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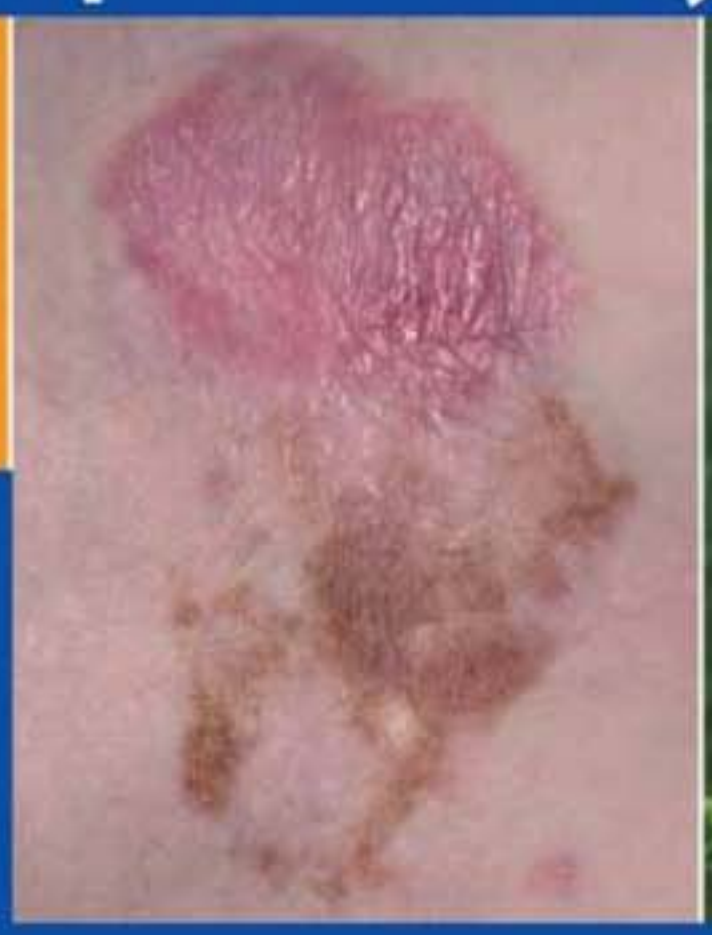
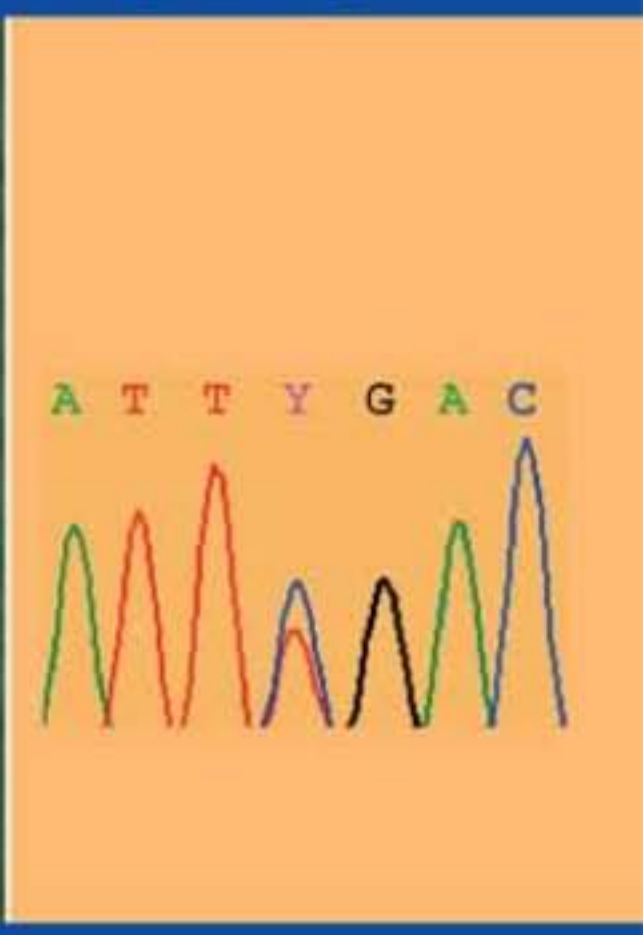
Life with Epidermolysis Bullosa (EB)





Etiology, Diagnosis, Multidisciplinary Care and Therapy

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Jo-David Fine
Helmut Hintner (eds.)

Life with Epidermolysis Bullosa (EB):
Etiology, Diagnosis, Multidisciplinary Care and Therapy

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Forewords

As a patient

Questions, answers.

I often hear people saying: 'Wow, this is such a challenge.' And it's true. When a child grows up, having EB, where every day is not like the day before, it takes several steps until it learns to master this lifetime challenge. You have to accept it somehow, what turns out to be not very easy. Asking questions like 'why does it happen to me?' is ok, but until you can find an answer there's a long way to go. A life with EB is full of questions. So I think this book is very important, because it is able to give some answers, to the parents, the docs and nurses, maybe also to friends and partners. It helps to figure out what to do, when you realise that your skin is just not working right. And then time goes by and every child, every growing up, every man and woman learns how to live with EB. For me it was a very important step, when I was able to fix my skin on my own. When I realised, that now I can control EB, and that it's not longer controlling me. These moments are the ones in which you grow, finding your own ways, finding self confidence, losing fears, learning to trust yourself. Now I'm not a child anymore. Why I have to live with this challenge is not longer the primer question. EB is a part of the person that is me. Still every day is not like the day before, but in the end, this is, what keeps you awake in life, isn't it?

May this book be helpful to find answers for your skin (and this will make it easier to find the answers for your mind).

Thanks to all those who worked on this book! You are the ones, who take the steps with us. We can move faster forward together!

Ianina Ilitcheva
EB sufferer

As the mother of a patient

Being a mother of a child with eb is like a journey . . .

. . . a journey from the hurt of my child to the hurt of my "inner child", from pain to compassion, from disappointment to satisfaction, from rage to love, from guilt to forgiveness, from "Oh, my God!" to "Thanks God"!

My son is 15 years old now and lives with junctional EB.

When he was born in a village near Salzburg, nobody immediately knew what he had. We were lucky that by that time, Professor Dr. Hintner already was an EB-specialist located in Salzburg. So he told us about EB and recommended us to meet a mother with an EB-child in Italy, to hear something about what life is like with EB. I was shocked, but also full of hope, because the child we met was a positive and lively child.

The first years with my son where filled up with sorrows, rage and guilt. I didn't look at my son, but only at his skin. Some lost years. I am still sad about it, that I wasn't able to be in a close positive emotional contact to my son.

Soon I started to realize that the skin of my son hides a secret message for me. EB is not only a genetic condition or a medical problem. For me, it has got a deeper meaning, makes sense and enriches my life. I learned that when looking at my son it was like looking into a mirror, in which I could recognize myself and my feelings of being less worthy, dirty, not loveable,. . . And by coming into touch with my own pain, I came into contact with my heart and then longed for ability to love myself and my child.

But it was a very heard and painful time. Pain caused by around 100 blisters daily, but also psychical pain, full of open questions. Will my son ever be able to run? Will he be able to go into a normal school? Will he have friends? Will his fingers grow together? Will he loose his hair and nails?

The first time, I did everything to make EB gone away, to compensate the handicap, to make everything better than any other mother in the whole world.

Today living with EB has become much easier for my son as well as for me.

Firstly because of the tremendous media-work of the patient support group debra-austria, many doctors and nurses and nearly all of Austria know now about this genetic condition, so we feel understood and we are not asked all the time about whether my son has been burnt, and not told any longer which cream could help.

Secondly, the skin of my son became much better and he can do a lot more than we have ever thought he can do. Today he is a boy who runs on his feet, goes to a higher school, has friends, didn't loose his hair and nails, plays football, skates, plays the guitar, the drums, table-tennis and tennis, rides the bike, opens a Coke, eats what he likes, wears jeans and belts and carries his satchel.

Thirdly, life became easier, because of our belief in Love – in God. Love and compassion heals all wounds, that's what we believe, and what my son's skin has told us.

I want to thank Prof. Dr. Helmut Hintner and his medical and research-team and Dr. Rainer Riedl and his debra-team for their work for the debra-families! I want to thank the father of my son for his support the years after birth. I want to thank my husband, that he accepts his step-son and that he drove on our spiritual way. And I want to thank Valentin for teaching me mindfulness.

Last but not least I want to thank all who took part of this book. I am sure it will be a help for all people living with EB, parents, and for all professionals who are concerned with Epidermolysis Bullosa Hereditaria.

Stefanie Zauchner-Mimra

As a patient support group

debra-austria

"Was lange währt wird endlich gut" ("the longer it takes the better it will be") is a famous German saying. Writing a book does not happen over night, and the work put into it can not be measured, especially for this book of complete guidelines and standards of care and therapies for people with Epidermolysis bullosa (EB). The making of this book involved bringing together and coordinating the work of many first class authors.

"**Life with Epidermolysis bullosa**" has turned out well. It has brought together the state of the art knowledge in the care and treatment of a rare birth defect which at the time is as yet incurable. It reflects the long years of practical experience from many clinical experts. This book gives future clinicians across the world an excellent textbook with guidelines for the treatment of patients with EB.

It has only been in the last few years that many of the children and the parents of those affected by EB have been acknowledged. They are known as the "butterfly children", the label given to the little patients. The knowledge that has since been accumulated about this disease, its causes, its natural history and the possibilities to make treatments easier, were scarce at one time. Through the creation of patient support groups the situation has really improved. Worldwide there are around 40 DebRA self-help groups. The self-help group debra-austria has also made a substantial contribution. They have held publicity campaigns to familiarize the public with the needs and concerns of people affected by EB. They have raised funds (most of which comes from donations) which provide medical care and maintenance, research for a cure, and assistance in coping with the financial burden of this disease. It all began at the end of 2005 with the opening of the "eb-haus Austria", a unique clinical center within the Department of Dermatology of the Paracelsus Private Medical University Salzburg (PMU)/ Salzburger Landeskliniken (SALK). This center provides medical care, scientific

research, education and seminars for both people affected by EB and experts learning more about the illness. All this is provided under one roof.

"**Life with Epidermolysis bullosa**" is lastly a labor of love, the result of many years of work with cooperation between various experts, researchers, doctors, therapists and nurses worldwide. We at the "eb-haus Austria" and numerous clinicians have cooperated together to produce a first class medical reference, in an effort to improve the quality of life for people living with EB. The initiative first started from two outstanding research doctors, Prof. Jo-David Fine and Prof. Helmut Hintner. They made it possible to put this valuable knowledge into book form and to make it available for a large audience. Therefore, as chairman of the debra-austria and father of a "butterfly child", I would like to thank everyone who has contributed to this book.

I have three wishes for the future of this book:

- Even though EB is rare, I hope that other clinicians across the world, not just the relevant experts, will gain knowledge about the disease.
- That this book will contribute to improving the care of people affected by EB worldwide.
- That in periodic intervals new issues should be published to update the practical and clinical knowledge which will lead to improvement of therapies.

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DEBRA International

It is a great pleasure to have been invited to write this foreword on behalf of DEBRA International for this most valuable addition to the literature on epidermolysis bullosa (EB), edited by two of the most eminent clinicians and researchers in the field. It will provide an excellent source of information for years to come.

DEBRA International is the worldwide grouping of national EB support groups. All member national groups are controlled by people living with EB. Initially, DEBRA Europe was founded in 1992, to promote cooperation and communication between European associations of people with EB, as a joint initiative by EBAE (France) and DebRA UK. From the very beginning, however, non-European groups participated and the focus of activity swiftly moved to the international stage, as DEBRA International.

The objectives of DEBRA International are:

- to promote equality of access and opportunity to all aspects of civil society by people with EB
- to assist member groups to provide services in their own countries
- to promote and coordinate research into EB
- to interact with international institutions for the benefit of people living with EB

Current activities include:

- active participation in several major international disability organisations, particularly those concerned with genetic or rare disabilities, such as Eurordis and the European Disability Forum.

- coordination of EB research via a single system of peer review for all research grant applications received by any member group worldwide
- strategic partnerships with the research community, including the funding of task forces on particular issues, such as cancer in EB and transitional research
- workshops and conferences with researchers to define priorities in line with the wishes of people with EB
- coordination of research funding from national EB groups and the establishment of an international fund for EB research
- partner in three current European Union Sixth Framework research programmes
- conferences and workshops of people living with EB
- organisational development programme
- professional education via conferences and web-based forums, creating international networks of doctors, nurses, dietitians, etc.
- website that seeks to be the definitive source of information on EB, www.debra-international.org
- advice and assistance to individuals where no national EB association exists
- directory of specialist clinical centres worldwide
- seed funding for the development of services, for example specialist nurses, particularly in Eastern and Central Europe
- exceptionally, fundraising for specific projects, e.g. a national treatment centre in Chile

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PREFACE

Over the past few decades increasing numbers of health care professionals worldwide have become interested in and actively involved in the day to day care of children and adults with inherited epidermolysis bullosa (EB). Although not too many years ago the care of the EB child was provided primarily by consulting dermatologists and pediatricians, major advances in care during neonatal life and infancy have led to the survival in adulthood of most EB children, even those with the most severe forms. As a result, nearly every medical and allied health practitioner now plays a critical role in the care of these patients, and multidisciplinary care has become the norm. One very visible example of this is the creation of the eb-haus Austria (as discussed in Chapter 3.2), a freestanding, independently funded unit devoted solely to the multidisciplinary management of EB children and adults. It is hoped that similar facilities will be forthcoming elsewhere in the world, so as to best meet the many needs of these patients and their parents.

Within the same timeframe, major advances have been forthcoming which have given great insight into EB at the clinical, epidemiological, cellular, and molecular levels. As the result of nearly 20 years of epidemiological data collection and analysis by the American National EB Registry, for example, we now know for each major EB subtype its prevalence and incidence, the range of cutaneous manifestations, the risk of extracutaneous complications, and its natural history. At the same time, the molecular basis of each major EB subtype has now been elegantly elucidated, setting the stage for gene therapy. Similar advances in our detailed understanding of wound healing should also lead to the development of more effective treatment strategies for our patients, which will be greatly needed until a cure becomes a reality.

The goals of this monograph are several-fold. First, our co-authors have attempted to meticulously summarize the clinical literature pertinent to EB so as to better educate healthcare workers and patients about diagnosis, classification, surveillance, and management. Whenever possible they have made recommen-

dations, based on their experiences and that of the published literature, as to how best to approach and treat patients with this disease. Second, the research literature has been comprehensively summarized, so as to more clearly explain how this disease arises at the level of the gene, and how those mutations result in dysfunctional behavior by cells within the skin.

We hope that this monograph will serve as a valuable primer for those who are not yet intimately involved in clinical care or bench research on this disease, and that it will stimulate new ideas and approaches that will ultimately lead to improved care and eventually the cure of all patients with inherited EB.

We would like to thank our publisher, Springer-Verlag, for agreeing in the timeliness of such a publication, to debra-austria for its generous financial assistance in helping to make the eb-haus Austria a reality, and to DEBRA International for its continued encouragement and support of research world-wide. As co-editors we are indebted to our many authors who have taken valuable time from their own clinics and laboratories to produce their very up to date and authoritative chapters. We also wish to thank Dr. Rudolf Hametner for his excellent photographs. The editors are also grateful to their families, most notably Elisabeth, Catherine, and Tygg, for all of their understanding during the past year as this work was being planned, written, and edited.

And finally, and most importantly, we would like to thank each of the thousands of EB patients and families with whom the editors and authors have worked and from whom they have learned over nearly three decades. They have patiently taught us how to approach and learn from each new patient, and through their own many insightful clinical observations, to apply that knowledge to the betterment of care for each succeeding generation of children born with inherited EB. It is ultimately to our patients and their remarkable families that this monograph is most rightfully dedicated.

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Major abbreviations

EB	epidermolysis bullosa
EBS	EB simplex
EBS-K	EBS, Koebner subtype
EBS-DM	EBS, Dowling-Meara subtype
EBS-MP	EBS with mottled pigmentation
EBS-WC	EBS, Weber-Cockayne subtype
EM	electron microscopy
IFM	immunofluorescence antigenic mapping
JEB	junctional EB
JEB-H	junctional EB, Herlitz subtype
JEB-nH	junctional EB, non-Herlitz subtype
JEB-I	junctional EB, inversa
JEB-PA	junctional EB with pyloric atresia
DEB	dystrophic EB
DDEB	dominant DEB
RDEB	recessive DEB
RDEB-HS	RDEB, Hallopeau-Siemens subtype
RDEB-nHS	RDEB, non-Hallopeau-Siemens subtype
RDEB-I	RDEB, inversa

1. General aspects

1.1 Definition

Christoph M. Lanschuetzer

The term epidermolysis bullosa (EB) hereditaria comprises a group of genetically and clinically heterogeneous diseases which are characterized by the formation of blisters and erosions on skin and mucous membranes following minor traction or trauma (i.e. “mechano-bullous diseases”). The name EB was first used by Koebner in 1886 [6] although cases consistent with this diagnosis were described earlier by others [4]. Our current nomenclature reflects the ongoing efforts of many clinicians and researchers throughout the twentieth century whose published reports on new clinical variants and diagnostically useful laboratory findings have allowed EB patients to be distinguished phenotypically, ultrastructurally, antigenically, and now most recently, molecularly.

Numerous extracutaneous manifestations, like blistering and erosions of the cornea and mucosal tissues, enamel hypoplasia, stenoses or strictures of respiratory, gastrointestinal, and urogenital tracts, pylorus atresia, muscular dystrophy, and cancer may complicate the different entities of EB.

In infancy, hereditary forms of mechano-bullous diseases must be differentiated from epidermolysis bullosa aquisita [5], which may occasionally affect children as well as adults [1]. This acquired autoimmune bullous disease is caused by injury induced by autoantibodies against type VII collagen, which is the major constituent of dermal anchoring fibrils [7]. This is of importance, since its inherited counterpart, dystrophic EB, is characterized by mutations within the gene encoding for type VII collagen [8].

Hereditary variants of EB are currently classified into three major groups according to the level within which blisters arise within tissues: epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JEB) and dystrophic epidermolysis bullosa (DEB). EBS is characterized by cytolysis within keratinocytes, JEB by clefting within the lamina lucida (i.e. hence the term "junctional"), and DEB by blistering below the lamina densa (i.e. dermal separation) of the dermo-epidermal basement membrane zone (BMZ) [2, 3].

Mutations have now been identified in more than ten genes that encode for structural proteins within keratinocytes or within mucocutaneous basement membranes. A unifying feature of these proteins is that they all contribute to the adherence of epithelium to the BMZ or underlying extracellular matrix. Although phenotype-genotype correlation is somewhat variable within some of the major EB types, the kinds of mutations, and in some situations, the locations of the mutations, may at least partially account for the severity of different clinical manifestations seen among different EB variants.

Recent advances in our understanding of the genetic basis of each of these diseases is increasingly enabling us to quickly establish reliable diagnoses. Furthermore, the insights gained from mutational analysis, as will be discussed elsewhere, are prerequisites for efficient genetic counselling (Chapter 1.4.3), prenatal DNA-testing and DNA-base preimplantation diagnosis (Chapter 1.4.2.3), and finally, gene therapy (Chapter 3.6).

Although the initial concern of pediatric dermatologists and neonatologists is to establish an accurate diagnosis and implement appropriate care for the skin, treatment of extracutaneous complications becomes increasingly important over time, in order to ensure the best quality of life for these patients. Given the rather protean manifestations that arise in hereditary EB, optimal care is performed in a specialised multidisciplinary center staffed by physicians and therapists of all medical disciplines who are well familiar with this disease.

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1.2 Classification and molecular basis of hereditary epidermolysis bullosa

Christoph M. Lanschuetzer and Jo-David Fine

Epidermolysis bullosa (EB) encompasses a number of diseases all having blistering and mechanical fragility as common features. Each type and subtype is classified based on phenotype, mode of inheritance, and genotype [8]. Currently, EB is separated into three major types – simplex, junctional, and dystrophic – based on whether blisters arise within the epidermis ("epidermolytic"), within the lamina lucida ("lamina lucidolytic") of the dermo-epidermal junction (DEJ) (Fig. 1.2-1), or within the uppermost papillary dermis ("dermolytic"), just beneath the DEJ.

Over 30 distinctive EB subtypes have now been described. In this chapter, we review the clinical features of the major subtypes which are most often encountered in clinical practice, emphasizing a classification system which was published in 2000 [8] and is still used by physicians, geneticists, and researchers worldwide. A newly revised classification system [14], which was published in June 2008, will also be discussed. More detailed descriptions of each of the known EB subtypes can be found within each of these two classification papers, as well as within a variety of dermatology textbooks, review articles, and several monographs [17, 21, 26]. In addition, the risk and treatment of each of the major extracutaneous complications of EB will be found elsewhere in this monograph, as will detailed discussions on etiology, laboratory diagnosis, and potential molecular therapies for the future.

