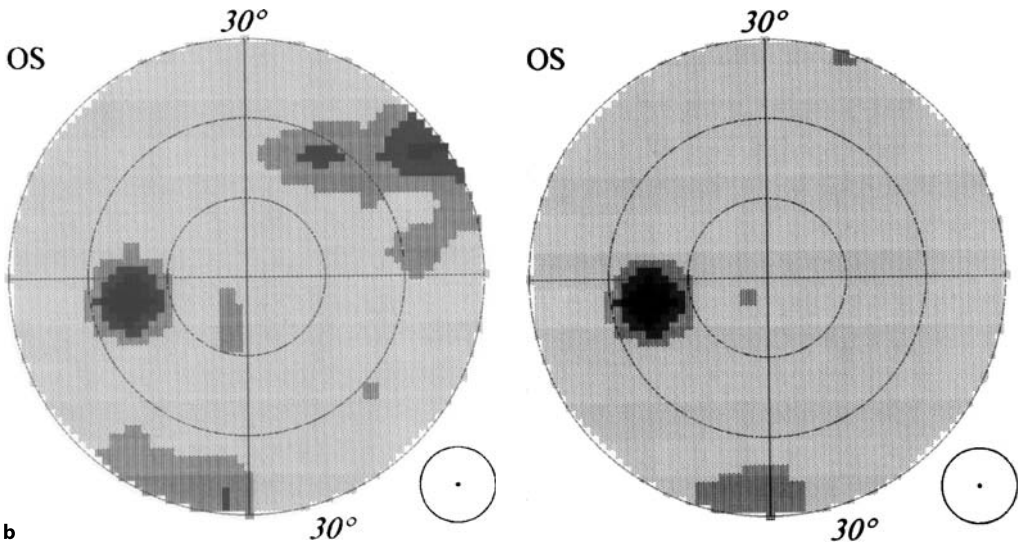


Fig. 14.1 Patient with demyelinating optic neuritis: a 22-year old man who had had blurred vision in his left eye for 2 days with weird sensations, visual acuity OD 20/16; OS 20/20. MRI: multiple white matter lesions. **a** Swollen optic disc in OS; **b** 30° threshold perimetry (Octopus perimeter 101). *Left panel*: minor visual field defects. *Right panel*: 7 days later. **c** Pattern reversal visual evoked potentials. OD (*left panel*): slightly broadened P100 complexes, OS (*right panel*): prolongation of P100 peak latency (approximately 130 ms in OS vs. 110 ms in OD)

The minimum tools for the diagnosis of typical ON performed by the ophthalmologist should include the following:

1. Measurement of visual acuity
2. Swinging flashlight test
3. Examination of smooth pursuit eye movements
4. 30° visual field
5. Fundoscopy

The VEP may be helpful for the diagnosis of typical optic neuritis, in particular in cases in which other optic neuropathies cannot be ruled out. It has to be emphasized that the amplitude of the P100 component of the pattern reversal VEP