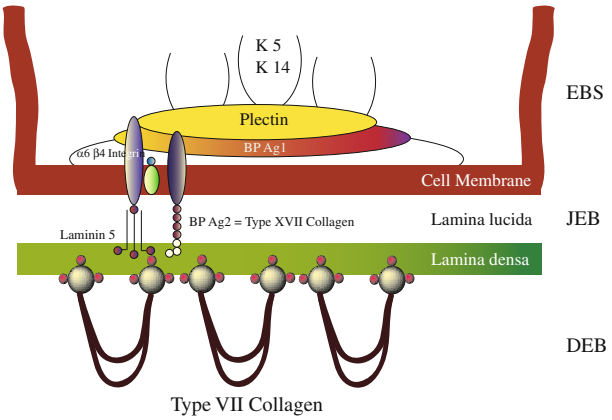

Fig. 1.2-1. Schematic diagram of the dermo-epidermal junction, depicting the ultrastructural localization of specific structural proteins pertinent to EB

Classification system 2000 (Table 1.2-1) [8]

Epidermolysis bullosa simplex (EBS)

The vast majority of EBS subtypes are inherited in an autosomal dominant fashion. The most common EBS subtype, a localized variant known as Weber-Cockayne (EBS-WC) disease, has blistering mainly limited to the palms and soles (Fig. 1.2-2) although blisters can still arise in any other site if the skin is severely traumatized. EBS-WC usually first becomes clinically obvious during infancy but in rare cases may not present itself until early adulthood. Extracutaneous manifestations, other than clinically insignificant blisters within the oral cavity [37, 38] during early childhood, are rare.

The two major generalized forms of EBS are EBS Koebner (EBS-K) and EBS Dowling-Meara (EBS-DM). In EBS-K, blistering is usually present at birth. It may involve any skin site (Fig. 1.2-3). When blisters arise on the extremities, palms and soles are usually spared, in contrast to what is seen in EBS-WC. The most severe variant of autosomal dominant generalized EBS, the Dowling-Meara subtype (EBS-DM) [7], is characterized by the presence of blisters or vesicles which arise in arcuate grouping (hence the older name "EBS herpetiformis", since some lesions may mimic those of herpes simplex) (Fig. 1.2-4). Extensive or confluent palmoplantar hyperkeratosis, nail dystrophy, atrophic scarring, milia and mucosal involvement are regularly seen [8]. EBS-DM may be associated with growth retardation, laryngeal stenosis [31], and premature death [2, 15].

Most EBS subtypes are caused by mutations in the genes encoding for keratins K5 and K14 [19]. These defects inhibit the formation of keratin filaments

Table 1.2-1. Current classification system for the most common types and subtypes of inherited EB*

Major EB type	Major EB subtype	Proteins targeted for mutation
EB simplex	EBS, Weber-Cockayne (EBS-WC)	K5, K14
	EBS, Koebner (EBS-K)	K5, K14
	EBS, Dowling-Meara (EBS-DM)	K5, K14
	EBS with muscular dystrophy (EBS-MD)	plectin
Junctional EB (JEB)	JEB, Herlitz (JEB-H)	laminin-332
	JEB, non-Herlitz (JEB-nH)	laminin-332; type XVII collagen
	JEB with pyloric atresia (JEB-PA)	α6β4 integrin
Dystrophic EB (DEB)	dominant DEB (DDEB)	type VII collagen
	recessive DEB, Hallopeau-Siemens (RDEB-HS)	type VII collagen
	recessive dystrophic EB, non-Hallopeau-Siemens (RDEB-nHS)	type VII collagen

* Based on Fine et al. [8]

("tonofilaments") which in turn leads to enhanced fragility of the cytoskeleton resulting in the cytolysis of basal keratinocytes. Among all of the types of EB, the best phenotype-genotype correlation is seen in EBS [33]. The location of the point mutations within K5 and K14 genes determines the severity of the disease: the severe subtype EBS-DM is caused by mutations in the part of the gene encoding for the highly preserved N- and C-terminal end-domains of the proteins with the

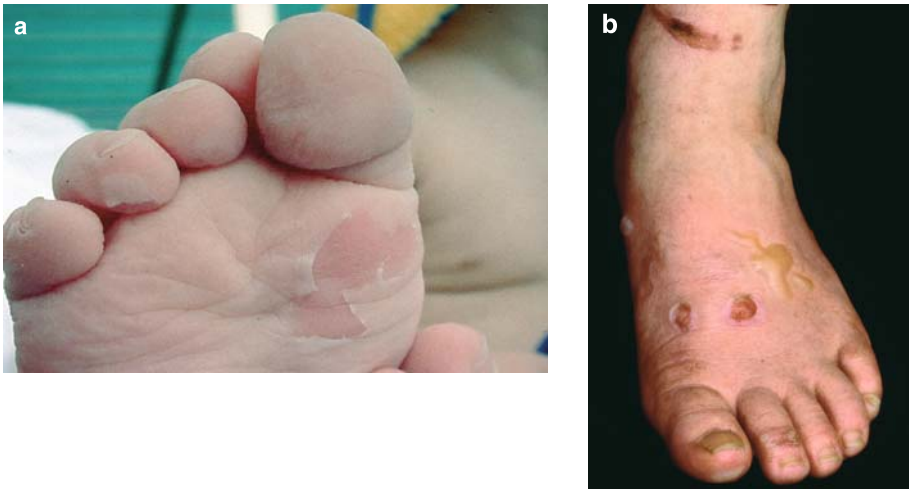

Fig. 1.2-2. **a** and **b** Blisters and erosions in EB simplex Weber Cockayne

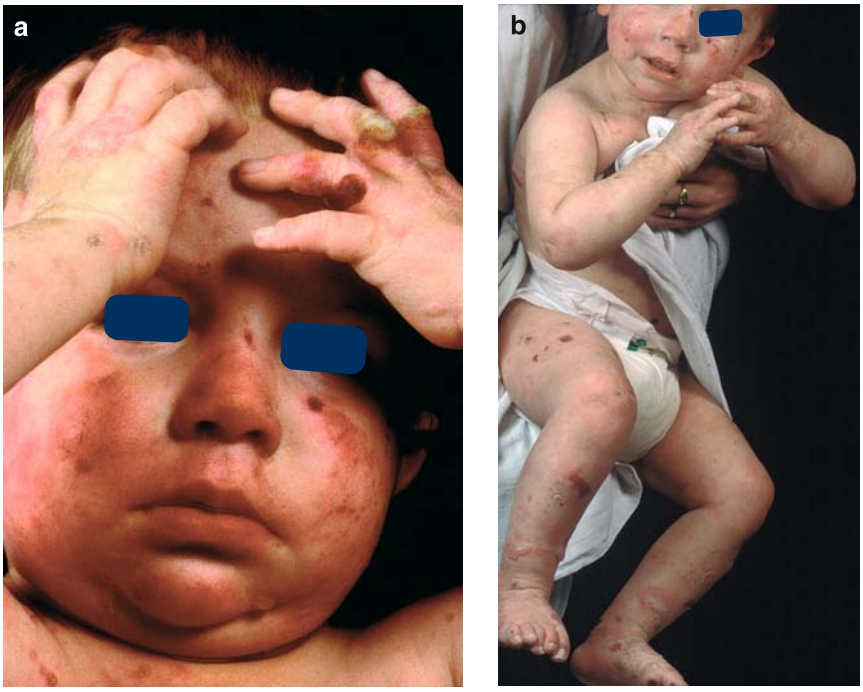

Fig. 1.2-3. **a** and **b** Generalized blistering and ist's sequelae in EB simplex Koebner

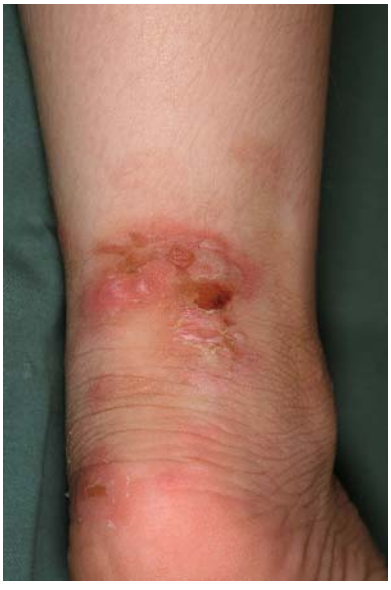

Fig. 1.2-4. Blisters in typical herpetiform distribution in EB simplex Dowling – Meara

consequence that an end to end aggregation of keratin filaments is inhibited. On the other hand, mutations in the gene sections encoding for the rod-domain of the protein cause milder variants (EBS-K, EBS-WC) where the formation of keratin filaments, although impaired, is still possible [3]. As expected, the severity of the disease is also influenced by homozygozity (severe manifestations) or heterozygozity (milder manifestations) of the genetic defect. In addition, the kind of the point mutation also affects disease severity. For instance a conversion of methionine to valine at position 119 (=W119 V mutation) in the keratin 14 gene causes the milder variant EBS-K, whereas a W119T-mutation (methionine to tyrosine) causes the considerably more severe EBS-DM variant [1, 3].

A rare EBS type of EBS is named EBS with muscular dystrophy (EBS-MD) (Fig. 1.2-5). This variant is caused by premature termination codons within the PLEC1 gene, that encodes for the protein plectin, which is a structural protein within the hemidesmosome and the "z-disks" of muscle cells [20, 27]. Blistering is present from birth on, whereas muscular dystrophy manifests later (2–35 years of life) [25].

Most patients with EBS have a normal live expectancy, although an elevated mortality rate has been reported in children with EBS-DM and patients with EBS-MD, the latter of whom may succumb from their muscle involvement within the third or forth decade of life.

Junctional EB

There are two major subtypes of junctional EB. Both are autosomal recessive in transmission. The more severe generalized variant is named Herlitz JEB (JEB-H) [23]; in some older literature it used to be named EB letalis, due to the high risk for premature death. Most patients with generalized JEB represent the non-Herlitz variant (JEB-nH), formerly also named generalized atrophic benign EB (GABEB) [4, 24].

The Herlitz variant is caused by homozygous mutations in the genes LAMA3, LAMB3 and LAMC2, that each encode for one of the three chains of the heterotrimer laminin-332. Nonsense mediated mRNA decay of one of the three chains causes a complete loss of this structurally vital basement membrane protein [30, 34].

Typical findings in JEB-H include extensive blistering, erosions, and atrophic scarring of the skin, onychodystrophy (eventuating in complete loss of nail plates (Fig. 1.2-6) and severe scarring of the nail beds), milia, severe soft tissue involvement of the oral cavity, and enamel hypoplasia and severe caries of the teeth. A pathognomonic finding in JEB-H is excessive granulation tissue which arises symmetrically around the mouth, central face, and nose (Fig. 1.2-6), upper

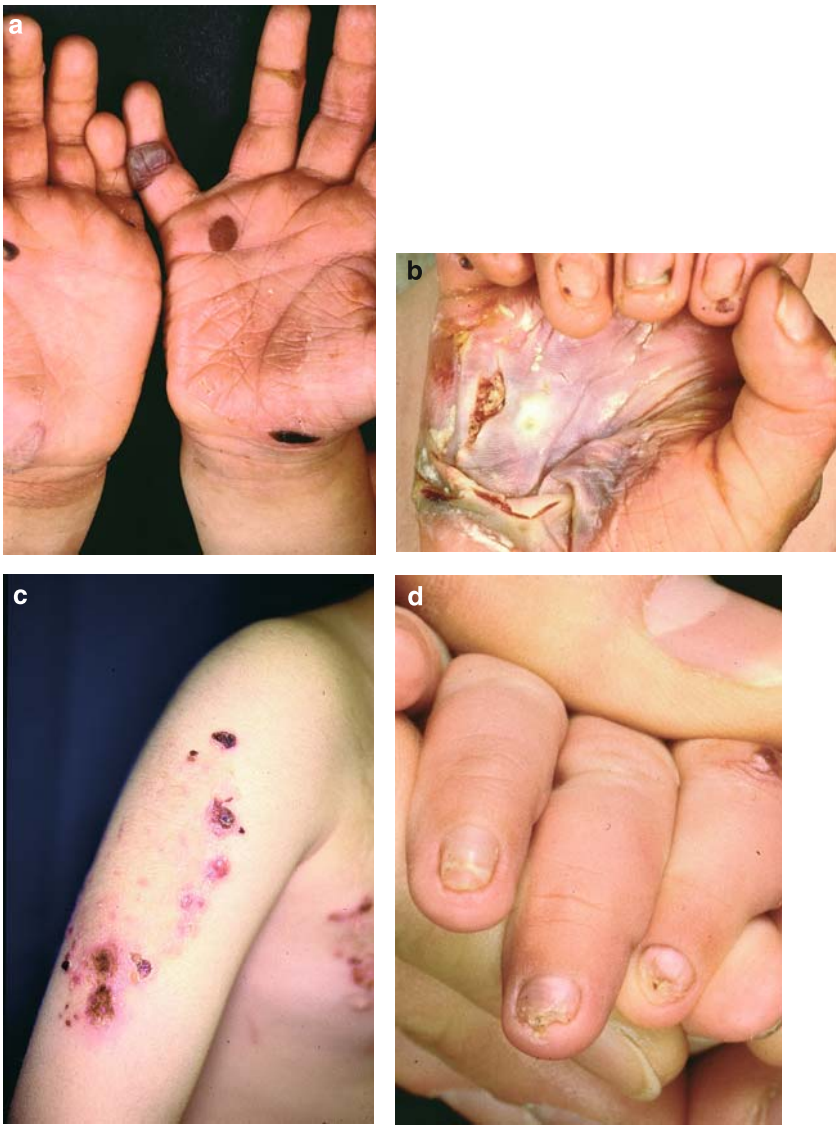

Fig. 1.2-5. **a** to **d** Small to very large, partly hemorrhagic blisters and erosions as well as early onychodystrophy in an infant with EB simplex with muscular dystrophy

back, axillary vaults, and nail folds. Severe multifactorial anemia, growth retardation, erosions and strictures in the gastrointestinal tract, and involvement of mucous membranes of the upper respiratory and the urogenital tracts, kidney, external eye, and rarely the hands [12, 22] are possible systemic complications. Mortality is very high, especially within the first few years of life, as a result of failure to thrive, sepsis, pneumonia, and tracheolaryngeal obstruction [2, 15].

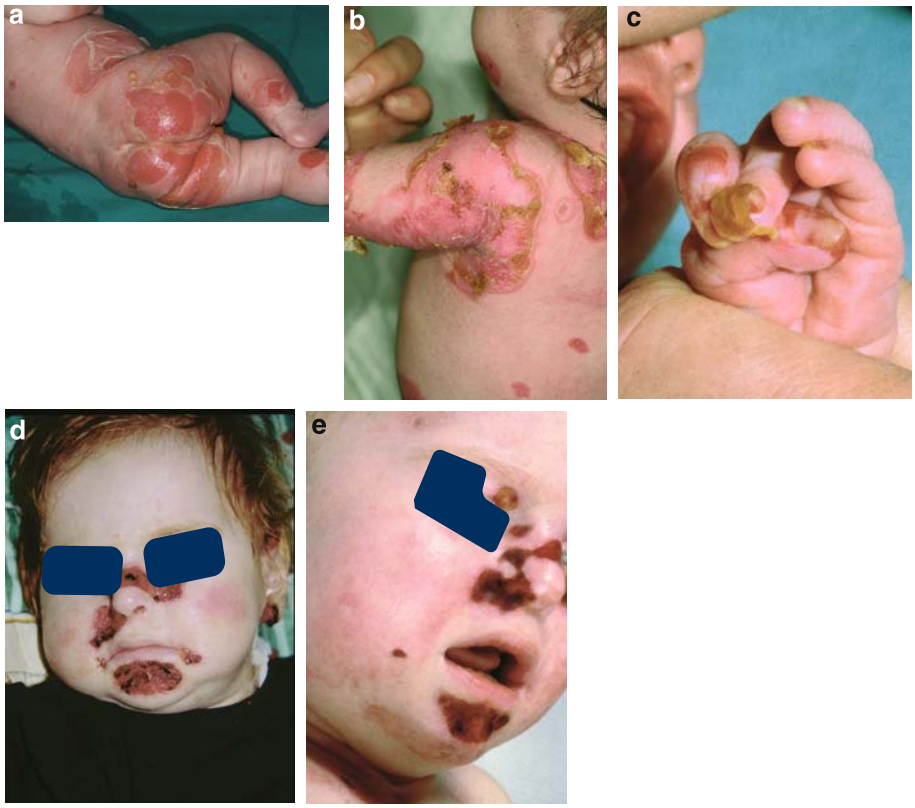

Fig. 1.2-6. Herlitz JEB. **a** and **b** Generalized detachment of the epidermis in a newborn with Herlitz JEB. **c** Onycholysis and erosions on the fingers. **d** and **e** Typical non-healing erosions with exuberant granulation tissue on the face

JEB-nH variants of EB are caused either by combined heterozygous mutations in the laminin-332 genes, or by homozygous nonsense mutations [5], missense mutations, or inframe deletions [6] within the COL17A1-gene that lead to complete loss or reduced expression of type XVII-collagen, a constituent protein of anchoring filaments [29]. Patients with JEB-nH have generalised blistering, erosions, and crusting of the skin, atrophic scars (Fig. 1.2-7), scarring ("male pattern") alopecia (Fig. 1.2-8), nail dystrophy or loss, and enamel hypoplasia and caries. In contrast to JEB-H, extracutaneous manifestations, other than tracheolaryngeal stenosis [13], are rare, and the live expectancy is usually normal.

A very rare variant of junctional EB is JEB with pylorus atresia (JEB-PA). Affected individuals genetically lack functional α6β4 integrin in the hemidesmosomes of epithelial cells, Schwann cells, thymocytes, and neuronal fibroblasts. Due to the severe course of the skin disease and systemic involvement (to include pylorus atresia and in many, genitourinary tract anomalies), children with JEB-

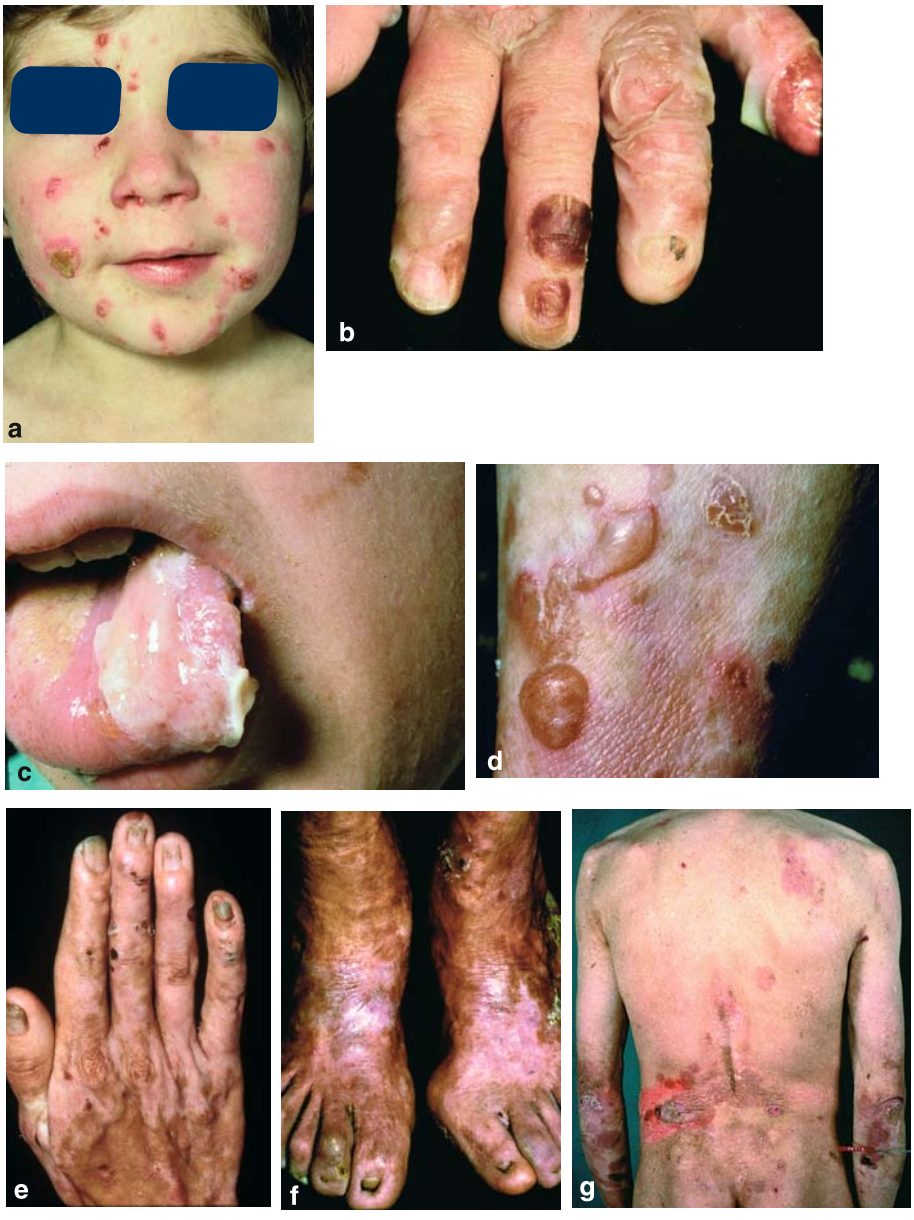

Fig. 1.2-7. Non-Herlitz junctional EB. **a** Cosmetically disturbing blisters and partly superinfected erosions in the face of a child. **b** Partly hemorrhagic blisters, erosions and onychodystrophy. **c** Large erosion on the tongue covered with fibrinogen. **d** Tense blisters and crusts on largely atrophic skin of an adult patient. **e** and **f** Ruptured blisters, erosions and partly hemorrhagic crusts besides onychodystrophy and anonychia on one hand and the feet of an adult patient. **g** Patient with patches of blistering, erosions, atrophy and scarring but normal stature. Shagreen-like, palm sized eb naevus on the back with large perilesional erosion after removal of an adhesive dressing

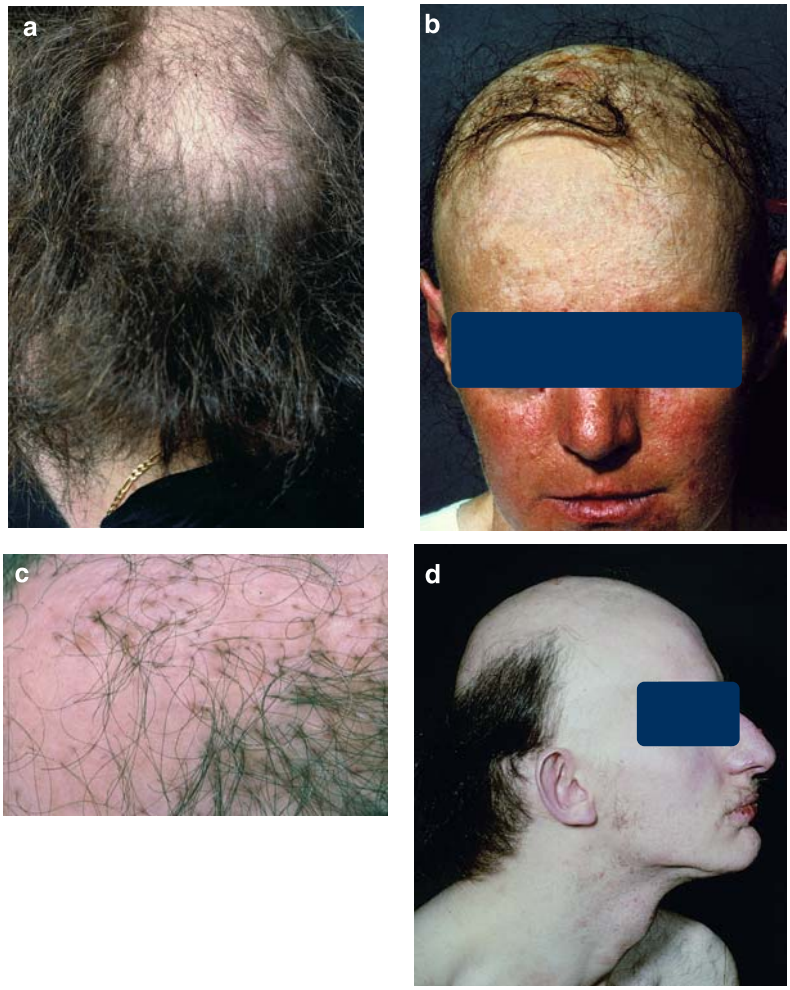

Fig. 1.2.-8. Junctional EB non-Herlitz, male pattern baldness. **a** In a 19 year old and **b** 37 year old women; **c** a close up of the scalp demonstrated marked scarring with bundles of remaining hairs; **d** in a 26 year old man

PA often die within the first few months of life. Recently, a non-lethal, milder variant caused by a compound-heterozygous mutation was described as a new variant [28].