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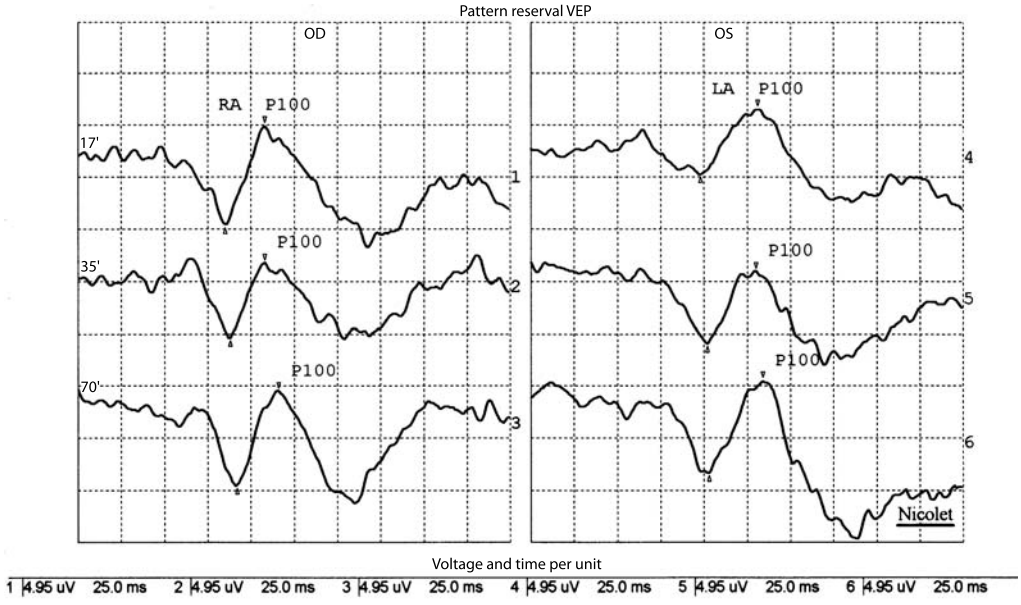

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does not render any meaningful information. In contrast, the P100 peak latency may be helpful. However, it must be realized that a prolongation of this latency may occur in a variety of optic neuropathies and retinal disorders as well (see Fig. 14.3). Based on our experience the amount of prolongation is crucial and should exceed 30% of the normal value or of the P100 peak latency of the fellow eye. It should be emphasized that in principle the diagnosis of ON does not depend on the VEP, as it is clinical. The measurement of contrast sensitivity or low contrast visual acuity is certainly sensitive in ON [2] and a decrease in contrast sensitivity may bother the patient much