Dystrophic EB

Dystrophic EB (DEB) is caused by mutations in the gene COL7A which encodes for type VII-collagen, the major constituent of basement membrane anchoring fibrils [35, 36]. There is a remarkably high number of different mutations within

the COL7A1 gene. The mutations and the allelic combinations of each individual patient is family specific. Although phenotype-genotype correlation is variable, the type of the mutation as well as its localisation within the COL7A1 gene help to define the severity of clinical manifestations.

DEB is separated into two main subtypes according to the mode of inheritance. It may be transmitted in either autosomal dominant (DDEB) or autosomal recessive (RDEB) manner [8]. The latter is subdivided into the severe RDEB Hallopeau-Siemens (RDEB-HS) variant and the less severe RDEB non-Hallopeau-Siemens (RDEB-nHS) variant.

Clinically, DDEB is characterized by recurrent blistering, milia, and atrophic scarring (especially on extremities; (Fig. 1.2-9), as well as nail dystrophy and eventual loss of nails. In most DDEB patients, skin involvement is generalized. Extracutaneous manifestations, other than esophageal involvement, are uncom-

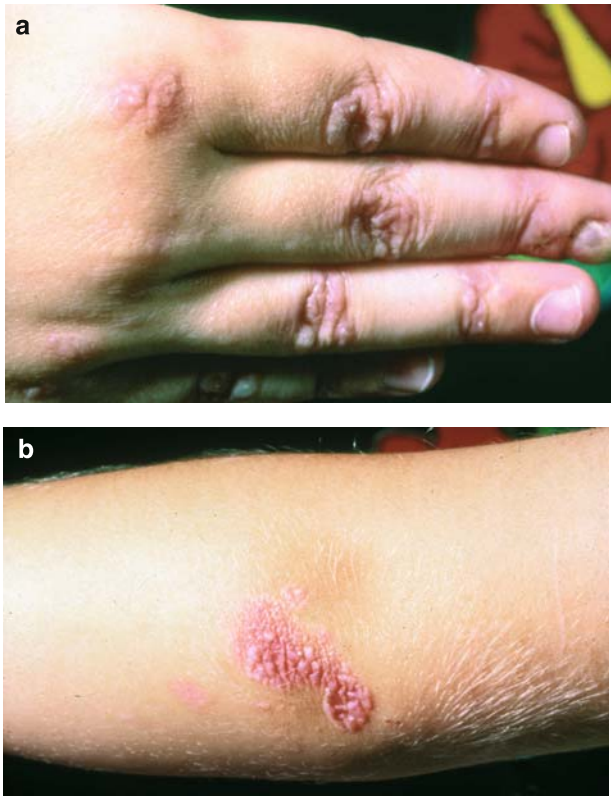

Fig. 1.2-9. Dominant dystrophic EB with atrophy and milia formation on (**a**) fingers and (**b**) elbow of a young girl

mon. The underlying genetic defect in DDEB is a glycine-substitution in the collagenous domain of the pro-α-chain of the homotrimer type VII collagen that in turn destabilizes the triple-helix [35]. Since the defective as well as the wildtype alleles are expressed equally, 12.5% of anchoring fibrils are functionally intact, accounting for the relatively mild course of DDEB.

Genetic defects like missense mutations or in-frame deletions, which underly milder non-Hallopeau-Siemens variants of RDEB, disturb the assembly and aggregation of type VII collagen polypeptides into anchoring fibrils. The resultant anchoring fibrils are found to be either reduced in numbers or altered in morphology in the skin of these patients.

Premature termination in both alleles, resulting in a complete loss of type VII collagen and anchoring fibrils, underlies RDEB-HS. Generalised blistering, erosions, crusts, atrophic scarring, onychodystrophy and loss of nails, mutilating pseudosyndactyly of hands and feet [12], and functionally disabling contractures in hands, feet, elbows and knees, characterize this severe EB variant (Fig. 1.2-10). Extracutaneous complications are common in RDEB-HS, including gastrointestinal [16] and urogenital [9, 10] involvement, external eye [11], chronic anemia, growth retardation [16], and a markedly elevated risk for the development of aggressive squamous cell carcinomas [18] (Chapter 2.1.3).

Classification system 2008 [14]

An international panel of EB experts convened in Vienna in May 2007 to critically review the currently used classification system. The intent of that meeting was to revise the system so as to accommodate inclusion of several newly described EB subtypes, to embrace several other conditions sharing common features with inherited EB, and to facilitate whenever possible the further focusing of future revisions towards the underlying ultrastructural and molecular basis of each EB subtype. In addition, some previously described entities were subdivided whenever clinical and genetic findings dictated that this be done so as to most accurately portray them to the practicing clinician. A consensus report, summarizing the recommended changes to the current classification system has just been published [14]. Tables 1.2-2 to -5 summarize this newly proposed classification system where there are several new key features.

First, Kindler syndrome [32] has been added as a distinct fourth major subtype of EB, in addition to EBS, JEB, and DEB, since the ultrastructural level of blister formation in Kindler syndrome may involve multiple levels of the skin, and since this disease typically presents as an inherited mechanobullous disease.

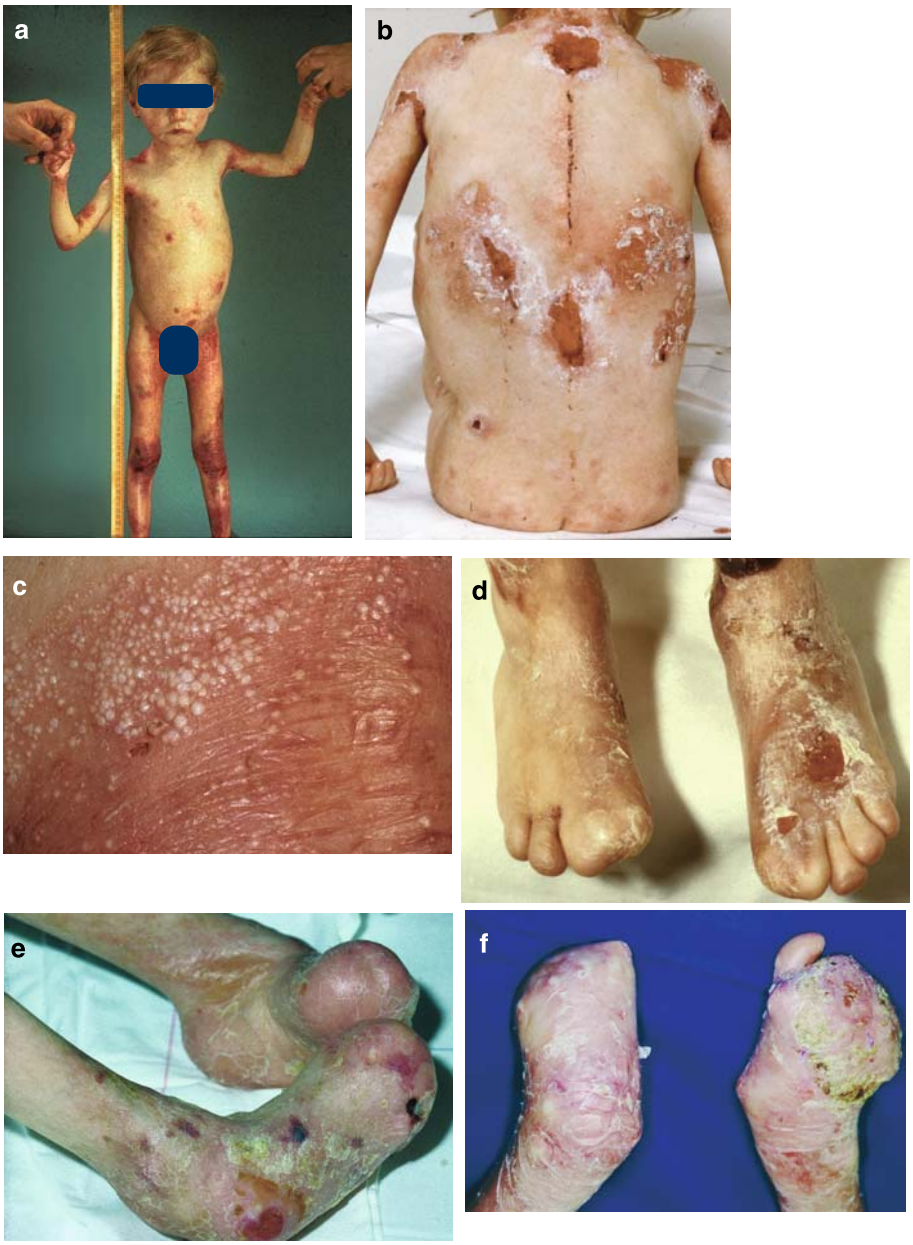

Fig. 1.2-10. Hallopeau-Siemens recessive dystrophic EB. **a** Growth retardation in a 9 year old girl; **b** Large non-healing wounds and ulcerations on the back and shoulders; **c** Aggregation of numerous milia in atrophic skin; **d** Mitten formation of toes one to three of the right and toes one and two of the left foot and anonychia; large, partly hemorrhagic blisters and erosions on the feet; **e** Severe contractures of both feet with mitten formation of all toes make walking for the patient impossible. Hemorrhagic blisters, erosions and crusts are also present; **f** Complete pseudosyndactyly of both hands with severe contractures

Table 1.2-2. New classification scheme for the major EB subtypes*

Major EB type	Major EB subtypes	Targeted protein(s)
EB simplex (EBS)	Suprabasal EBS Basal EBS	plakophilin-1; desmoplakin; ? others keratins 5 & 14; plectin; α6β4 integrin
Junctional EB (JEB)	JEB, Herlitz (JEB-H) JEB, other	laminin-332 (laminin-5) laminin-332; type XVII collagen; α6β4 integrin
Dystrophic EB (DEB)	dominant DEB (DDEB) recessive DEB (RDEB)	type VII collagen type VII collagen
Kindler syndrome	–	kindlin-1

* Based on Fine et al. [14]

Table 1.2-3. New classification scheme for all known EB simplex subtypes*

Major types	EBS subtypes	Targeted proteins
EBS suprabasal	*lethal acantholytic EB*	desmoplakin
	plakophilin deficiency	plakophilin-1
	EBS superficialis (EBSS)	?
basal	EBS, localized (EBS-loc)[a]	K5; K14
	EBS, Dowling-Meara (EBS-DM)	K5; K14
	EBS, other generalized (EBS, gen-nonDM)[b]	K5; K14
	EBS with mottled pigmentation (EBS-MP)	K5
	EBS with muscular dystrophy (EBS-MD)	plectin
	EBS with pyloric atresia (EBS-PA)	plectin; α6β4 integrin
	EBS, autosomal recessive (EBS-AR)	K14
	EBS, Ogna (EBS-Og)	Plectin
	EBS, migratory circinate (EBS-migr)	K5
(rare variants in italics)		

*Based on Fine et al. [14]
[a]Previously called EBS, Weber-Cockayne
[b]Includes patients previously classified as EBS-Koebner

Second, EBS has now been subdivided into basal and suprabasal subtypes, to include and highlight three clinically and molecularly distinct entities – lethal acantholytic EB, plakophilin deficiency, and EBS superficialis – in which blisters arise within the uppermost portions of the epidermis rather than within the basal keratinocyte layer.

Table 1.2-4. New classification scheme for all known junctional EB subtypes*

	Major JEB subtype	Subtypes	Targeted protein(s)
JEB	JEB, Herlitz (JEB-H)	–	laminin-332
	JEB, other (JEB-O)	JEB, non-Herlitz, generalized (JEB-nH gen)[a]	laminin-332; type XVII collagen
		JEB, non-Herlitz, localized (JEB-nH loc)	type XVII collagen
		JEB with pyloric atresia (JEB-PA)	α6β4 integrin
		JEB, inversa (JEB-I)	laminin-332
		JEB, late onset (JEB-lo)[b]	???
		LOC syndrome (laryngo-onycho-cutaneous syndrome)	laminin-332 α3 chain
(rare variants in italics)			

*Based on Fine et al. [14]
[a]Formerly known as generalized atrophic benign EB (GABEB)
[b]Formerly known as EB progressiva

Table 1.2-5. New classification scheme for all known dystrophic EB subtypes*

	All subtypes	Targeted protein(s)
DDEB	DDEB, generalized (DDEB-gen)	type VII collagen
	DDEB, acral (DDEB-ac)	
	DDEB, pretibial (DDEB-Pt)	
	DDEB, pruriginosa (DDEB-Pr)	
	DDEB, nails only (DDEB-no)	
	DDEB, bullous dermolysis of the newborn (DDEB-BDN)	
RDEB	RDEB, severe generalized (RDEB-sev gen)[a]	type VII collagen
	RDEB, generalized other (RDEB-O)	
	RDEB, inversa (RDEB-I)	
	RDEB, pretibial (RDEB-Pt)	
	RDEB, pruriginosa (RDEB-Pr)	
	RDEB, centripetalis (RDEB-Ce)	
	RDEB, bullous dermolysis of the newborn (RDEB-BDN)	
(rare variants in italics)		

*Based on Fine et al. [14]
[a]Previously called RDEB, Hallopeau-Siemens

Third, the laryngo-onycho-cutaneous syndrome (LOC syndrome) has now been formally included among the subtypes of JEB, given the presence of blister formation within the lamina lucida, its distinctive clinical features, and its association with laminin-332 α3 chain mutations.

Fourth, EB with pyloric atresia has been separated into EBS and JEB subtypes, now that two different ultrastructural levels of skin cleavage have been observed. Although originally EB with pyloric atresia was felt to be exclusively a variant of JEB, it has since been shown that rare patients actually have blister formation within basal keratinocytes and yet have clinical findings that are similar or identical to those with more typical junctional cleavage.

Fifth, some EB subtypes, for example JEB-nH, have been separated into generalized and localized variants.

Additional changes include (i) separation of DEB pruriginosa and bullous dermolysis of the newborn into both DDEB and RDEB variants; (ii) addition of a subtype of DDEB having only nail involvement (DDEB, nails only); (iii) addition of a rare EBS subtype characterized by migratory lesions (EBS, migratory circinate), and (iv) the substitution of clinically more descriptive names for two common entities currently identified by eponyms (Weber-Cockayne; Hallopeau-Siemens).

This system also again reinforces the position stated in 2000 [8] that the term hemidesmosomal EB should no longer be used when classifying patients with EB. This position has been taken since hemidesomosal EB conceptionally has too many practical limitations. As an important correlate, this name incorrectly suggests that those GABEB patients with COL17A1 mutations, who have been separated from other forms of JEB under the heading of hemidesmosomal EB, are phenotypically or prognostically different than those patients with JEB-nH in whom disease is caused instead by mutations within the laminin-332 genes.

This proposed revision in the classification system, which hopefully will be in use by 2009, more accurately describes each of the known EB subtypes than was possible in 2000, as well as being based, to some extent, on newer concepts pertaining to their underlying molecular and ultrastructural pathology. Furthermore, this proposed revision is an attempt to broaden the boundaries of EB wherever appropriate so as to encompass other inherited conditions which share sufficient clinical features with EB, most notably mechanical fragility and blister formation, to seriously merit inclusion among the other more traditionally accepted forms of EB.

Of considerable practical importance, and in reflecting the unanimous consensus of the international committee which produced this newly pro-

posed classification scheme, this system continues to rely on clinical findings (phenotypic and genetic), as well as those of diagnostic immunohistochemical and ultrastructural studies, rather than the results of DNA mutational analysis. Although the latter information will be ultimately essential for the implementation of any gene therapy in the future, and it does provide the most precise means of confirming the genetic mode of inheritance in those patients lacking a positive family history of other identically affected relatives, it is believed at this time that there is insufficiently strong phenotype-genotype correlation (with the possible exception of EBS) to merit the use of DNA testing as the *primary* means of subclassification of these patients. In addition, mutational analysis is as yet unfortunately unavailable or unaffordable for EB patients in every country, making it impractical to hinge the classification of an EB patient exclusively on the basis of mutational analysis. This is indeed a remarkably powerful laboratory technique, however, which can provide extremely useful information, especially as it relates to the pathophysiology of this disease. Furthermore, it does remain the hallmark laboratory test by which prenatal and preimplantation diagnosis is currently performed. Whether it will be more heavily employed in future classification systems is as yet unknown.

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1.3 Epidemiology of inherited epidermolysis bullosa

Jo-David Fine

Little is still known about the epidemiology of most skin diseases, in part because, with the rarest of exceptions, there are no strict enforceable nationally based requirements for the reporting of dermatological conditions other than selected communicable ones, such as syphilis. In addition, many skin diseases are so transient in nature or sufficiently mild as to prevent their being evaluated systematically by physicians or other health care providers. As such, a true estimate of the extent of those diseases becomes impossible. Finally, accurate diagnosis of skin diseases is oftentimes difficult, requiring the degree of skill possessed only by fully trained dermatologists. Clinically dependent data generated by others may be easily tainted by misclassification. As but one recent example, there are considerable reported differences in the prevalence of nephrogenic fibrosing dermopathy (NFD), depending on whether these research studies involved participation by dermatologists, since some of the cases touted as having this entity may have actually been variations of stasis dermatitis or lipodermatosclerosis which were simply misclassified as NFD by physicians lacking dermatological training.

Accurate prevalence and incidence data on inherited EB became possible only after the National EB Registry (NEBR) was established in 1986 [1–3]. Funded by the National Institutes of Health, this project, now into its 21st year of existence, had as one of its major goals the identification, recruitment, enrollment, and evaluation of all patients with inherited EB residing with the Continental United States. Systematic case finding and data collection were employed, using conventional rigorous epidemiological principles, and data were subsequently analyzed by a biostatistical team headed by a prominent researcher with a particular interest in population demographics. Details of the methodology

employed, as well as specifics of the project's extensive data instruments, have been described previously [4, 5].