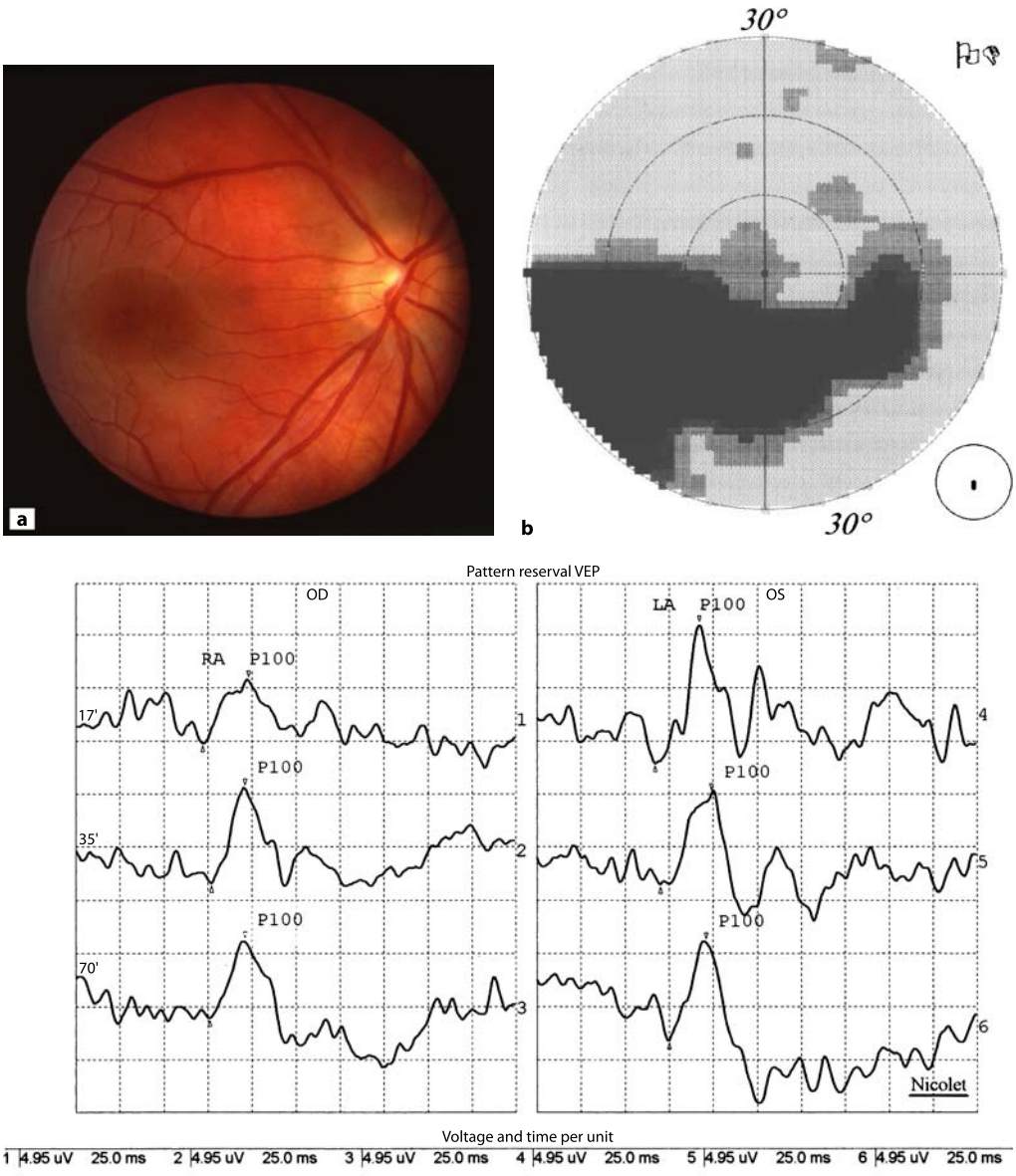


Fig. 14.2 Patient with atypical optic neuritis: 18-year-old woman who had had "shadow" OD for 4 days, with no pain or unusual sensations in the right eye, visual acuity 20/20 OU. Serum antibodies for toxoplasmosis. **a** Mildly, sectorially swollen disc in OD. **b** Absolute nerve fiber layer defects in OD (Octopus perimeter 101). **c** Pattern reversal visually evoked potential (*VEP*) with somewhat smaller P100 amplitudes in OD (*left panel*) compared with OS (*right panel*), but without P100 peak latency shift

longer than decreased visual acuity. However, standardization of tests for contrast sensitivity is not yet satisfactory. Therefore, this kind of testing cannot currently be recommended as a baseline investigation for ON.

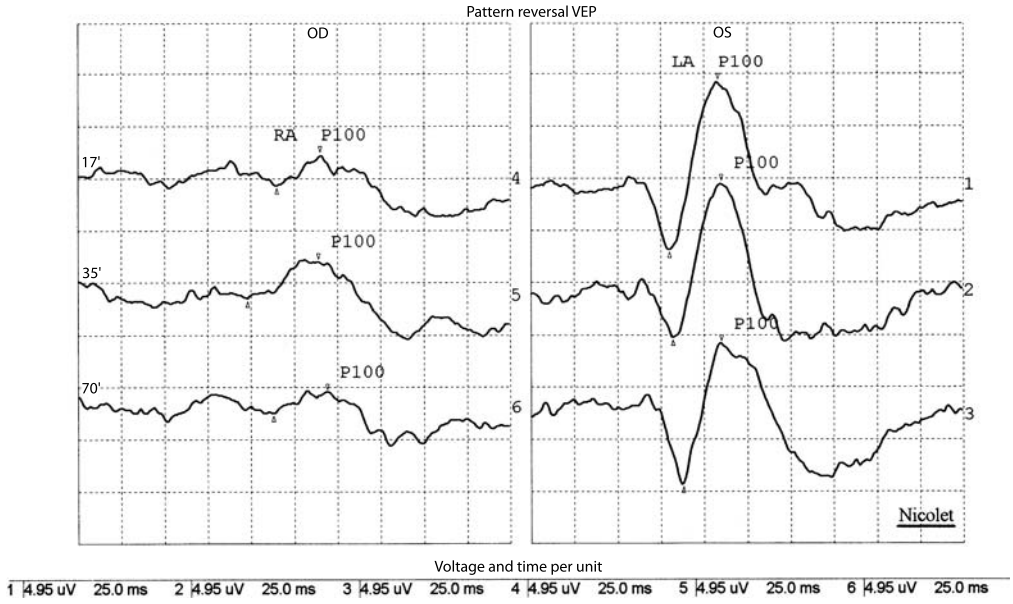


Fig. 14.3 Pattern reversal VEP of a patient suffering from an optic nerve sheath meningioma in OD. The P100 of the right eye (*left panel*) is markedly prolonged, the left eye (*right panel*) is unremarkable (approximately 140 ms in OD vs. 110 ms in OS)

Atypical optic neuritis should be considered in cases of:

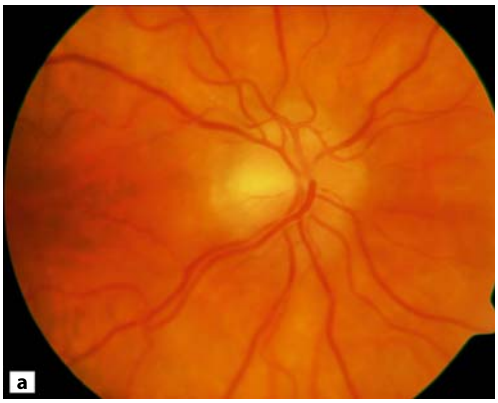
1. Bilateral disease;
2. Marked optic disc swelling and/or retinal hemorrhage and/or retinal exudates;
3. Lack of pain;
4. No light perception;
5. Age over 50 or below 20 years.

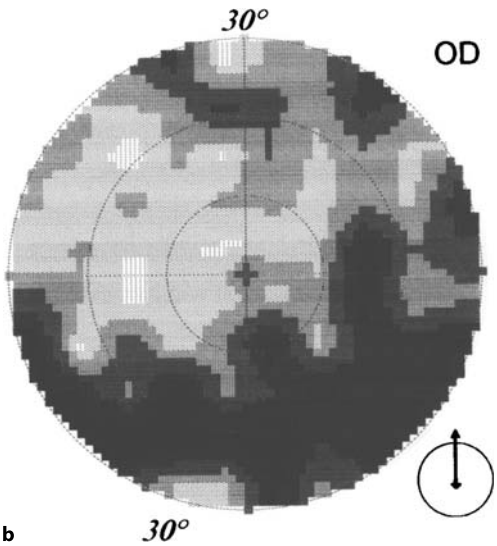
Clinical symptoms may be comparable to those of demyelinating ON. However, resolution of vision is sometimes prolonged, a feature that may be indicative of atypical ON per se. Theoretically, anterior ischemic optic neuropathy (AION) may be a differential diagnosis for ON. However, the different age of manifestation (age <50 in ON, >50 in AION), the frequently different visual field defect (central scotoma and others in ON, altitudinal field loss in AION), and the aspect of the optic disc (no alterations or homogenous swelling in ON, sectoral swelling with hemorrhage in AION) allow in many cases to clearly differentiate these two disease entities (Fig. 14.4). In people older than 50 years it may, however, be difficult to discern vascular optic neuropathy from atypical optic neuritis.

In addition to typical optic neuritis other manifestations of demyelinating disease may occur, which may be first encountered by the ophthalmologist, but these are much less frequent than ON. In our experience the most common early ophthalmological symptoms of MS apart from ON are:

1. Afferent defect in parts of the visual pathway other than the optic nerve (chiasm, optic tract, optic radiation, and visual cortex);
2. Uveitis (frequently intermediate uveitis or periphlebitis);
3. Internuclear ophthalmoplegia (INO);
4. Gaze-evoked nystagmus and nystagmus on eccentric gaze;
5. Spontaneous nystagmus (occult or overt).