A number of false assumptions about EB existed within the medical literature prior to analysis of data collected by the NEBR. These were the understandable result of clinical impressions formed by physicians who reported on their own experiences with limited numbers of EB patients, most of whom had severe disease. It was believed, for example, that there might be significant racial predilections for EB, based on the paucity of case reports of affected African–Americans. This was later proven to instead reflect marked differences in access to health care and, most notably, to referral to dermatologists or medical geneticists, rather than any inherent genetic difference in risk of disease across racial lines. It was similarly incorrectly believed that most cases of junctional (JEB) and recessive dystrophic EB (RDEB) represented Herlitz (JEB-H) and Hallopeau-Siemens (RDEB-HS) subtypes, respectively, and that these two subtypes were very frequently lethal in early childhood. It was also thought that EB simplex (EBS) was not as common as we now know it to be, since only a minority of EBS patients routinely sought dermatological evaluation and care.

Pre-NEBR epidemiological data

Prevalence estimates

Prior to analyses based on the NEBR study population, data on much more limited numbers of EB patients were generated from Scandinavia, Northern Ireland, Croatia, Japan, and British Columbia. Many of these estimates varied considerably, in part a reflection of much smaller sample sizes and far less rigorous methods of patient recruitment. Prevalence estimates in 1995 in Norway for EBS (all types), EBS-K, and EBS-WC were 23–42, 1.4–6.9, and 14.5–20 per million, respectively, depending on how the EBS subtypes were classified [7]. In contrast, from 1962–1984 the prevalence of EBS in Northern Ireland was believed to be about 28 per million [6]. In 1995, the prevalence in Norway of JEB, dystrophic EB, RDEB, and RDEB-HS was estimated at approximately 2, 10, 2.3, and 0.7 patients per million, respectively [6]. In 1990 the prevalence of RDEB-HS in Croatia was believed to be 9.6 per million [9]. In Japan, using hospital survey data obtained in 1983 on less than 400 patients, it was reported that the prevalence of EBS, JEB, DDEB, and RDEB was 0.29–0.40, 0.015–0.020, 0.11–0.15, and 0.15–0.20 per million, respectively.

Possibly the most accurate pre-NEBR estimate was obtained in British Columbia, using a governmental health surveillance-based registry. In that latter population during the period 1952–1989, the overall prevalence of EB was calculated by Sybert and colleagues to be 9.9 per million [5].

Incidence estimates

In Norway in 1983, the incidences of Koebner, Dowling-Meara, and Weber-Cockayne subtypes of EB simplex were reported to be two, 1.7, and at least six per million live births, respectively. From 1965–1994, incidences of JEB (all subtypes) and Herlitz JEB in Norway and Sweden were estimated at 7 and 4.6 per million live births, and >8 and >7.1 per million live births, respectively. From 1947 to 1994, the incidence of RDEB was estimated at 5.6 per million live births. In a report from Croatia in 1990, the incidence of RDEB-HS was suggested at 19.2 per million live births (one in 52,000). In British Columbia, the incidence of EB, all types, during the period 1952–1989 was estimated at 17.9 per one million live births.

Demographics of enrollees in the NEBR

As of June 1, 2002, when the NEBR closed its systematic enrollment of new patients into its database, data had been collected on 3280 patients. About 10% of these patients could not be classified as to EB type; their data were excluded from all subsequent analyses. Of importance, the demographics of the entire NEBR study population were identical to that of the first 1700 patients who had been enrolled from 1986–1996, and on whom prevalence and incidence estimates were calculated [5].

About half of all NEBR enrollees had EBS. Of these, nearly two-thirds had EBS-WC, 6–7% had EBS-K or EBS-DM, and about 22% could not be accurately subclassified. Seven percent of NEBR patients had some form of JEB; of these, only about 20% had JEB-H. DDEB and RDEB each represented about 13% of the NEBR population. Among the RDEB enrollees, 33%, 63%, and 4% had RDEB-HS, RDEB-nHS, and RDEB inversa, respectively.

Prevalence and incidence by EB type and subtype (NEBR data)

Table 1.3-1 summarizes prevalence and incidence data, as stratified by both EB type and subtype, for the United States. These data represent analysis of approximately 1700 patients who were recruited to the NEBR during its first 10 years of existence. Prevalence estimates were based on the number of NEBR enrollees alive during the 1990 calendar year, to permit use of data from the 1990 United States Census. Incidence estimates were based on the five year period, 1986–1990. Given the clinical severity of JEB and RDEB, as well as the availability of free diagnostic testing by the NEBR, it is likely that virtually every patient with these two major types of EB was identified and enrolled in the project from 1986–1996. Similarly, it is probable that most patients with DDEB were included within this study population, as were most patients with severer subtypes of EBS. Localized EBS, however, is oftentimes clinically rather mild, and therefore many

Table 1.3-1. Prevalence and incidence of EB, stratified by type and subtype (based on the NEBR study population)

EB type or subtype	Prevalence[a]	Incidence[b]
EBS (all subtypes)	4.60	10.75
EBS-WC	3.14	6.81
EBS, all others	1.46	3.95
JEB (all subtypes)	0.44	2.04
JEB-H	0.07	<0.41
JEB, all others	0.37	<2.04
DDEB	0.99	2.86
RDEB (all subtypes)	0.92	2.04
RDEB-HS	0.42	0.41
RDEB, all others	0.49	1.63
EB, all types & subtypes[c] (including unclassified patients)	8.22	19.60

[a]EB patients per one million (1990)
[b]EB births per one million live births (1986–1990)
[c]Including unclassifiable cases

of these patients did not come to the attention of the NEBR. In calculating prevalence and incidence on EBS, it was assumed that no more than 5–10% of all EBS patients had been captured by the NEBR. Even this may be an overestimate of the success of patient enrollment for this major EB type.

The overall prevalence for inherited EB within the American population was 8.22 per million. Prevalences for EBS, JEB, DDEB, and RDEB were 10.75, 2.04, 2.86, and 2.04 per million, respectively. Prevalences of the major subtypes are listed in Table 1.3-1. It should be noted that there were too few patients within most of the EB subtypes to accurately estimate prevalences. Similarly, the incidence for inherited EB within the American population was 19.60. These data, however, are based on the assumption that we have captured essentially all EB patients, regardless of subtype, within the NEBR study population. If, however, only 10% and 5% of all EBS patients have been identified, then the prevalence of EBS was instead 46.00 and 91.99, respectively, and the overall prevalence of EB was 49.62 and 95.61 per million, respectively. Even with the latter rather stringent assumption, there would have been only 23,780 EB patients alive in 1990 within the entire continental United States, based on the 1990 census.

Other reported demographic data

Several EB registries have now been established in other countries, to include Italy [10], Germany, Scotland [8], and Australia. As of 2002, the Italian registry

had 697 classifiable enrollees, the majority of which had non-simplex disease (JEB, 10%; dystrophic EB, 62%). Within the Italian cohort the overall prevalence and incidence during the period 1997–1998 were 10.1 per million and 20.1 per one million live births, respectively. Although the distribution of EB types enrolled in Italy differs from that in the United States, the overall rates are remarkably close to those generated by the NEBR, suggesting the likelihood that the American data are indeed comparable to at least Western Europe, if not worldwide.

It is still possible, though, that differences may be found in other cohorts or in other countries. There are several reasons to explain these possible disparities. First, the accuracy of epidemiological data is highly dependent on the degree of success by which patients are identified. Less than extremely rigorous case finding will result in lower estimates of prevalence and incidence, as will also misdiagnosis and misclassification. Second, prevalence and incidence estimates will also be impacted upon by restricted patient access to registries or their physicians. As we have already learned from our experience in the United States, some ethnic groups may indeed have less access to specialty health care than others. A complication of a health care system that is composed of numerous private medical insurance and more limited governmental health care plans, well over 40 million American citizens lack any health care coverage. Although most children with EB would still be at least partially covered by federal or state funded health systems, this is not necessarily the case with adults. Third, there may be differential enrollment across the spectrum of EB. Patients with mild autosomal dominant forms of EB, for example EBS-WC, may choose not seek medical evaluation for their skin diseases in the absence of effective therapies, based on the experiences of other affected family members. As a result, those cases would not be identified as having EB and therefore not be included in prevalence estimates. On the other hand, much higher prevalence and incidence rates of severe autosomal recessive EB subtypes would be found in smaller countries in which, for a variety of cultural or economic reasons, consanguinity may be a far more common occurrence.

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1.4 DIAGNOSIS

1.4.1 NON-MOLECULAR TECHNIQUES

1.4.1.1 Routine histopathology in epidermolysis bullosa

Martin Laimer

Introduction

Based on landmark electron microscopy studies performed over 45 years ago by Pearson [5], three major groups of epidermolysis bullosa (EB) can be differentiated according to the level of split formation within the dermo-epidermal junction.

In the traditional EB simplex variants, blister formation occurs within the epidermis (i.e. epidermolytic) due to cytolysis of basal keratinocytes, the result of mutations in the genes encoding for the major cytoskeletal proteins keratin 5 and 14, and for a hemidesmosomal protein, plectin. Three very rare intraepidermal heritable blistering diseases – EBS superficialis, lethal acantholytic EBS, and plakophilin-1 deficiency – which have their cleavage planes within suprabasal keratinocytes, have now been proposed for inclusion in the spectrum of EBS [1]. In two of these, molecular mutations have been proven to occur within the genes for desmoplakin and plakophilin-1.

Junctional EB (JEB) is characterized by blistering within the lamina lucida (i.e. junctional cleavage) of the basement membrane zone (BMZ), and reflects molecular defects within the genes (LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4) encoding for several structural proteins responsible for the integrity between basal keratinocytes and the dermis (each of the three chains of laminin-332 (formerly named laminin-5); type XVII collagen; both heterodimeric chains of integrin $\alpha 6\beta 4$).

The hallmark of the third major group of hereditary mechanobullous diseases, dystrophic EB, is blistering just beneath the lamina densa of the BMZ (i.e. dermolytic). It is caused by alterations in quantity or structure of anchoring fibrils, and is due to mutations in the gene (COL7A1) encoding for type VII collagen, the constitutive protein of anchoring fibrils.

Limitations of light microscopy in the diagnosis of EB

In general, routine light microscopy is of little value in the diagnosis of hereditary EB, since in the majority of cases, i.e. in junctional and dystrophic EB, the major finding is just a cell-poor "subepidermal" blister. As such, light microscopy is not routinely performed unless other unrelated blistering diseases (for example, intrauterine herpes simplex) are being considered in the differential diagnosis. Although intraepidermal cleavage, secondary to cytolysis through the level of the basal keratinocytes, may be visible in some routine histopathologic specimens taken from patients with EBS, at other times it may even be difficult to distinguish between EBS and non-EBS types, since only focal blistering may occur just above the level of the basal keratinocyte cell membrane, with only tiny remnants remaining attached to the skin BMZ. At the light microscopic level it may be difficult or impossible to visualize these fragments. Similarly, although cleft formation within the upper epidermis may suggest a suprabasal subtype of EBS, other unrelated disorders may need to be included in the differential diagnoses.

In order to precisely determine the anatomic level of blister formation it is necessary that electron microscopy or immunofluorescence antigenic mapping be performed, as discussed in Chapters 1.4.1.3 and 1.4.1.2.

Light microscopic features in inherited EB

EB simplex

This major group of EB is characterized by intraepidermal cleavage, usually through the basal cell layer of the epidermis (Fig. 1.4.1.1-1). As noted previously, the tissue separation may be so low in the epidermis that in routine paraffin sections the blister may appear to be "subepidermal", suggesting JEB or DEB. In some instances, however, in thin plastic-embedded sections it may be possible to observe fragments of basal keratinocytes on the base of the blister, where the PAS-positive basement membrane is also found. Another hint to the intraepidermal nature of the blisters may be found in cytolysis [6] or vacuoles within basal keratinocytes adjacent to the blister. In the Dowling-Meara variant of EBS, a number of eosinophils, although not specific for this variant, may

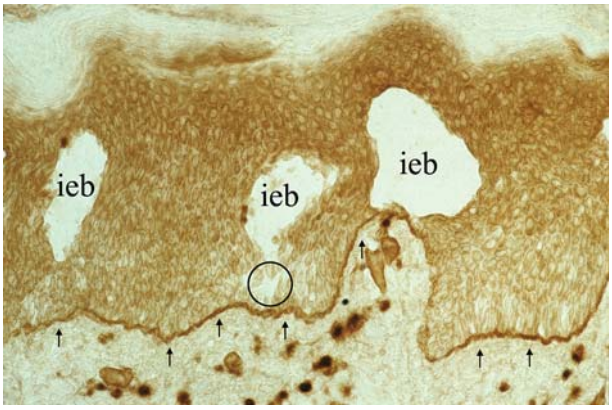

Fig. 1.4.1.1-1. EB simplex skin, stained by immunoperoxidase technique using an anti-type IV collagen antibody. Focal cytolysis is visible (circle) as well as several "intraepidermal blisters" (ieb) of variable size, with type IV collagen staining (arrows) of the basement membrane present solely along the blister floor

sometimes be found in the papillary dermis. In EB simplex with muscular dystrophy, blistering always occurs just above the hemidesmosomes. In EBS superficialis subcorneal splitting, which can mimic peeling skin syndrome, has been reported [2]. Another rare subtype has been reported as having enlarged dyskeratotic basal cells which have eosinophilic clumps in the cytoplasm and show some atypical mitosis [4].

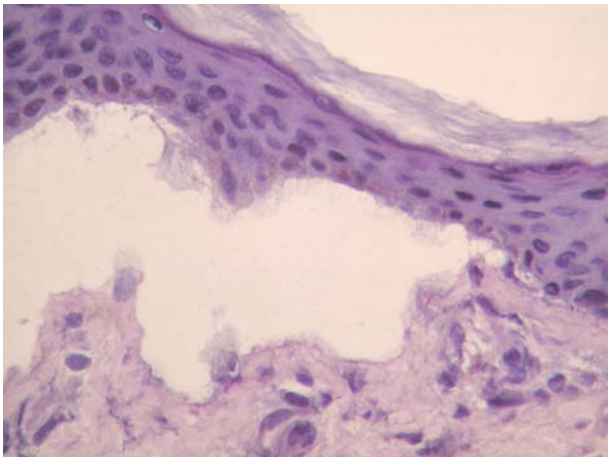

Fig. 1.4.1.1-2. Junctional EB skin, stained with PAS. A subepidermal blister is present, with the PAS positive basement membrane found along the blister floor

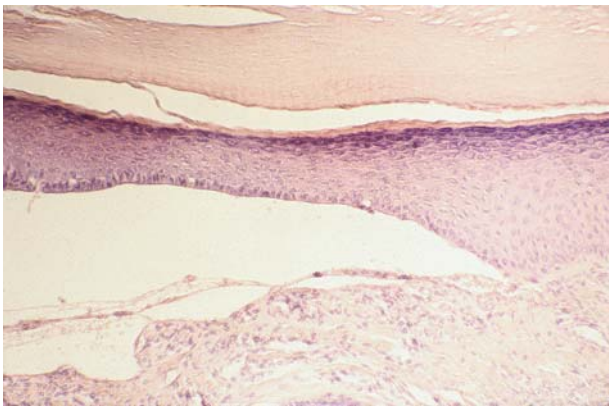

Fig. 1.4.1.1-3. Dystrophic EB skin, stained by conventional hematoxylin-eosin technique. A subepidermal blister is present, in the absence of a dermal inflammatory infiltrate

Junctional EB [3, 7]

Junctional EB variants appear as "subepidermal" cell-poor blisters with the PAS-positive basement membrane detectable along the floor of the blister (Fig. 1.4.1.1-2). If cutaneous atrophy is present, there is thinning of the epidermis and flattening of the rete ridges. Dermal fibrosis is visible in cicatricial areas.

Dystrophic EB [3, 7]

Cell-poor, "subepidermal" blisters are visible within dystrophic EB skin (Fig. 1.4.1.1-3). Uncommonly, sparse eosinophils have been observed in the dermis. Superficial dermal scarring and milia are often present. The PAS-positive basement membrane is found on the roof of the blister.

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1.4.1.2 Antigen mapping

Gabriela Pohla-Gubo, Elke Nischler, and Helmut Hintner

Antigen mapping (AM) is principally a method to evaluate the expression of antigens (mainly structural proteins) in tissue sections by immunohistochemistry (mostly via immunofluorescence microscopy). In the skin of patients with hereditary epidermolysis bullosa (EB) the presence, absence, or reduced expression of structural proteins of keratinocytes or of the dermo-epidermal junction as well as their distribution within a natural blister or an artificial split formation is determined [1, 3]. Thereby the level of blistering (epidermolytic; intra-lamina lucida ("junctional"); dermolytic) can be determined. The AM serves as the primary non-molecular diagnostic tool for EB and as the results are available within half a day, it can give fast information about the classification (major types) and thereby prognosis about the outcome and course of the disease [5]. The results also provide an essential basis for mutation analyses in that they point out which of the different genes encoding for structural proteins of keratinoytes or of the dermo-epidermal basement membrane zone (BMZ) should be targeted for study [7, 9].

Materials and methods

Biopsy

A four to six millimeter punch biopsy (or one part of an excision biopsy for routine histology, AM and electron microscopy) should be taken preferentially from lesional/perilesional skin including parts of a fresh blister. From patients with the more severe forms of EB that blister readily, clinically normal looking skin (inner aspect of the upper arm) can be biopsied since the trauma caused

Table 1.4.1.2-1. Michel's medium

Buffer A	
1.0 M Potassium citrate buffer pH 7.0	2.5 ml
0.1 M Magnesium sulphate	5.0 ml
0.1 M n-Ethylmalemide	5.0 ml
Distilled water	87.5 ml
Buffer B (transport medium)	
Buffer A	100.0 ml
Ammonium sulphate (adjusted to pH 7.0)	55.0 g

by the punch or excisional biopsy is almost always sufficient to cause an "artificial" split at the respective level. If there are no "new" blisters, one can also try to gently rub or apply rotary traction with a pencil eraser to the skin and then take the biopsy [10]. Taking the biopsy, attention has to be paid that the often minute and fragile epidermis is not kept in the punch instrument, or gets lost during the final embedding procedure of the sample. The biopsy is then placed in a small transport tube containing Buffer B from Michel's medium [8] (for formula see Table 1.4.1.2-1) which allows the sample to be safely maintained for at least 7 days at room temperature. In this medium the biopsy can then be sent to the appropriate laboratory. If the immunofluorescence (IF) laboratory is at the same institution where the biopsy is taken, the sample can be wrapped in aluminum foil, placed in a small tube (with no liquid) and then immediately transferred to a freezer (-20° or -70°C). Alternatively, the biopsy can be mounted in Tissue Tec O.C.T. compound (Miles Inc., USA), frozen in the cryostat, and then stored as described above. Samples transported in Michel's medium Buffer B have to be washed in the laboratory for a few hours in Buffer A (for formula see Table 1.4.1.2-1). Otherwise the samples may turn brown during the actual two-step antibody staining procedure. Then they are frozen as described above and can be stored for years in a freezer.

Immunofluorescence (IF)/immunoperoxidase (IP) visualization of antigens

(A) Determination of the level of split formation

In the laboratory an appropriate number of slides with frozen sections (4–6 microns thick) are prepared of normal human skin (NHS) and patient's skin (lesional/perilesional with part of a natural blister or an artificially induced split) (Fig. 1.4.1.2-1). The NHS serves as a positive control, i.e. to demonstrate the normal

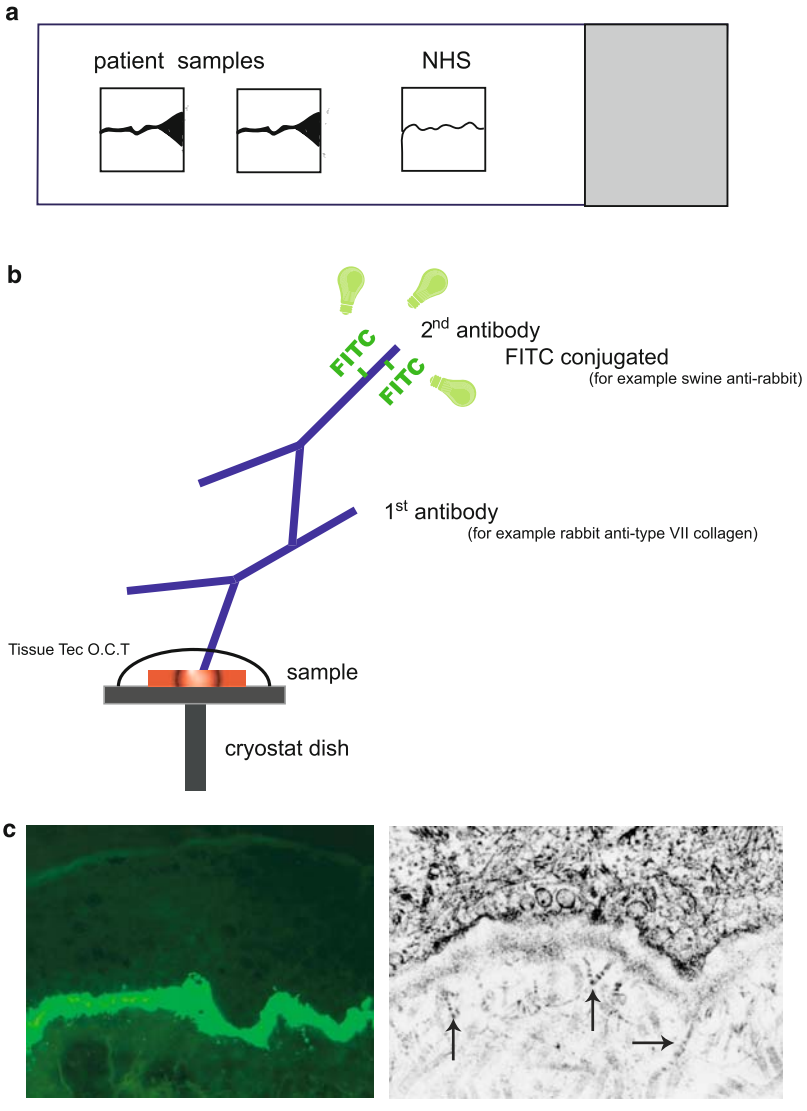

Fig. 1.4.1.2-1. Schematic depicting the steps involved in performing antigen mapping. **a** Normal human skin (NHS) and EB patient's samples on a microscopic slide. **b** Indirect immunofluorescence microscopy is used to detect the relative expression of an antigen (i.e. type VII collagen) in NHS and EB patient samples. **c** Left: immunofluorescence micrograph depicting a strong linear band of type VII collagen along the dermo-epidermal basement membrane zone of normal human skin. Right: electron micrograph correspondingly showing anchoring fibrils (arrows) in normal human skin

expression of the respective structural protein or antigen. The number of slides depends on the number of antibodies used to detect the respective antigen (as for example, type IV or VII collagen; serum of a patient with bullous pemphigoid with

Table 1.4.1.2-2. List of antibodies commonly used in the laboratory of the Department of Dermatology, Paracelsus Private Medical University Salzburg, Austria

1st antibody	2nd antibody	Source
Pan Keratin	anti-mouse	1
Keratin 1	anti-mouse	1
Keratin 5	anti-mouse	1
Keratin 8	anti-mouse	1
Keratin 10	anti-mouse	1
Keratin 14	anti-mouse	1
Plectin (5B3)	anti-mouse	2
BPAG1 (230KD)	anti-rabbit	2
Integrin Alpha-6	anti-rat	1
Integrin Beta-4	anti-mouse	1
BPAG2 (HD 18)	anti-mouse	2
Laminin-332 (GB3)	anti-mouse	1
Type IV collagen	anti-mouse	1
Type VII collagen (LH7:2)	anti-mouse	1

1 = Commercially available; 2 = obtained for diagnostic purposes from selected research laboratories

autoantibodies to BPAG1 (as proven by immunoblot, immunoprecipitation, or ELISA) (see Table 1.4.1.2-2)). It includes also slides for negative controls (i.e. by replacing the first antibody with phosphate buffered saline (PBS)).