This list also implies that the ophthalmologist who cares for a patient with optic neuritis should not omit looking at the efferent visual pathway.





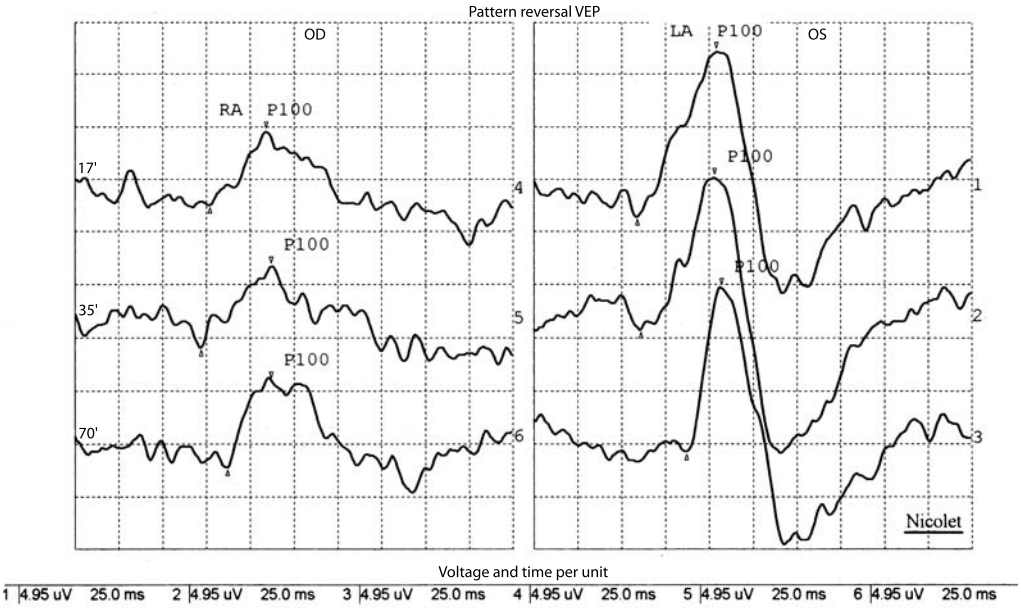


Fig. 14.4 Patient with anterior ischemic optic neuropathy: 67-year old woman who had had visual deterioration with sudden onset in OD for 2 days. Visual acuity OD 20/50, OS 20/25. **a** Sectoral swelling of the optic disc (in OD), pronounced in the upper half. **b** Nerve fiber layer defects in the 30° threshold perimetry (Octopus perimeter 101). **c** Pattern reversal VEP P100 amplitude reduction in the affected eye (OD, *left panel*), but no P100 peak latency prolongation

14.3.2 Non-Ophthalmologic Diagnostic Tools

The extent of the use of additional diagnostic tools that should be employed in cases of typical optic neuritis has been defined by the ONTT [5]. It was stated that routine blood tests, chest X-ray, brain MRI, and lumbar puncture are not necessary for the diagnosis of typical ON. However, bearing in mind early therapeutic options and the possibilities of patients obtaining medical information via the internet, additional diagnostic tools nowadays have to be measured. For ophthalmologists the most important ancillary test in patients with ON is brain MRI, not least in their collaboration with neurologists. If MRI is performed the main parameter is the presence and extent of white matter lesions in the brain. The MRI appearance of the optic nerve itself is again not necessary for the diagnosis. Moreover, it is still not clear whether the appearance of the optic nerve as seen on orbital MRI in patients suffering from acute ON tells us anything about the prognosis or the effect of corticosteroid therapy [15, 20, 23]. As stated in Sect. 14.2.2 brain MRI helps to define the probability of developing MS. This issue should be discussed with the patient. Moreover, if a brain MRI is initiated for a patient with ON there has to be a plan outlining how to deal with the potential result. In the literature, up to 70% of patients with a first episode of ON show periventricular white matter lesions on MRI consistent with demyelination [14] and the patient has the right to be told the significance of this finding.

The ONTT also looked at the justification of lumbar puncture in ON patients [27]. In a subgroup of 83 patients of the ONTT the most prevalent alteration of the CSF was oligoclonal bands. Thirteen of these 83 patients developed MS after 2 years, 11 having had oligoclonal bands in the CSF at the baseline examination. Although 2 of these 11 patients had normal brain MRI the authors do not believe that CSF analysis is helpful in patients with typical ON, but analysis at later time points of the ONTT is still lacking. From the perspective of a neurologist, however, the analysis of cerebrospinal fluid might become important as a diagnostic criterion for MS in patients who have suffered from ON at any time.

In conclusion, if the patient is willing to know about the risk of having or developing MS, brain MRI and eventually an analysis of CSF should be performed. In our experience, information on early therapeutic options drives the vast majority of patients to undergo the ancillary tests mentioned above. This is even true when the ophthalmologist, based on the respective recommendations, does not believe that early immunomodulation therapy is applicable in that particular patient. Although as an ophthalmologist there is no great need for these ancillary tests, it must be realized in collaboration with neurologists that the decision regarding the definite diagnosis of MS should be based on a broad and partly redundant spectrum of diagnostic tools.

In cases of atypical optic neuritis the VEP may be helpful, as shown in Fig. 14.2. In any case, in atypical optic neuritis the work-up has to be more comprehensive. In particular, serological investigations should be initiated (e.g., lues, borreliosis, toxoplasmosis, cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), human immunodeficiency virus type 1 (HIV1), possibly Bartonella henselae). In addition, analysis of antinuclear antibody (ANA), anti-neutrophil cytoplasmic antibody (ANCA), angiotensin converting enzyme (ACE), C-reactive protein (CRP), erythrocyte sedimentation

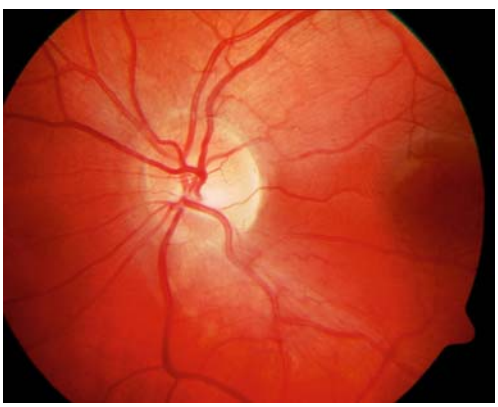


Fig. 14.5 Optic disc (OS) of a 15-year-old boy with Leber's hereditary optic neuropathy (LHON). Small as well as large vessel abnormalities can be detected. Visual acuity in the affected left eye was 20/400, in the hitherto unaffected right eye: 20/20 (homoplasmatic mutation G to A at position 11778 of the ND4-gene)

rate (ESR), serum vitamin B_{12} and a blood count should be performed. With regard to imaging techniques, brain MRI and a chest X-ray should be carried out. If there is no resolution of vision in atypical cases and/or the second eye is also involved, molecular genetic diagnostic tools should be used to rule out Leber's hereditary optic neuropathy (LHON). Ophthalmologists recognize this disease by the peripapillary alterations of small and large vessels (Fig. 14.5).

Summary for the Clinician

- The diagnosis of ON is based on the typical age (20–50 years), the typical history, and typical ophthalmological symptoms.
- Typical ophthalmological symptoms are: pain on eye movement, unilateral loss of vision, visual field defects, and relative afferent pupillary defect.
- The visual evoked potential (VEP) is hampered by the fact that there is often no pattern response in acute disease.
- If the patient wants to know more about the disease MRI is the most important non-ophthalmological procedure.
- In order to confirm or to refute the suspicion of MS additional tests such as CSF analysis may be useful in collaboration with neurologists.