In the case of intraepidermal blistering (indicative of EB simplex), all three antigens are found on the blister floor [1, 4]. Intra-lamina lucida split formation (indicative of junctional EB) is characterized by reactivity of the BPAG1 serum on the blister roof and of antibodies to type IV or VII collagen on the blister floor [1]. Dermolytic blistering (diagnostic of dystrophic EB) is demonstrated by the presence of all three antibodies binding to the blister roof (Fig. 1.4.1.2-2) [1, 2, 6].

(B) Evaluation of the expression of various antigens or structural proteins within keratocytes and along the dermo-epidermal junction

The same method (i.e. indirect IF) is also used to detect the intensity and pattern of staining of various structural epidermal and basement membrane zone proteins with mostly commercially available antibodies (Tables 1.4.1.2-2 & -3). Substrate is again in most cases a biopsy of clinically normal appearing skin of the EB patient; NHS serves as the positive control.

In the first 30 min incubation period, the slides are covered with different antibodies at optimized dilutions (Table 1.4.1.2-2). After washing the slides in

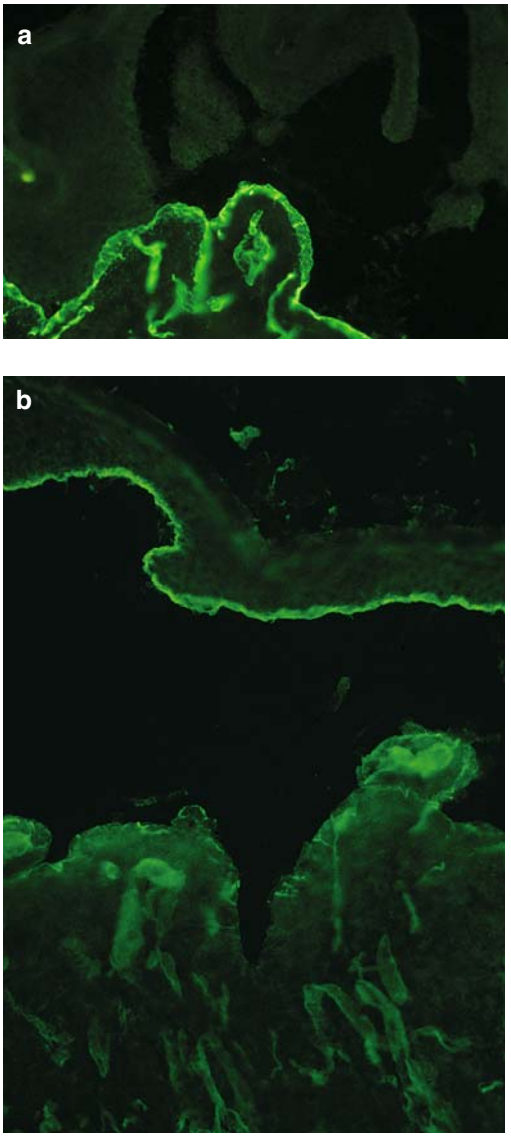

Fig. 1.4.1.2-2. Determination of the level of split formation by antigen-mapping. Substrate: The biopsy of normal appearing skin of a patient with non-Herlitz junctional EB (**a**) and a patient with Hallopeau-Siemens recessive dystrophic EB (**b**) leads to tissue separation within the lamina lucida (i.e. junctional blistering) in (**a**) and beneath the lamina densa (i.e. dermolytic blistering) in (**b**). Anti-type IV collagen (present in the lamina densa) antibodies bind (**a**) on the floor and (**b**) on the roof of the split induced by the trauma of the biopsy itself

Table 1.4.1.2-3. The most common patterns of expression of antigens within the skin of selected EB types and subtypes

Antigen	EBS	JEB-H	JEB-nH	DDEB	RDEB-HS	RDEB-nHS
Keratin 14	N[a]	N	N	N	N	N
Plectin	N[b]	N	N	N	N	N
Laminin-332 (laminin-5)	N	absent or markedly reduced	reduced (in the majority of JEB-nH)	N	N	N
Collagen XVII	N	N	absent or reduced (in a minority of JEB-nH)	N	N	N
α6β4 integrin	N[d]	N	N[d]	N	N	N
Collagen VII[c]	N	N	N	N	absent	reduced

N = normal

[a]Absent or markedly reduced in severe autosomal recessive EBS

[b]Reduced or absent in EBS with muscular dystrophy and in EBS with pyloric atresia, and reduced in EBS-Ogna

[c]Patients with the rare dystrophic EB subtype named bullous dermolysis of the newborn characteristically have intracytoplasmic granules of collagen VII within keratinocytes, as well as reduced or absent expression along the dermo-epidermal junction, only during the time in which blister activity is still present

[d]Absent or reduced staining in JEB with pyloric atresia and in EBS with pyloric atresia

phosphate buffered saline (PBS, 2×15 min), the second 30 min incubation is performed with species-specific, mostly anti-IgG antibodies from different animal sources (i.e. swine, rat, rabbit) depending on the antibody species used for the first step. These second antibodies are conjugated with a fluorescent dye, most commonly fluorescein-isothiocyanate (FITC). Substituting the first antibody with PBS yields a suitable negative control. After washing the slides again (PBS, 2×15 min), the skin sections are covered with glycerol and a coverslip and then read with an IF microscope at 450–490 nm.

Fig. 1.4.1.2-3. Antigen mapping using immunoperoxidase (**a**, **b**) and immunofluorescence microscopy (**c**–**j**): The examples (**a**, **c**, **e**, **g**, **i**) demonstrate the presence of various structural proteins of keratinocytes and the dermo-epidermal basement membrane zone in normal human skin (NHS) and their absence in the skin of patients with different subtypes of EB (**b**, **d**, **f**, **h**, **j**): Anti-keratin 14: NHS (**a**) and EB simplex Koebner (**b**); Anti-plectin: NHS (**c**) and EB simplex with muscular dystrophy (**d**); Anti-laminin-332: NHS (**e**) and Herlitz JEB (**f**); Anti-type XVII collagen: NHS (**g**) and non-Herlitz JEB (**h**); Anti-type VII collagen: NHS (**i**) and Hallopeau-Siemens RDEB (**j**)

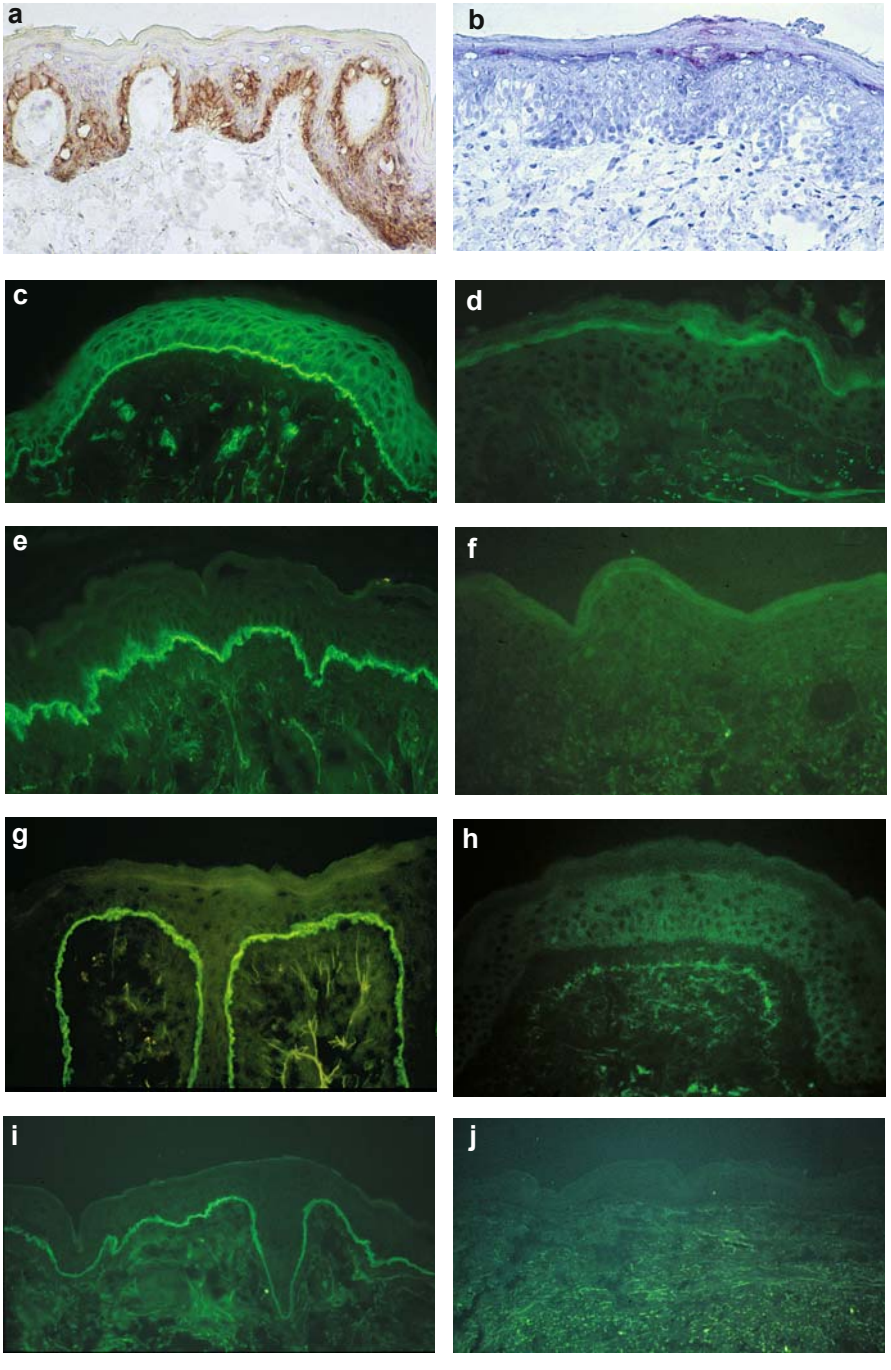
a
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Table 1.4.1.2-3 summarizes the antigen mapping findings in each of the major EB types and subtypes. Illustrative findings are depicted in Fig. 1.4.1.2-3.

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1.4.1.3 Electron microscopy for the diagnosis of epidermolysis bullosa

Robin A. J. Eady

Electron microscopy, or more specifically, transmission electron microscopy (TEM), has played a major role in underpinning the modern classification of epidermolysis bullosa (EB). In the early 1960s, Pearson [31] was the first to show that two of the previously recognized types of EB, namely, EB simplex and dystrophic EB, were characterized by levels of blistering that could be clearly and reproducibly distinguished by TEM. Other authors [1, 5, 37] confirmed that the level of skin splitting in different subtypes of dystrophic EB was consistently just beneath the lamina densa of the dermo-epidermal junction (DEJ), and described a possible primary defect in anchoring fibril formation. It also became evident that the more recently identified third major type of EB, junctional EB (then known as 'EB letalis'), was associated with yet another specific ultrastructural plane of tissue separation, namely the lamina lucida [4, 17, 32, 38]. Moreover, another component of the 'anchoring complex', known as hemidesmosomes, was also ultrastructurally abnormal. These key ultrastructural findings have been further described in a number of reviews [3, 10, 36].

This chapter aims to provide a summary of the main TEM findings in the major types of EB and briefly to describe the ultrastructural features of some of the disorders that have been recently added to the EB classification [11]. Finally, it will include a short discussion on the advantages and disadvantages of using TEM for EB diagnosis in a contemporary setting.

Preparation of skin samples

The importance of proceeding through each step correctly, in a sequence involving biopsy sampling, preparation and analysis, cannot be emphasized too strongly.

Biopsy site: Optimally, both non-separated (non-blistered) and blistered skin should be available for TEM examination. Remember that clinically visible blisters often show microscopic changes of tissue necrosis and wound repair that can produce misleading and equivocal information. It is always better, where possible, deliberately to induce fresh tissue separation by gently rubbing normal-appearing skin. However, this manoeuvre can be very difficult to achieve successfully in certain patients, especially those with the milder, more localized subtypes of dystrophic EB. For the critical evaluation of various subcellular components, such as anchoring fibrils and hemidesmosomes (see descriptions below) it is essential to have available sections containing non-separated skin.

Biopsy procedure: Since the pathology in all types of EB is confined to the epidermis or dermo-epidermal junction, only superficial skin samples are needed to establish the diagnosis. My preference is to take two or three slivers of tissue using a shave biopsy technique achieved by raising a bleb of (anesthetized) skin with a needle, and gently slicing off the sample with a small scalpel blade.

Sample collection and processing: The sample should be immediately immersed in appropriate TEM fixative. Take care to prevent it from drying out since this may result in a massive degradation of cell membranes and other cell components, thus rendering the sample suboptimal or useless for critical examination. Later, each sample will have to be subdivided into several smaller subsamples to allow adequate penetration of the fixative and, eventually, embedding medium, which is usually a type of plastic. However, these subsamples should also be large enough to allow for the provision of informative semithin (~1 μm thick) sections for light microscopy (see below) an essential step before trimming the blocks further for TEM examination.

At the same time, it may be convenient to obtain a further sample, for immunofluorescence analysis (IF), which will involve the use of a different fixative or transport medium. If the preference is to subdivide a single biopsy sample for both TEM and IF, take great care to prevent the epidermis from detaching from its dermal base. This may occur even with gentle manipulation, including cutting with a scalpel or razor blade. Processing for TEM normally takes several days, although rapid methods have been developed for urgent diagnosis, especially involving the processing of fetal skin samples for prenatal diagnosis [9].

Light microscopy

Before trimming the resin blocks for ultramicrotomy, the mechanism used for cutting ultrathin sections for TEM, semithin sections (0.5–1.0 µm in thickness) should be taken for light microscope examination. These sections, when appropriately stained, provide valuable information about the tissue preservation and orientation. They may also provide important clues to the level of blistering, and other processes, such as intracellular filament aggregation in the Dowling-Meara subtype of EB simplex (Fig. 1.4.1.3-1).

Electron microscopy

In order to understand the relevance of the different ultrastructural levels of tissue separation to EB diagnosis, one should first become familiar with the detailed ultrastructure of the dermo-epidermal junction, especially the region of the anchoring complexes (Fig. 1.4.1.3-2). Irrespective of the quality of the tissue preservation or other steps necessary for adequate TEM analysis, a major downside is that TEM can only provide two-dimensional images of extremely thin sections of fixed tissue, which may, at best, be a poor representation of the living skin. However, as in diagnostic histopathology, the experienced microscopist should be able to distinguish 'normal' from 'abnormal' and seek other clues to allow him or her to draw conclusions key to an accurate diagnosis.

EB simplex: In the most common subtype, EB simplex Weber-Cockayne, the blisters arise at the level of the basal epidermal keratinocytes, beneath the cell nuclei [14, 16], although sections may show more extensive disruption extending higher into the epidermis. Clumping or aggregation of keratin filaments

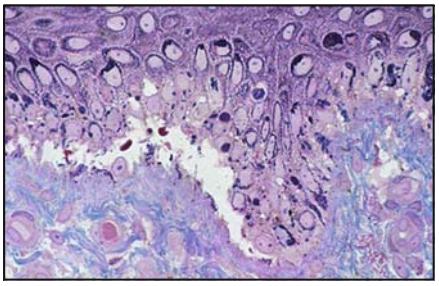

Fig. 1.4.1.3-1. Semithin section of skin biopsy from a patient with EB simplex of the Dowling-Meara subtype. Note a split has occurred at a very low intra-epidermal level, beneath the basal cell nuclei. Numerous intracytoplamic dark blue inclusions in the basal and lower suprabasal cells represent keratin filament aggregates, more clearly resolved at higher magnification by TEM (See Figs. 1.4.1.3-3 and -4). Stained with methylene blue and Azure II

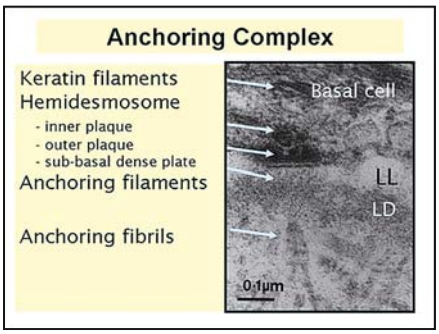

Fig. 1.4.1.3-2. High magnification image of an anchoring complex, which encompasses a hemidesmosome plaque (both inner and outer components), sub-basal dense plate, anchoring filaments, and anchoring fibrils. The inner hemidesmosome plaque is associated with tonofilaments (keratin filaments), and the anchoring filaments traverse the lamina lucida (LL) which lies between the basal cell plasma membrane and the lamina densa (LD)

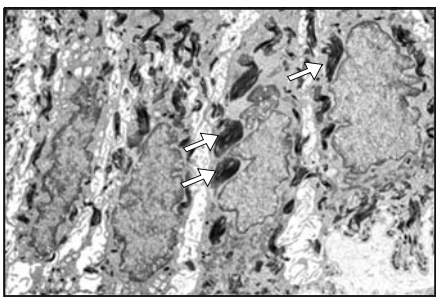

Fig. 1.4.1.3-3. EB simplex (Dowling-Meara). Non-blistered skin. Many dense keratin filament aggregates (arrows) are seen in the perinuclear cytoplasm of adjacent basal epidermal cells

is pathognomonic of the Dowling-Meara subtype of EB simplex [2, 24, 29] (Figs. 1.4.1.3-3 and -4; also see Fig. 1.4.1.3-2). ImmunoTEM has clearly shown that he abnormal aggregates preferentially label for keratins K5 and K14 [22]. In the form of EB simplex associated with muscular dystrophy resulting from a plectin deficiency, the early changes occur very low in the basal cells, just above the basal plasma membrane and associated hemidesmosome plaques [13, 15, 28, 35] (Fig. 1.4.1.3-5). EB simplex superficialis is characterized by a clefting high up in the epidermis, at the junction between the strata granulosum and corneum [12]. In the rare subtype of autosomal recessive generalized EB simplex, usually caused by homozygous nonsense mutations in keratin 14, the basal cell keratin filaments are severely reduced in number or totally unrecognisable [7, 33] (Fig. 1.4.1.3-6). EB simplex associated with mottled pigmentation shows abnormal aggregates of melanosomes [6, 8] and an abnormality of the keratin filament network [39].