14.4 Treatment

14.4.1 Natural Course

Since the publication of the results of the Optic Neuritis Treatment Trial in 1992 [4], high-dose methylprednisolone therapy has become the standard therapy for ON in Germany and in other countries all over the world. However, the benefits for the patient are limited as the visual outcome of the disease is good even without any therapy. Clinical experience in untreated patients tells us that within days or weeks first the pain and then the vision loss resolve. In most cases there is further improvement over the next few months. Over a long period visual acuity was 20/40 or better in 92% of the affected eyes of 319 ONTT patients followed for 9.9 to 13.7 years and it was worse than 20/200 in only 3% [9]. Visual loss beyond 20/200 was only observed in eyes experiencing recurrent episodes of ON. This overall outcome of visual acuity was independent of therapy. These numbers are of great importance for counseling the patients presenting with a first episode of ON. Also important for this purpose is the fact that in this cohort of patients 35% developed recurrent neuritis within the time frame mentioned above. Although recurrent ON was observed more often in patients developing MS the fact of recurrence alone is not sufficient to make the diagnosis of MS.

14.4.2 High-Dose Methylprednisolone

One of the most important results of the ONTT was that the application of oral corticosteroids at dosages of 1 mg/kg bodyweight increased the risk of recurrence of ON (27% risk with oral prednisone, 13% with placebo or intravenous methylprednisolone). This kind of management was well established, at least in Germany, before 1992, although there had not been sufficient published evidence. This important outcome remains true after an observation period of about 10 years (43% risk of recurrence in patients treated with oral prednisone, 29% in the intravenous methylprednisolone group and 31% in the placebo group) although statistical significance was lacking [9]. However, daily experience shows that there are still ophthalmologists who prescribe oral prednisone at dosages between 1 and 1.5 mg/kg bodyweight in cases of typical ON. It remains obscure whether this attitude is related to a lack of knowledge or whether there is genuine skepticism concerning published study results [17].

In neurology, corticosteroids have been regularly applied in patients suffering from relapsing–remitting MS during acute episodes, first to ameliorate the acute symptoms and secondly in the hope of positively influencing the future course of the disease. During the time straight after the publication of the ONTT the expectations asso-

ciated with the application of high doses of intravenous methylprednisolone, i.e., 250 mg every 6 h for 3 days followed by oral prednisone 1 mg/kg/day for 11 days, were high for several reasons. First, the improvement of visual loss was faster in the intravenously treated patients, at least within the first 15 days. This difference compared with patients treated with placebo became smaller after 6 months and finally disappeared after 1 year of observation [5, 6]. In spite of this equalization the effect is an important point for ON patients when they are informed of their disease and the therapeutic options when the first episode occurs. This is particularly the case in younger patients. In addition, our own experience shows that the almost immediate disappearance of the ocular pain should not be underestimated when counseling patients about their options.

The 2-year results of the ONTT were promising with regard to a possible decrease in the risk of developing MS by the application of high doses of methylprednisolone after the first event of ON [6]. In those patients with two or more white matter lesions on MRI the risk of developing MS was 16% in the patients with the intravenous treatment and 36% in the placebo group. The significance of this difference disappeared at the 3-, 5-, and 10-year follow-up [4, 8, 25]. In addition, the 2-year results were not observed in other studies, for example in those patients with two or more lesions visible on MRI [12, 18]. In summary, it does not seem to be justified counseling the patient with lesions on MRI that his/her risk of developing MS can be reduced if intravenous methylprednisolone is administered after the first attack of ON. However, it has to be conceded that a single intravenous methylprednisolone treatment might not be sufficient. Zivadinov et al. found in a study including 88 patients with relapsing–remitting (RR)-MS that regular pulse therapy (every 4 months for 3 years and every 6 months for 2 years) may be of benefit to the patients [31]. Compared with patients with RR-MS receiving intravenous methylprednisolone only in the case of a relapse the regularly treated patients showed a slowed development of T1 black holes on MRI and a delay in brain atrophy. In addition, disability progression was also delayed. They stated that additional studies are warranted to clarify this question.

Therapy by intravenous methylprednisolone is characterized by its route of administration (i.e., intravenously) and the dosage. Although there is still no convincing evidence, dosage is probably the critical parameter. For this reason, the "Quality Standards Subcommittee of the American Academy of Neurology" recommended the application of at least 500 mg of prednisolone either orally or intravenously [19].

14.4.3 Immunomodulation

Immunomodulation other than corticosteroids has been a therapeutical option for RR-MS for many years. The recent development of interferons has led to the recommendation to start immunomodulatory therapy as soon as the diagnosis of MS is established. For ophthalmologists the point of view is different. The question is whether a patient with a first episode of ON should immediately or soon receive immunomodulatory agents (IMAs). The results of the already cited 383 patients comprising industry-sponsored CHAMPS imply that interferon beta-1a should be started in patients after the first episode of optic neuritis or other acute demyelinating events (incomplete transverse myelitis, or a brain-stem or cerebellar syndrome), who are at a high risk of developing MS [18]. This high risk was defined by the presence of demyelinating lesions on brain MRI. All patients received intravenous methylprednisolone according to the ONTT ahead of interferon beta-1a. Looking at the 192 patients with ON [12] after at least 22 months of follow-up, 28% of the interferon beta-1a group versus 37% of the placebo group developed MS (adjusted rate ratio in the treated patients was 0.58 [95% confidence interval, 0.34–1.00; p=0.05]). Basically, MS was defined by a second demyelinating event. Combined with the MRI criteria (change in T2 lesion volume, number of new or enlarging T2 lesions, number of gadolinium-enhancing lesions) the respective numbers are 61 and 75% (adjusted rate ratio 0.50 [95% confidence interval, 0.34–0.73; p<0.001]). Although different in design, the results of the CHAMPS have basically been confirmed by the "Early Treatment of Multiple Sclerosis Trial" (ETOMS) [16].

Independent of his/her own attitude toward such studies as CHAMPS they force the ophthalmologist to inform the ON patient about the relationship between ON and MS. In addition, this study enhanced the role of MRI and led to more intensive cooperation with the neurologist. Again, it has to be recognized that the internet makes studies like the ONTT and CHAMPS easily accessible to patients or their relatives, especially in cases of younger patients suffering from ON.

The decision in favor of IMAs is certainly difficult for the patient as there are some concerns:

1. It is not a definite therapy.
2. In the case of interferon beta-1a it has to be administered intramuscularly once a week.
3. In the long run the therapy would not have been necessary for many patients (44% in the ONTT with no MS after 10 years)
4. Therapy is associated with high costs.
5. Therapy forces the diagnosis of MS into the daily life of patients and, if applicable, into that of their family.

The near future will probably bring better alternatives. The example of natalizumab (Tysabri), a selective adhesion molecule, which inhibits binding of leukocytes and prevents their transmigration across the endothelium into inflamed parenchymal tissue, showed that substances with a greater therapeutic potential are conceivable. Unfortunately, this drug has been taken off the market because of serious side effects. Certainly, the decision of the patient to use IMAs is mainly biased by the physician who is taking care of the patient. As this decision is difficult for the patient we recommend that even after a first episode of optic neuritis there should be patient contact with a neurologist, not least because they have more experience in the management of MS patients and in the administration of IMAs.

Summary for the Clinician

- The benefit of intravenous methylprednisolone therapy in ON patients is a faster recovery of visual loss and an early disappearance of ocular pain.
- The application of oral corticosteroids at a dosage of 1 mg/kg bodyweight is obsolete.
- The decision to take immunomodulatory agents other than steroids (e.g., interferons) must be carefully discussed with the patients and should follow the effective consensus.

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Subject Index

D

E

F

G

H

I

J

O

P

R

S

T

U

V

W

X