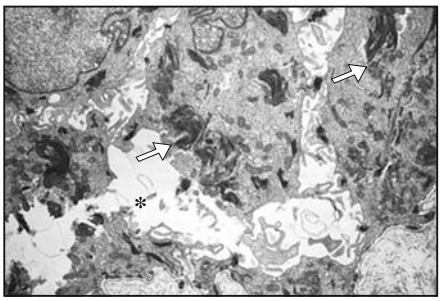

Fig. 1.4.1.3-4. EB simplex (Dowling-Meara). Early blister. The fracture plane (asterisk) is in the sub-nuclear cytoplasm of the basal cells which contain keratin filament aggregates (arrows)

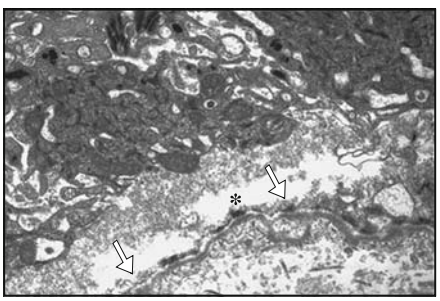

Fig. 1.4.1.3-5. EB simplex associated with muscular dystrophy. The split has occurred very low in the basal cell layer, immediately above the hemidesmosome plaques, which may be diminutive (arrows)

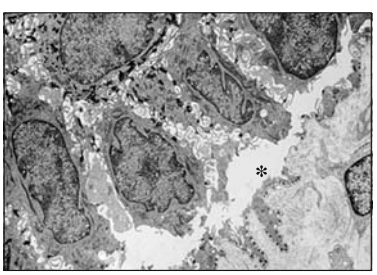

Fig. 1.4.1.3-6. Autosomal recessive EB simplex. Here, the split (asterisk) is again very low in the basal layer, but in contrast to the cells in Figs. 1.4.1.3-3 and -4, no keratin filaments, either clumped or normal, can be identified in the basal cells

Junctional EB: In all patients with junctional EB (JEB), irrespective of the genes involved or the specifics of underlying mutations, the cleavage occurs through the lamina lucida (Fig. 1.4.1.3-7) which is located between the basal cell plasma membrane and the underlying lamina densa (see Fig. 1.4.1.3-2). JEB may occur as

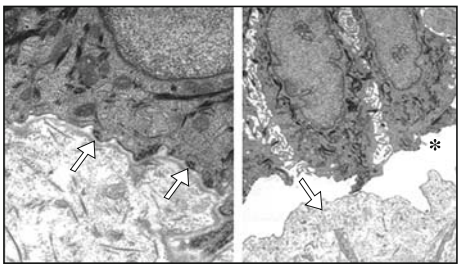

Fig. 1.4.1.3-7. Herlitz junctional EB. Left panel: non-blistered skin. Small hemidesmosome plaques are seen (arrows) Right panel: blister. The cleavage (asterisk) has caused a massive expansion of the lamina lucida, beneath the basal cells and lamina densa (arrow)

various subtypes, including Herlitz JEB, non-Herlitz JEB, and the form of JEB associated with pyloric atresia. In the first and third subtypes, the hemidesmosome plaques are often very small [4, 10, 17, 32, 36, 38, 40], and the close association with keratin intermediate filaments is reduced [26] (Fig. 1.4.1.3-7). In non-Herlitz JEB, the hemidesmosomes are variable in both size and number, and may appear normal [17, 19, 38]. A word of caution – patients with non-Herlitz JEB due to mutations in collagen XVII may show blisters arising in the lower epidermis [21, 30], thus mimicking cases of EB simplex. The same cleavage level may also be seen in JEB associated with pyloric atresia arising from mutations in integrin $\alpha 6\beta 4$ (Eady RAJ, personal observation, 2008).

Dystrophic EB: In this major type of EB the split will invariably appear immediately beneath the lamina densa (Fig. 1.4.1.3-8). The difference between the blister levels in dystrophic EB (DEB) and JEB is a fraction of a micrometer (see Fig. 1.4.1.3-2) and well beyond the resolution of a light microscope. In the autosomal recessive, severe generalized subtype of DEB, often given the eponym of 'Hallopeau-Siemens', normal anchoring fibrils are unrecognizable [5, 37].

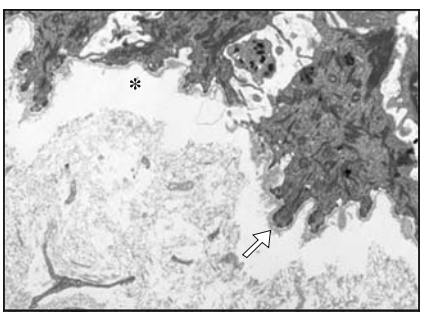

Fig. 1.4.1.3-8. Recessive dystrophic EB. The level of blistering (asterisk) is only a fraction of a micron deeper than in junctional EB, namely immediately beneath the lamina densa where anchoring fibrils are normally seen (see Fig. 1.4.1.3-2). Arrow indicates lamina densa at the roof of the split

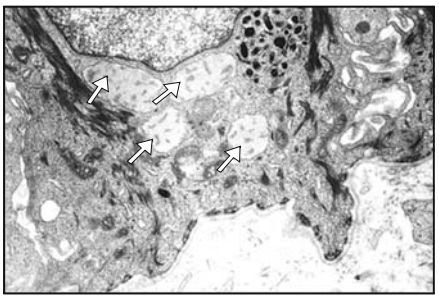

Fig. 1.4.1.3-9. Transient bullous dermolysis of childhood: Characteristic inclusions (arrows) are present in the perinuclear cytoplasm of a basal cell. These inclusions have been shown by immunoEM studies to express type VII collagen

However, anchoring fibrils are present in reduced numbers in the dominant and milder recessive subtypes of DEB [37]. In the variant of DEB known as 'transient bullous dermatosis of childhood' inclusions with a characteristic appearance are seen in the basal epidermal cells [14, 18] (Fig. 1.4.1.3-9) which have been shown by immunocytochemical methods to stain for type VII collagen, the major constituent of anchoring fibrils.

Findings in disorders recently classified as types of EB

A recently revised EB classification [11] decided to include a number of hereditary disorders with similar features to the established forms of EB, including the appearance of blisters or erosions after mild mechanical trauma. The most well-known disorder in this group is Kindler's syndrome, in which the level of splitting at the TEM level is not fixed; it can occur through the basal epidermis, the lamina lucida or the sub-lamina densa zone [20, 34]. On occasion, one may see all these cleavage planes within the same thin section. Another important feature is the reduplication of the lamina densa (Fig. 1.4.1.3-10) and the presence of dermal colloid bodies.

The same newly revised EB classification has divided EB simplex into basal and suprabasal categories. We have already reviewed the principal changes seen in the basal subtypes (EB simplex). Apart from EB superficialis (see above), the suprabasal category includes two distinct autosomal recessive diseases resulting from mutations in desmosome related molecules. The first is caused by mutations in plakophilin 1, a component of the desmosome plaque. The resulting plakophilin 1 deficiency results in the disorder also known as 'skin fragility with ectodermal dysplasia" [25, 27]. The main TEM abnormalities are mainly confined to the mid-epidermal or spinous cell layer. These include marked widening of the intercellular spaces, retraction of the keratin filaments into a perinuclear

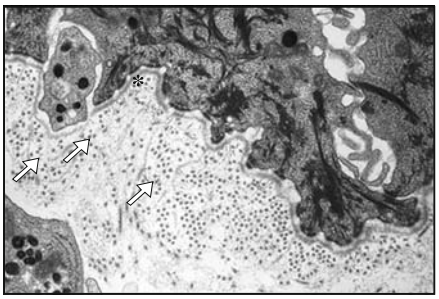

Fig. 1.4.1.3-10. Kindler syndrome: Multiple layers of lamina densa (arrows) can be seen in non-blistered skin

distribution, and the presence of diminutive desmosomes (Fig. 1.4.1.3-11). At higher magnification, the abnormal desmosomes are seen to be associated with finger-like projections on the cell periphery. A second desmosome disorder resulting from loss of the tail domain of another plaque component, desmoplakin, shows rather similar ultrastructural changes to those in plakophilin deficiency. This clinically more severe disease has been named 'lethal acantholytic EB simplex' [23].

Advantages and disadvantages of electron microscopy in EB diagnosis

TEM has for many years been the gold standard laboratory test for EB diagnosis. It has been crucial to devising the modern classification of EB [11] and to the discovery of newer disorders, such as plakophilin 1 deficiency, which has recently become part of the EB family. It still retains a pre-eminent position in determining the precise level of tissue separation. It is also unrivalled in allowing a detailed

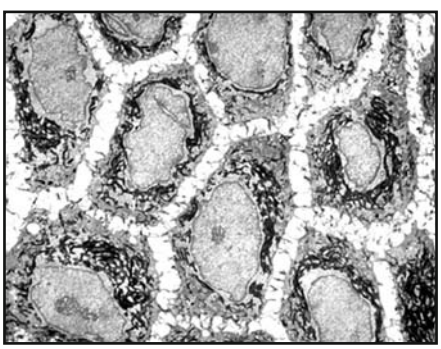

Fig. 1.4.1.3-11. Plakophilin deficiency. Keratinocytes in the spinous layer are widely separated and the keratin filaments are condensed into a perinuclear distribution

examination of cells and organelles (e.g. mitochondria, endoplasmic reticulum, lamellar granules) and other subcellular components (e.g. tonofilaments, hemidesmosomes, anchoring fibrils and plasma membrane). However, with the availability of well-characterized monoclonal and polyclonal antibodies recognizing the key components of the dermo-epidermal junction and other intraepidermal molecules involved in EB pathogenesis and diagnosis, indirect immunofluorescence has largely replaced TEM as the primary means for EB diagnosis (Chapter 1.4.1.2).

Yet, it is recognised [11] that diagnostic TEM should remain available in at least a few reference centres to assist with the diagnosis of more complex cases. A final word of caution: more important than the equipment available is the skill and, in particular, the experience, of the electron microscopist analysing the samples and providing the report.

Conclusion

TEM has played an essential part in EB diagnosis. It will continue to have a role, even though other approaches have now attained an increasingly prominent position in the laboratory.

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1.4.2.1 Mutation analysis

Alfred Klausegger and Johann Bauer

Definition of mutation analysis

Mutation analysis refers to testing of any gene for families with previously identified mutations associated with common or rare genetic conditions, e.g. epidermolysis bullosa or testing of a target gene for a patient with unidentified mutations associated with a distinct phenotype.

Who can benefit from mutation analysis?

- Patients with the diagnosis epidermolysis bullosa would like to find out about the ultimate cause of the phenotype of the disease.
- Patients with a mutation identified in a research laboratory and who would like to have clinical confirmation for management or diagnostic purposes.
- Families in which a mutation was identified previously in an affected family member in a research laboratory, and the family is seeking prenatal or carrier testing.
- Extension of the mutation database of a given gene makes a substantial contribution on genotype/phenotype relationship and provides prognostic markers for the progress of the disease.
- The exact knowledge of the mode and location of the mutation is a precondition for a genetherapeutic approach. Predefined cell culture models are necessary for basic research.

Introduction

Since the discovery of the major genes responsible for the different forms of epidermolysis bullosa, molecular testing has become possible. Although epidermolysis bullosa is mainly a clinical diagnosis, certain situations may occur in which molecular analysis for EB genes is wanted, either for diagnostic, management or genetic counseling purposes. The diagnosis based on clinical parameters alone can be challenging in patients not yet fulfilling the diagnostic criteria. In addition, molecular testing allows the geneticists to offer prenatal or preimplantation genetic diagnosis to couples, an option for which demands are steadily increasing.

Nevertheless, molecular analysis is at present a laborious and expensive analysis which can not be offered on a routine basis. The efficiency of the mutation detection in EB associated genes depends on a number of factors, such as the type of mutation, the technique used and, above all, the accuracy of the clinical diagnosis. Today, mutation screening techniques allow a mutation detection rate approaching about 90%, providing the clinical selection is made appropriately.

Procedure of mutation analysis

The first step in EB diagnostics is performing a skin punch biopsy (4 mm) from the patient and collecting blood or buccal cells from the patient and her/his relatives. The punch biopsy is used to prepare frozen skin sections for antigen mapping with a panel of antibodies directed against most of the structure proteins of the skin. As soon as the cleavage plane and/or loss of expression of protein is determined, the candidate gene for mutation analysis can be selected for further procedure (Fig. 1.4.2.1-1).

Usually genomic DNA is isolated from blood of patients and their parents with a commercially available kit. We use Puregene^{TM} DNA Isolation Blood Kit (gentra, Minneapolis, USA). Another possibility is to use buccal cells, especially when newborns are being tested or if a patient is not willing to provide sufficient blood. In the case of prenatal diagnosis chorionic microvilli sample (CVS) and cultured amnion cells are used as sources for DNA.

Regions of the genes of interest are amplified by exon-specific polymerase chain reaction (PCR). Wherever possible, primers are chosen to amplify two or three neighbouring exons with the flanking intronic sequences to reduce the total number of fragments. In addition to standard criteria or primer design, the maximum sequence length for reliable reading, the minimum distance between primer binding site and start of reliable reading, the total length of PCR fragments

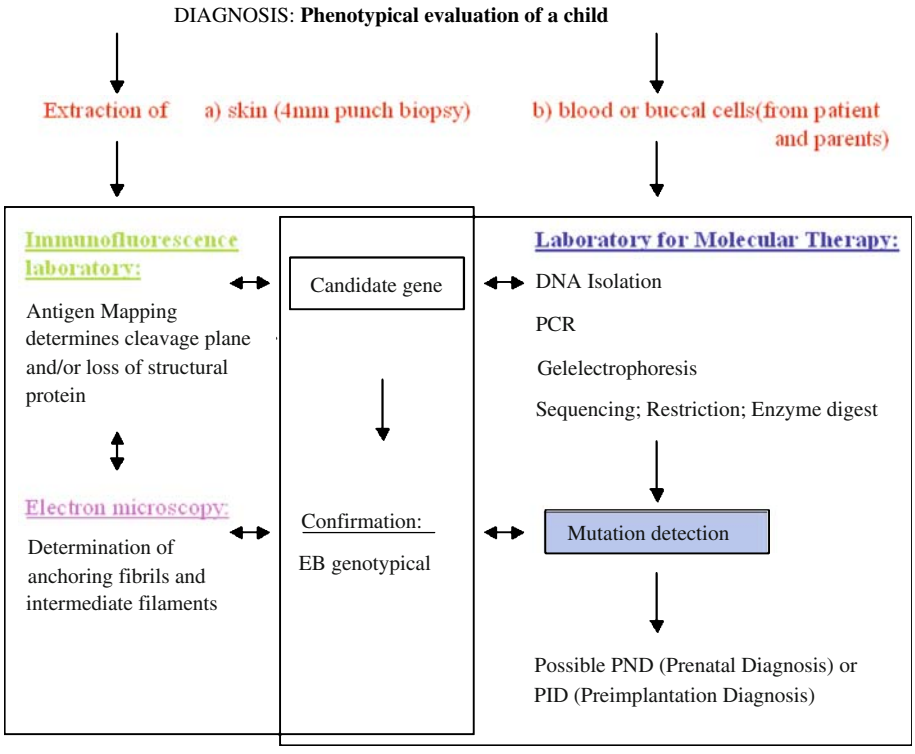

Fig. 1.4.2.1-1. Pathways in molecular diagnostics of EB. Once the cleavage plane has been determined and the candidate gene is identified by antigen mapping, the procedure follows the common methods for mutation analysis. In case of carrier detection in families with known mutations, only **b**) is necessary

and the size of the exons and introns should be taken into account. The primers are then used for sequencing the product in 5′ and eventually in 3′ direction.

Restriction enzyme analysis (see Figs. 1.4.2.1-3 and 1.4.2.1-4) is often used to confirm the sequencing result and is a very quick method to disclose a wildtype, mutant heterozygous or homozygous status.

Guidelines for shipping of blood samples for mutation analysis

For adults we need 2–5 ml blood in an EDTA tube (NOT heparin or citrate). For infants at least 0.5 ml blood in an EDTA tube or buccal swabs with a cytobrush in PBS can be taken. Please do not freeze blood samples! Storage of blood samples at 4 °C is possible for several months to years.

For sending of blood samples be aware of the guidelines by law and put the samples into a container and a small box and send them at room temperature by regular mail. Supporting us with clinical information and a clinical photograph of the patient as well as a pedigree facilitates the interpretation of results.

Please give notice if you send a sample to avoid transport challenges: Tel. 0043 662 4482 3110; Fax. 0043 662 4482 3125; a.klausegger@salk.at

Types of mutations in EB

Single-base substitutions

A single base is replaced by another. Single base substitutions are also called point mutations. If one purine [A or G] or pyrimidine [C or T] is replaced by the other, the substitution is called a transition. If a purine is replaced by a pyrimidine or vice-versa, the substitution is called a transversion.

Missense mutations

With a missense mutation, the new nucleotide alters the codon so as to substitute a different amino acid in the protein product (Fig. 1.4.2.1-2a). This kind of mutations is usually involved in mild forms of epidermolysis bullosa.

Nonsense mutations

With a nonsense mutation, the new nucleotide changes a codon to a STOP codon. (TAA, TAG, or TGA) (Fig. 1.4.2.1-2b). Therefore, translation of the messenger RNA transcribed from this mutant gene will stop prematurely. The earlier in the gene this occurs, the shorter the protein product is and the more likely it is that it will be unable to function. Therefore nonsense mutations are involved mainly in severe forms of epidermolysis bullosa.

Silent mutations (single nucleotide polymorphisms – SNPs)

Most amino acids are encoded by several different codons. For example, if the third base in the CTA codon for leucin is changed to any one of the other three bases, leucin will still be encoded (Fig. 1.4.2.1-2c). Such mutations are called to be silent and defined as single nucleotide polymorphisms (SNPs) because they cause no change in their protein product. But we also know SNPs involving a change of an amino acid with no phenotypical appearance, that can sometimes be hardly distinguished from real mutations. Comparison of the found SNP/mutation with

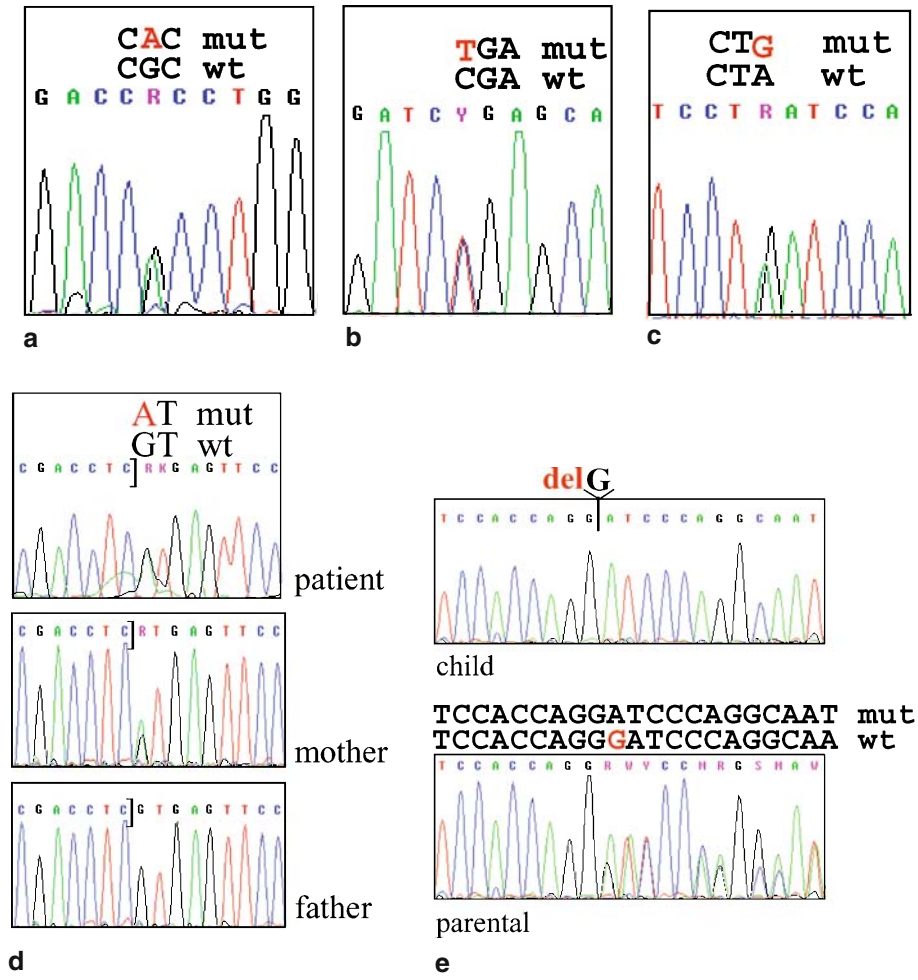

Fig. 1.4.2.1-2. **a** 374G>A; **b** 1903C>T; **c** 522A>G; **d** 682 + 1G>A; **e** 3561delG

SNP databases and listed SNPs in literature, as well as screening of at least 100 alleles of wildtype DNA samples facilitates the indentification of a SNP or a mutation.

Splice site mutations

Nucleotide signals at the splice sites guide the enzymatic machinery to remove intron sequences from pre-mRNA with great precision to be processed to mature mRNA. If a mutation alters one of these signals (Fig. 1.4.2.1-2d), complete exons can be skipped, or cryptic splice sites are used leading to insertions/deletions of parts of the introns/exons in the final RNA molecule. The translation of its

sequence alters the sequence of the protein product, and in most cases a frameshift leads to a preterminal stop codon.

Insertions and deletions (indels)

Extra base pairs may be added (insertions) or removed (deletions) (Fig. 1.4.2.1-2e) from the DNA of a gene. The number can range from one to thousands. Collectively, these mutations are called indels. Indels involving one or two base pairs (or multiples thereof) can have devastating consequences to the gene because translation of the gene is "frameshifted". The mRNA is translated into new groups of codons and the protein specified by these new codons will be nonfunctional. Frameshifts usually create new nonsense mutations to prematurely stop the useless protein. Indels of three nucleotides or multiples of three may be less serious because they preserve the reading frame.

The nomenclature for mutations

The nomenclature for mutations should be chosen according to previously published recommendations [3, 4]. The DNA mutation numbering is based on the gene's cDNA sequence referred to GenBank, and +1 corresponds to the A of the ATG translation initiation codon of the sequence.

Attention should be drawn on gene COL17A1! Previous publications do not use the common nomenclature for mutations at cDNA level, because the authors started with an old nomenclature and did not use +1 but a location −105 nucleotides in 5′ direction from ATG translation initiation codon. This calculation with a difference of 105 basepairs should be taken into account when working with mutations in COL17A1!

Mutational hotspots in EB

R635X and R42X in JEB

The mutation rate in the three genes LAMA3, LAMB3 and LAMC2 is approximately equal, with unique mutations appearing in each family. However, two recurrent mutations in LAMB3, R635X and R42X, account for almost 60% of the mutant LAMB3 alleles in the Herlitz type of junctional epidermolysis bullosa. The most prevalent mutation, R635X, was noted for nearly 50% and R42X has been observed in about 10% of mutant LAMB3 alleles [9].

These nonsense mutations occur at CpG dinucleotide sequences and convert an arginine codon (CGA) to a premature termination codon (TGA), suggesting

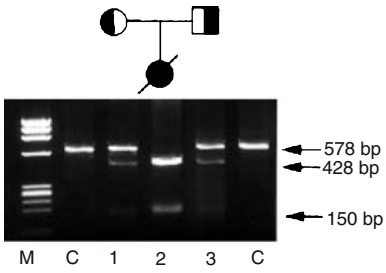

Fig. 1.4.2.1-3. Detection of the mutation R635X by restriction enzyme digest. The proband (lane 2) demonstrates digestion of the 578 bp fragments into 428 bp and 150 bp fragments, indicating homozygosity for the mutation R635X. The parents are heterozygous carriers for this mutation [9]

hypermutability of 5-methylcytosine to thymine. Additional evidence suggested that R635X and R42X represent mutational hotspots rather than propagation of common ancestral alleles, because R635X was based on two different genetic backgrounds in a homozygous patient and at least four different British backgrounds demonstrated by haplotype analysis. R42X arose *de novo* as a result of maternal germline mutation [1, 9].

Identification of these two predominant gene defects has led to a mutation-detection strategy for JEB that first analyzes the LAMB3 gene for the presence of these common mutations by using restriction enzyme digests and if no mutation is disclosed, the remaining exons have to be examined by sequencing. A restriction enzyme digest can be used to screen very easily for known mutations. As a consequence of the mutation R635X in exon 14 a new restriction enzyme site BglII is created resulting in complete digestion of the 578 bp-PCR product into two new digestion products of 428 bp and 150 bp in case of homozygosity. Heterozygous carriers show the wildtype allele in the presence of an additional undigested 578 bp band (Fig. 1.4.2.1-3).

As a consequence of the mutation R42X in exon 3 a new restriction enzyme site DdeI is created resulting in digestion of the 273 bp-PCR product into three wildtype digestion fragments of 103 bp, 85 bp and 38 bp and two additional new fragments of 241 bp and 32 bp in case of homozygosity. Heterozygous carriers show the wildtype allele in the presence of an additional undigested 273 bp band (Fig. 1.4.2.1-4).

Unusual mode of inheritance – uniparental isodisomy (UPD)

UPD (uniparental isodisomy or meroisodisomy) with homozygosity of recessive alleles is being increasingly recognized as the molecular basis for several

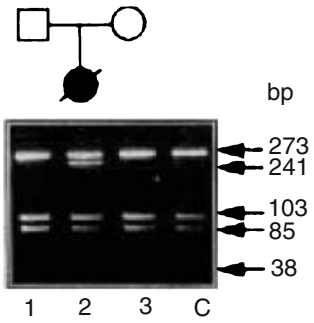

Fig. 1.4.2.1-4. Detection of the mutation R42X by restriction enzyme digest. The proband (lane 2) demonstrates digestion of one of the 273 bp fragments in 241 bp and 32 bp (invisible) fragments, indicating heterozygosity for the mutation R42X. The parents do not carry this mutation, suggesting that this mutation is a *de novo* event [9]

autosomal rezessive disorders [20]. In fact, there have been over 35 reported cases of recessive diseases resulting from UPD [12]. To date there are six reported cases of UPD in epidermolysis bullosa (Table 1.4.2.1-1), four of them resulting in JEB involving the LAMB3 gene in three cases and the LAMC2 gene in one case [6, 15, 18, 19]. Another report describes UPD in lethal EBS associated with pyloric atresia (PA) involving the PLEC1 gene [14]. The most recent reports deal with a HS-RDEB phenotype due to UPD in the COL7A1 gene [5, 8].

Uniparental heteroisodisomy refers to the presence of a pair of chromosome homologues, whereas uniparental isodisomy (UPD) describes two identical

Table 1.4.2.1-1. Reported cases of autosomal recessive disorders in eb, with genes located on chromosomes 1, 3, and 8, resulting from UPD

Gene	Disorder	Chromosome	Mutation	Uniparental	Nature of disomy	Literature
LAMB3	Herlitz JEB	Chr1	Q243X	maternal	mero isodisomy[1]	[15]
LAMB3	Herlitz JEB	Chr1	Q936X	maternal	mero isodisomy[1]	[19]
LAMC2	Herlitz JEB	Chr1	C553X	paternal	complete isodisomy	[18]
LAMB3	Herlitz JEB	Chr1	R635X	paternal	complete isodisomy	[6]
PLEC1	EBS-PA	Chr8	R1189	paternal	segmental isodisomy[2]	[14]
COL7A1	HS-RDEB	Chr3	345insG	maternal	complete isodisomy	[5, 8]

[1]Uniparental primary heterodisomy + partial isodisomy
[2]With additional maternal heterozygote mutation Q2538X

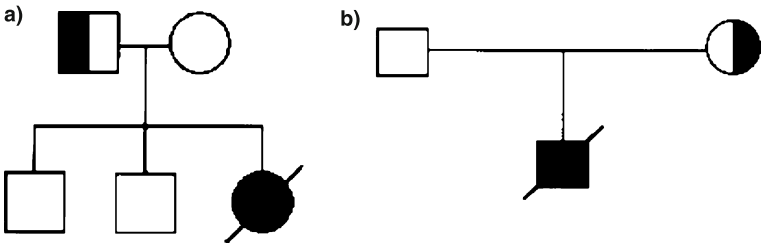

Fig. 1.4.2.1-5. Examples for **a**) paternal isodisomy; **b**) maternal isodisomy

copies of a single homologue, and meroisodisomy (partial isodisomy) is a mixture of these conditions [10]. That means, UPD allows two copies of a rezessiv mutation to be transmitted from a heterozygous carrier parent (Fig. 1.4.2.1-5).

The possibility of occurrence of UPD in epidermolysis bullosa has important implications for mutation screening and for the accuracy of genetic counseling.

Molecular analysis of each of the parents of a child affected with an autosomal recessive disease must be routinely performed. Parents of affected children are usually asymptomatic carriers with a 25% risk of recurrence in subsequent pregnancies. The exception is UPD, in which both homologues of a pair of chromosomes are inherited from just one parent. It can be expected that subsequent pregnancies will follow the Mendelian principles. Anyway, the majority of such cases appear to be associated with advanced maternal age. Delineation of *de novo* mutations that are not present in the parental germline, may substantially reduce, but not completely abolish, the risk of recurrence.

If the child is found to be homozygous for a certain mutation and one parental part is shown to be wild-type for this locus, haplotype analysis of the gene locus based on the inheritance of intragenic polymorphisms is useful to establish the origin of the child´s second mutant allele. To disclose the nature of disomy and find out how much of the chromosome is duplicated in the child, microsatellite markers spanning the entire chromosome can be genotyped (Fig. 1.4.2.1-6). The microsatellite markers can be amplified with carboxy fluorescein (FAM-6) labeled oligonucleotides and easily analyzed with an ABI Genetic Analyzer. Non-maternity, although extremely unlikely, or non-paternity should be excluded by analysing a battery of mikrosatellite markers on different chromosomes for discrepancies in the segregation of maternal and paternal alleles to the affected child.

UPD can also result in distinct phenotypes depending on the parental origin of the chromosomes, a phenomenon known as genomic imprinting. Epigenetic modification of a specific gene or a group of genes determines the parent-of-origin specific gene expression in a way that gene transcription is altered and only one inherited copy of the relevant imprinted gene(s) is

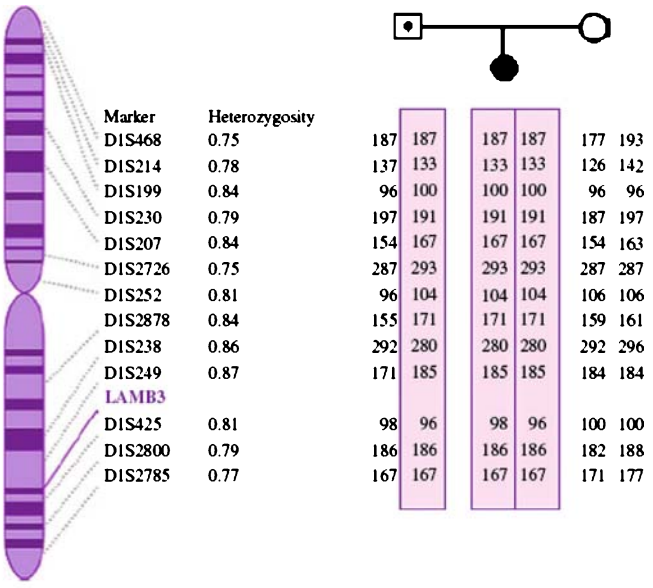

Fig. 1.4.2.1-6. Idiogram of chromosome 1 and genotype analysis of the affected child and both parents using mikrosatellite markers. The affected child is homozygous for all markers tested. All alleles have originated from the father [7]

expressed in the embryo [17]. Three phenotype abnormalities commonly associated with UPD for chromosomes with imprinting are intrauterine growth retardation, development delay, and reduced stature [2, 13]. Thus far there have been no reports of imprinted genes involved in EB and UPD.

The mechanism underlying UPD are diverse and include gamete complementation (non-disjunction in meiosis leading to a diploid gamete with two copies of a chromosome fertilized by a nullosomic gamete lacking the same chromosome), trisomy rescue (chromosome loss in trisomy), monosomy rescue (chromosome duplication in monosomy) and post fertilization error [11, 16].

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1.4.2.2 Mosaicism in epidermolysis bullosa

Martin Laimer

Introduction

Genetic mosaicism is defined as the presence of two or more genetically distinct cell populations that are derived from a single homogeneous zygote within one individual. Thereby, a mosaic contrasts to a chimera which is an organism composed of two genetically different cell populations originating from *different* zygotes [11]. Mosaic heterogeneous cell populations can arise after fertilization, during embryogenesis, fetal development, or postnatal life as a consequence of chromosomal abnormalities (e.g. aneuploidy, polyploidy) or mutations in single genes. The stage of development in which the mutation occurs will determine which tissue and what proportion of cells within the tissue will contain the change. Only the tissue derived from the precursor cell in which the alteration has occurred is affected and will mediate the extent of mosaic manifestations in endodermal, ectodermal and/or mesodermal tissue [12, 15]. Consequently, mutations which occur earlier in development would lead to more extensive cutaneous involvement and greater likelihood of other organ system involvement. Mosaicism thus provides explanations for asymmetric clinical distribution of lesions, lack of diffuse involvement of entire organs, discordant identical twins, sporadic occurrence and exclusively unaffected offsprings born to affected individuals [1].

In principal, a postzygotic mutation can either occur in gonadal or somatic cells, i.e. germline versus somatic mosaicism. Germline mosaicism implies putative transmission to the offspring as tissue giving rise to gametes is also mosaic. Classically, such genetic events occur *de novo* and convert normal cells to mutant cells ("forward mosaicism"). Alternatively, in "revertant mosaicism", an inherited

disease-causing mutation may be corrected by means of a second genetic event, resulting in complete or partial restoration of gene function in so-called "revertant" cells. Correction of a stem cell may lead, in this case, to life-long repair.

Molecular mechanisms involved in mosaic reversion

In molecular terms, the restoration of gene function may be obtained by different genetic mechanisms.

- True back or reverse mutation. This type of mutation retransforms a forward mutation back to the original wild-type sequence or to another one, thereby restoring the amino acid sequence of the wild-type polypeptide.
- Modification of splicing and mRNA editing [21]. The consecutive alternative transcript may mediate a structural and/or functional (partial) rescue of gene expression.
- Intragenic crossover. This can occur during meiosis if the same genes with homologous areas misalign, leading to a reciprocal exchange of part of a gene on one chromosome to part of a gene on the partner chromosome (single crossover).
- Mitotic gene conversion. This means the nonreciprocal transfer from one (parental) chromosome to another in such way that an internal portion of its sequence has been replaced by a homologous segment copied from another allele or locus [9, 16].
- Additional second-site mutations. These take either place inside or outside the mutated gene without changing the nucleotides of the original mutation and are able to restore the activity of the mutated gene, but frequently lead only to partial reversion. Molecular mechanisms to compensate for the effect of the primary inherited mutation include base pair addition or deletion, suppressor mutation and chromosomal loss or gain [10]. Thus, small changes in the amino acid sequence may occur [17].

Revertant mosaicism in compound heterozygotes, where germ-line mutations are located at different sites on both alleles in a gene, is characterized by loss of heterozygosity [9]. Revertant cells thereby become either homozygous at a certain locus during mitotic recombination, resulting from a (double) crossing-over, gene conversion, or reverse point (back) mutation, or hemizygous resulting from a non-disjunction or deletion. Likewise, only one allele corrected may be needed in recessive diseases. However, to achieve revertant mosaicism in patients who are homozygous for a mutant allele and, thus, in which a genomically corrective template is absent, different mechanisms such as back mutation or DNA polymerase slippage (i.e. transient dissociation of the nascent and template strands during replication followed by a misaligned reassociation of

the strands leading to either a gain or loss of sequence and frameshift mutations) are required [4].

Principally, also epi- and non-genetic phenomena should be considered as an underlying cause of mosaic traits, although these heritable changes in gene expression are not due to changes in the DNA sequence. In contrast, in epigenetic mosaicism, environmental mechanisms may alter the genetic information within a cell that then give rise to genetically distinct cell populations. For example, eukaryotic organisms posses a complex molecular apparatus that maintains portions of their genomes in a state that is refractory to transcription initiation. One of these regulative components are retrotransposons, DNA sequences of viral origin that are incorporated into the nuclear DNA and replicated. They may silence or activate gene expression through induction of DNA methylation and demethylation [12, 20]. Such chemical modifications influence the phenotype of cells through mediating a repressive chromatin configuration. Epimutations in the germ-line can be maintained in somatic cells and mimic an inactivating mutation. However, since epimutations are probably a stochastic event, created by errors that act in cis which mostly affect promoters and other transcriptional control elements, biallelic epimutations will be rare unless something like paramutation occurs.

Lyonization is considered a specialized form of epigenetic regulation of gene expression that is inherent and not environmental. To compensate for the presence of two (paternal and maternal) X chromosomes in females, one X chromosome in every cell of a developing female embryo is randomly but stably inactivated in the late blastocyst stage by way of alterations in chromatin structure and methylation states. All females are therefore functionally mosaic with respect to most genes on their X chromosome. This process thereby contributes to rescue of otherwise lethal phenotypes in dominant X-linked conditions such as incontinentia pigmenti. Another thesis of how mosaicism may counter lethal molecular states was proposed by Happle and his concept of “autosomal lethal gene syndromes”. He suggested that several genetic disorders that pathogenetically either arise from a postzygotic mutation occurring after fertilization, during blastogenesis or early embryogenesis, or from a half-chromatid mutation occurring before fertilization in one of the two gametes forming the zygote, are due to the action of a gene product that if present in the germline would be lethal in nonmosaic state [6, 7]. Diseases such as Proteus syndrome, encephalocraniocutaneous lipomatosis, Sturge-Weber and Klippel-Trenaunay syndrome, congenital cutis marmorata teleangiectatica or neurocutaneous melanosis have been implicated to be molecularly determined by this way. In these syndromes, a clinical manifestation would only be possible when the molecular aberration is present in a subpopulation of cells in close proximity to normal cells, thereby surviving through mosaicism. No transmission of the mutation to the offspring would be possible, as if

transmitted, the zygote would die *in utero*. Rare exceptions from this rule of nonheritability could include paradominant inheritance: heterozygous individuals for a paradominant mutation would be phenotypically normal and the allele could be transmitted unperceived through generations. In this case, the trait would become manifest when a postzygotic mutation of the corresponding allele occurs, thereby giving rise to a cell colony displaying allelic loss and forming a mosaic patch [6].

Revertant mosaicism in epidermolysis bullosa

Revertant mosaicism has been demonstrated for several different genetic diseases, including tyrosemia type I, Bloom syndrome, Fanconi anaemia, Wiskott-Aldrich syndrome, X-linked severe combined immunodeficiency, and adenosine deaminase deficiency, as well as hereditary epidermolysis bullosa [17]. Thereby, different cell types such as hepatocytes, lymphocytes, and in the case of EB, keratinocytes were retransformed.

For EB, several studies of mosaic traits have been reported that significantly contributed to the characterization of pathogenetics and paragenetics of mosaicism and molecular reversion (Table 1.4.2.2-1).

In pioneering work by Jonkman [9], revertant mosaicism in EB was first described in a patient with JEB-nH. The proband was compound heterozygous for two truncation mutations within the COL17A1 gene, i.e. R1226X (nonsense on the paternal allele) and 1706delA (frameshift on the maternal allele). Re-expression of BP180 in keratinocytes was caused by a mitotic gene conversion of COL17A1 on the maternal allele, i.e. the site surrounding the maternal mutation had been repaired with the normal sequence from the paternal allele by a non-reciprocal crossing-over in somatic precursor cell(s). The paternal R1226X remained present in all cell samples. Remarkably, prevention of blistering was reached although not more than 50% of the basal keratinocytes had reverted in clinically unaffected skin patches reexpressing type XVII collagen. These positive clusters were suggested to reflect the presence of cells supplied by divisions of revertant stem cells whose corrective rate seemed to be sufficient to normalize skin function.

Revertant mosaicism has also been implicated to be responsible for the commonly observed amelioration with age in EBS-DM, either by accumulation of multiple advantageous reversions occurring over time or by replacement of mutant cells with revertant cells under suitable selective pressure, in this case blistering [16]. Healing over time of an inherited disease probably depends on the benefit the stem cells experience from re-expressing the protein in their competition with mutant stem cells. Other mutations in different genes or in different cell

Table 1.4.2.2-1. Reported mosaic traits in epidermolysis bullosa

EB-(sub-) type, (mode of inheritance), reference	Summary
JEB-nH, (AR) [17]	• 2 unrelated probands with multiple compensatory somatic second-site mutations, all correcting the same germline mutation c.628G → A;pE210K in LAMB3 • In patient 1 (c.628G → A/c.1903C → T) the skin on left lower leg became progressively clinically healthy, with normal expression of laminin 5 on previously affected skin • Patient 2 (c.628G → A/c.628G → A) depicted revertant patches located on arms, shoulder, chest
JEB-nH, (AR) [16]	• 2 unrelated probands • Patient 1 with true back mutation 3781T → C (arm) and second-site mutation 4463-1G → A compensating for frameshift mutation 4425-5insC (middle finger) in COL17A1 • Patient 2 with two distinct gene conversions correcting 1706delA (arm, hands), second-site mutation 3782G → C neutralizing PTC mutation 3781C → T (R1226X)
EBS, (AD) [14]	• Proband 1 heterozygous for 1649delG in KRT 5, classified de novo as no mutation could be detected in parental peripheral blood mononuclear cells (PBMNCs) • Proband 2 (sister of proband 2) born with identical mutation • Reinvestigation revealed that mother's DNA from hair bulb and buccal cell samples had 1649delG heterozygously, thus representing somatic and gonadal mosaicism
EBS-DM, (AD) [21]	• Mutation analysis of cultured primary keratinocytes of unaffected area from EBS-DM patient revealed heterozygous R125C in KRT 14 • Second site mutation 242insG creating a PTC immediately downstream, thereby silencing the dominant-negative R125C allele • Second site mutation only present in DNA of keratinocytes and absent in PBMNCs DNA
JEB-H LAMB3, (AR) [3]	• Patient with 1094delA and hotspot mutation R635X in LAMB3 • 1094delA detected in clinically unaffected mother • R635X not found in PBMNCs DNA of either parent • Exclusion of non-paternity by microsatellite analysis using random markers • During prenatal diagnosis for a second pregnancy the maternal mutation 1094delA was not detected in fetal DNA, whereas R635X was present in chorionic villus DNA, consistent with paternal germline polymorphism for recessive R635X

(continued)

Table 1.4.2.2-1 (*Continued*)

EB-(sub-) type, (mode of inheritance), reference	Summary
EBS, (AR) [19]	• Patient homozygous for KRT 14 1842-2A → C splice site mutation • Germline mutation resulted in different aberrant transcripts containing PTCs, all leading to truncated K14 proteins • Basal keratinocytes in skin and culture lacked K14−/− • Culture from new biopsy of affected skin of the K14−/− patient showed spontaneously K14 expressing keratinocytes, confirmed by immunohistochemistry, electron microscopy and immunoblotting • Analyses of KRT14 mRNA isolated from mosaic skin/K14 positive keratinocytes revealed an additional splice variant/ in-frame transcript (1844T → T, 1845delta6) coding for an abnormal K14 polypeptide with an exclusive two residue in-frame deletion and one amino acid change • Partial revertant mosaicism accounted for antibody staining pattern and reappearance of intermediate filaments, although semifunctionality impaired reversion of the clinical phenotype
DEB [2]	• Child with mild DEB and G2003R in COL7A1 • G2003R not found in PBMNCs of clinically unaffected parents and sibling • G2003R present in fetus during second maternal pregnancy, reflecting maternal germline polymorphism
JEB-nH, (AR) [4]	• 1 large kindred with four patients homozygous for 4003delTC in COL17A1 resulting in a PTC/non-sense mediated mRNA decay • 1 of these patients (phenotypically identical to her affected siblings) showed focal expression of type XVII collagen in epidermal basement membrane by IF microscopy (Fig. 1.4.2.2-1) • RNA/DNA analyses of revertant cells revealed that the patient was mosaic for a unique second site frame-restoring mutation (4080insGG) on one allele, thereby correcting the reading frame proximal to the PTC. This mosaic partial correction of a germ-line deletion by a second, reading-frame-restoring insert mutation countered mRNA decay and allowed protein production of appropriate immunoreactivity and size that, however, was clinically not effective
JEB-nH GABEB, (AR) [13]	• Mosaic distribution of type XVII collagen positive and negative cells • Uncein concomitantly absent in BP180 negative cells • Cellular mosaicism only observed in biopsies from perilesional skin, in uninvolved skin both closely related BP180 and uncein showed normal continuous pattern

(continued)

Table 1.4.2.2-1 (*Continued*)

EB-(sub-) type, (mode of inheritance), reference	Summary
JEB-nH GABEB, (AR) [9]	• No molecular profiling of COL17A1 • Mosaicism caused by reverse mutation, i.e. mitotic gene conversion of COL17A1 in somatic precursor cell(s) • Patient compound heterozygous for two truncation mutations, R1226X (nonsense) and 1706delA (frameshift) • Reexpression of BP180 in keratinocytes caused by reversion of the mutation on the maternal allele with the normal sequence from the paternal allele • Paternal R1226X remained present in all cell samples • Prevention of blistering was reached although not more than 50% of the basal keratinocytes had reverted

types that are not advantageous for the cell may just get lost [17]. Consequently, it is formally possible that fibroblasts and haematopoietic progenitor cells in a revertant EB variant initially also contain reverse mutations. However, since these cells, in contrast to keratinocytes, do not express basement membrane components such as type XVII collagen, they are not subject to natural selection. Therefore, a revertant mutation in fibroblasts and blood cells, that both are important DNA sources for standard molecular analyses, may get lost over time.

The importance of a distinctive selective pressure in maintaining advantageous genetic reversions may be exemplified by epidermolytic hyperkeratosis, a genodermatosis associated with suprabasal tissue disruption due to mutated keratin 10. Mutant stem cells in the basal layer can escape destruction and thus will lack any stimulation by a "corrective pressure". In other words, since stem cells may be located in the hair follicle, interfollicular epidermis, and sebaceous glands, the spreading of revertant stem cells probably must also include destruction of the deeper-located stem cells. This would favor maintenance of corrected stem cells in most EB variants with a disruption of the basement membrane zone.

In 2005, a study by Pasmooij et al. [16] reported on two compound heterozygous, unrelated probands suffering from autosomal recessive JEB-nH with discrete patches of functionally restored skin. These revertant patches remained stable during life and did not expand. As revertant stem cells thus appeared to have no selection advantage (to spread), the authors concluded that the correcting mutations leading to the healthy skin patches of tens of square centimetres in size occurred during embryogenesis. Genetic investigations determined that multiple distinct somatic reversions of the pathogenic COL17A1 mutations had occurred in these patients. Moreover, both of the inherited mutations, paternal as well as maternal, reverted at least once by different reversion events.

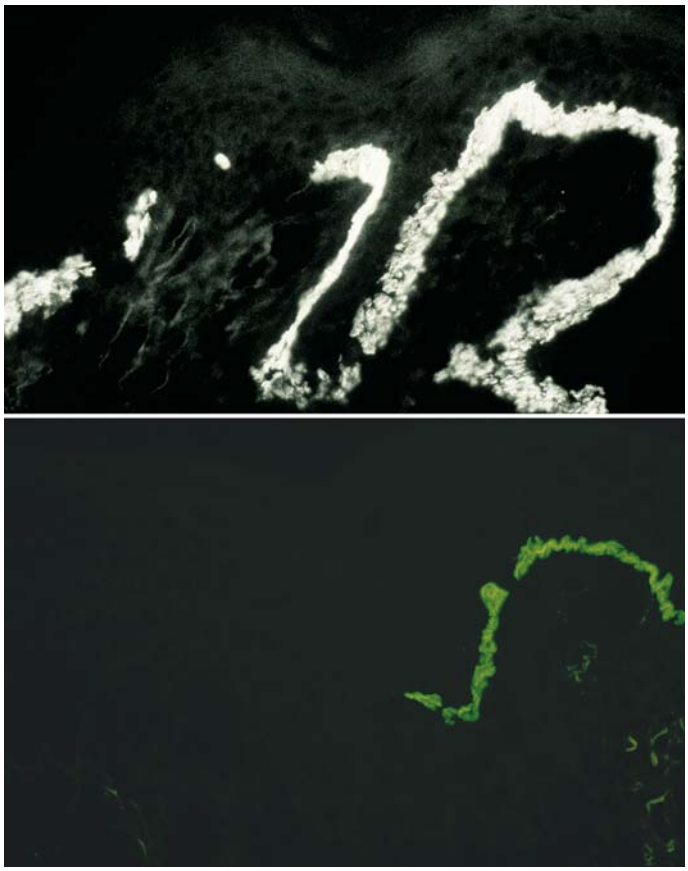

Fig. 1.4.2.2-1. Type XVII collagen was present only at focal sites within the epidermal basement membrane zone of a JEB-nH patient with mosaicism [4]

Recently, Pasmooij et al. [17] observed this phenomenon again in two unrelated probands with JEB-nH. In both, DNA analyses of LAMB3 showed multiple different second-site mutations in revertant keratinocytes that all corrected the same germline mutation c.628G → A;pE210K. c.628G → A led to an amino acid substitution within the N-terminal globular domain of the short arm of the laminin-332 beta3 chain, which has been postulated to be critical in the association of laminin-332 with other structural components of the basement membrane zone. A mutation based polarity change thus could have disturbed protein-protein interactions of laminin-332 leading to reduced epidermal-dermal adhesion in these patients. An even more profound effect, however, was observed on mRNA splicing, as the 628-nucleotide change took place in the consensus sequence at the 5′ splice site, thereby generating 4 additional aberrant transcripts.

While both probands had severe reduction of laminin-332 expression in affected skin, the occurrence of several compensatory somatic second-site mutations phenotypically correlated in patient 1 with skin on the left lower leg that became progressively clinically healthy. This body site of previously affected skin finally showed a normal expression of laminin-332. Patient 2 depicted several revertant patches located on arms, shoulders and chest. Notably, the reverant skin patch of patient 1 increased in size, while patient 2 had not noted extension of the healthy skin area. The difference of phenotypic expression in both probands was contributed to a likely higher level of laminin-332 production in deficient cells of patient 2, because one allele containing a nonsense mutation in patient 1 was not contributing to laminin-332 production. Therefore, expansion of reverted keratinocytes under selective pressure could indeed have been easier in patient 1 than in patient 2, as the deficient cells would be less able to compete.

According to these data, single individuals can undergo multiple reversion events. The authors concluded that the occurrence of multiple correcting mutations within the same patient implies that there is not a single preferred mechanism for the correction of a specific mutation. In addition, *in vivo* revertant mosaicism was thought to be a rare event, but these recent observations indicate that it might occur at a higher frequency than expected. The latter may be explained by an increased overall mutation rate due to accumulation of mutagenic metabolites, irradiation exposure (indicated by signature UV transition mutations C → T and G → A), mutational hotspots or a genetic defect that has an effect on the maintenance of genomic stability (i.e. inactivation of a caretaker gene) [6, 17]. Moreover, as mosaic patterns may be present in clinically unaffected and affected areas, it is likely that reversion may easily be overlooked and may happen even more often. Finally, obviously not all cells needed to be reverted in order to achieve clinical amelioration, as shown by the presence of interruptions in the immunohistochemical staining for type XVII collagen of the dermo-epidermal basement membrane in the biopsy specimens of unaffected JEB-nH patient's skin. This may open the possibility of applying revertant cell therapy in mosaic EB by using autologous naturally corrected keratinocytes, thereby bypassing the recombinant gene correction phase (Chapter 3.6).

Another suggested manifestation of somatic mosaicism represents cutaneous lesions following Blaschko's lines, which are S- or V-shaped, whorled, streaked, linear patterns not following any known nervous, vascular, lymphatic structures nor dermatomes of the skin. They are thought to trace dorso-ventral migratory pathways of the neuroectoderm originating in the neural crest during embryogenesis. For this cutaneous patterning, two types of mosaicism have been described [15]. In type 1, there are linear patterns of abnormal skin surrounded by normal skin. In contrast, type 2 mosaicism manifests in autosomal dominant disorders showing a diffuse cutaneous involvement with their genetic disease (representing a

heterozygous state) but linear patterns of exacerbation of the skin disorder. These areas follow the Blaschko's lines and reflect a homozygous state with loss of heterozygosity or a hemizygous state due to mutational events that lead to loss of normal alleles. For EB, however, such correlations have not yet been proven.

Nevertheless, in a patient with type 2 mosaicism and Hailey-Hailey disease, loss of the paternal allele with duplication of the mutated maternal allele, probably by postzygotic mitotic recombination, was observed [18]. Such postzygotic loss of the corresponding wild-type allele in a heterozygous embryo by somatic crossing-over could give rise to two different daughter cells homozygous for either the mutant or the normal allele (postzygotic/somatic recombination) [8].

Finally, Schuilenga-Hut et al. [19] reported on a patient suffering from recessive EBS who was homozygous for a KRT 14 splice site mutation (1842-2A → C). The germ-line mutation resulted in different aberrant transcripts containing premature termination codons, all leading to truncated K14 proteins. Basal keratinocytes in skin and culture lacked K14−/−. Interestingly, a culture from a new biopsy of affected skin of the K14−/− patient showed spontaneously K14 expressing keratinocytes. Immunohistochemistry and electron microscopy from this skin additionally revealed mosaic expression of K14 and reappearance of intermediate filaments in basal keratinocytes. Immunoblotting furthermore depicted a revertant K14 polypeptide with seemingly normal molecular weight. Analyses of KRT14 mRNA of K14 positive keratinocytes isolated from mosaic skin revealed an additional transcript (1844T → T, 1845delta6) coding for an abnormal K14 polypeptide with an exclusive two residue in-frame deletion and one amino acid change. This partial revertant mosaicism accounted for the antibody staining pattern and reappearance of intermediate filaments, although semifunctionality impaired reversion of the clinical phenotype. A second somatic modulating factor in the genome affecting the post-transcriptional processing of the mutant K14 pre-mRNA ("mRNA editing") was suggested to underlie this phenomenon. mRNA editing can involve enzyme-mediated insertion or deletion of nucleotides or substitutions of nucleotides at the RNA level [11]. The coding sequence is thereby altered from its genomically templated version.

Mosaicism and natural gene therapy

Natural gene therapy comprises the rescue of the disease-causing mutation by means of a naturally occurring secondary genetic phenomenon (Chapter 3.6). This correction may show a mosaic pattern when only subpopulations of cellular elements are affected. If the rescue occurs in cells of the germline carrying a

mutation, the revertant allele may also be transmitted to the offspring, which will not or mildly express the trait.

There are cases of dystrophic EB where an exon, containing a nonsense or frame-shift mutation, is spontaneously, or by activation of a splice enhancer, spliced out of the type VII collagen mRNA [11]. Out-splicing of the mutation-bearing exon restored the reading frame and thus partially rescued transcription, leading to the expression of a truncated protein and consequently milder disease than severe DEB-HS. Similar mRNA rescues have been found in patients with very mild forms of EB with exons containing premature termination codons (PTC) in both genes for laminin beta3, type XVII collagen or integrin beta4 [5, 11]. Four possible models for the skipping of PTC-containing exons have been postulated: (1) nuclear scanning, in which nuclear translation-like machinery removes exons containing a PTC; (2) nonsense-mediated pre-mRNA decay, which is assumed to use a form of transcriptional or splicing feedback which favors the generation of exon-skipped mRNA transcripts; (3) secondary pre-mRNA structure disruption, in which a local secondary RNA structure is required to promote exon inclusion. If a PTC disrupts the hairpin, the exon is skipped, and (4) finally, disruption of an exonic splicing enhancer causes the skipping of the PTC-containing exon. Amelioration of the EB phenotype can thus be achieved on the RNA level by *de novo* exon skipping accompanied by cellular reversion. However, this repair mechanism usually affects the whole body without a mosaic distribution.

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1.4.2.3 Prenatal and preimplantation genetic diagnosis in epidermolysis bullosa

Hiva Fassihi and John McGrath

Prenatal diagnosis: overview

Recent developments in clinical and molecular genetics have played an important role in the diagnosis and management of inherited disorders. Over the last 13 years the molecular abnormalities underlying the different subtypes of epidermolysis bullosa (EB) have been elucidated; however, as yet there are no effective treatments, despite recent progress in gene, protein and cell therapy [33, 37]. Therefore prevention of disease is the main option available for couples at reproductive risk. One of the major transitional benefits of research in inherited skin disorders has been the development of prenatal diagnostic testing, increasing options and choice for families at risk for recurrence of severe forms of disease. The purpose of prenatal diagnosis is the detection or exclusion of an inherited disorder *in utero*.

The techniques have changed over the years, from being heavily reliant on the analysis of fetal skin biopsy samples acquired during the second trimester, to the examination of DNA from first trimester chorionic villus samples. Furthermore, efforts continue to achieve simpler, less invasive methods of prenatal diagnosis that can be performed earlier in pregnancy, without compromising sensitivity of the assay and accuracy of the results.

In the absence of a cure, prenatal testing along with appropriate counselling is an integral part of the management of families at risk of recurrence of severe forms of EB.

Fetal skin biopsy (Fig. 1.4.2.3-1)

There has been considerable progress in testing for severe inherited skin disorders over the last 25 years [2]. Initially, ultrastructural examination of fetal skin biopsies (FSB) was established in a limited number of conditions. The first diagnostic examination of fetal skin for EB was reported in 1980, in a case of Herlitz junctional disease (JEB-H) [45], although this technique had once previously been used in the diagnosis of congenital bullous ichthyosiform erythroderma [23]. These initial biopsies were performed with the aid of a fetoscope to visualise the fetus [46]. This involved the insertion of a fibreoptic endoscope into the uterus, under sedation and local anaesthesia. However, with improvements in sonographic imaging, biopsies of fetal skin are now taken under ultrasound guidance.

Fetal skin biopsy samples obtained during the early 1980s could only be examined by light microscopy (Fig. 1.4.2.3-1A) and transmission electron microscopy [15, 46]. For EB, the diagnosis was made by finding a split at the dermal-epidermal junction by light microscopy, and then the precise level of cleavage was determined by electron microscopy. The introduction of a number of monoclonal and polyclonal antibodies to various basement membrane components during the mid-1980s led to the development of immunohistochemical tests (Fig. 1.4.2.3-1B) to help complement ultrastructural analysis in establishing an accurate diagnosis, especially in cases of EB [28]. LH7.2 monoclonal antibody, which binds type VII collagen, has been used for the rapid prenatal diagnosis of RDEB-HS [28]. Similarly, a number of other monoclonal antibodies may also be applied. These include GB3 (anti-laminin-332) and 19-DEJ-1 (anti-uncein) for JEB-H [20, 29], anti-α 6 and β4 integrin antibodies for junctional EB associated with pyloric atresia [34], anti-bullous pemphigoid antigen 180 (BP180) for JEB-nH [35], and anti-plectin for EB simplex associated with muscular dystrophy [22].

Fetal skin sampling is an invasive procedure with a $\sim$1% rate of fetal loss above the background incidence of spontaneous abortions. Sampling error, inadequacy of samples for analysis and difficulty in interpreting the morphological and immunohistochemical features can pose problems [31, 48]. From a practical perspective, fetal skin biopsies for EB cannot be performed before the 16th week of gestation, and the prospect for a second trimester termination of pregnancy of an affected fetus is often associated with considerable emotional and physical distress for the pregnant mother [6, 39, 42].

Chorionic villus sampling and amniocentesis (Fig. 1.4.2.3-1)

As the molecular basis of the different subtypes of EB has been elucidated, FSBs have gradually been superseded by DNA-based diagnostic screening using fetal DNA from amniotic fluid cells or chorionic villi.

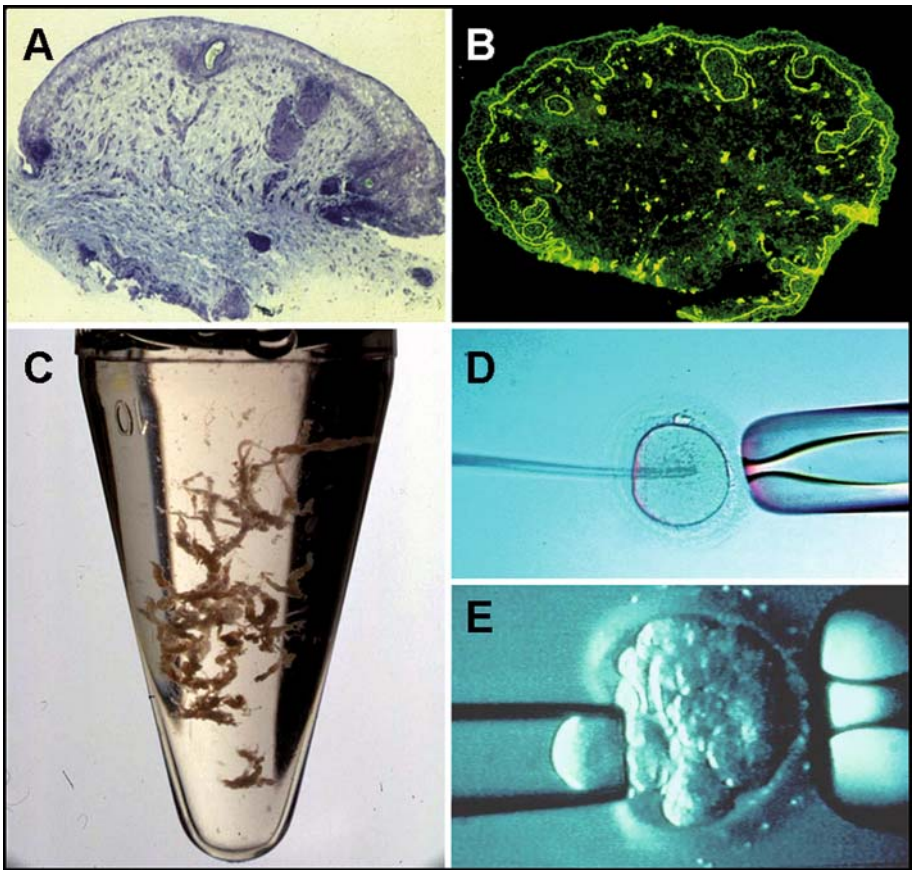

Fig. 1.4.2.3-1. Methods for prenatal testing for EB. **A** FSB taken at 18 weeks' gestation shows no evidence of EB; there is no blistering at the dermal-epidermal junction and the fetus is predicted to be clinically unaffected. **B** FSB stained with anti-collagen IV antibody. There is positive labelling at the dermal-epidermal junction and around dermal blood vessels and developing adnexal structures. Immunofluorescence microscopy can be used for antigen mapping (shows no blister in this case) or specific probes (e.g. to collagen VII or laminin-332) can be used to show normal or reduced immunostaining. **C** CVS taken at 11 weeks' gestation. After cleaning of maternal decidua, chorionic villi represent a source of fetal DNA. These villi can then be digested with proteinase K and the fetal DNA can be extracted and screened for the markers that indicate the presence or absence of EB in a particular family. **D** The first step of PGD testing involves injection of a single spermatozoon into an egg, a process known as intracytoplasmic sperm injection, ICSI. **E** Biopsy of a developing embryo involves breaching the zona pellucida, either by a laser beam or by a jet of acidified Tyrodes solution. Following this, a sampling pipette is introduced and a single nucleated blastomere is removed by suction for analysis. After genetic diagnosis on DNA from the single cell, suitable embryos can be transferred to the uterus on day 4 or day 5 of development

After implantation of the embryo, the chorion, the outermost layer of the embryonic sac, derived from the trophoblast layer of the blastocyst, attaches to the uterine wall. The chorion is lined by microscopic projections referred to as the chorionic villi. These are the fetal components of the placenta, containing the same genetic material as the fetus, and therefore a useful source of fetal DNA. Similarly the cells within the amnionic fluid, surrounding the fetus, are derived from fetal epidermis, as well as the gastrointestinal and genitourinary mucosae. Chorionic villus sampling (CVS) can be performed trans-cervically or trans-abdominally, both under ultrasound guidance. The position of the placenta determines which procedure is used; for example, transabdominally, if the placenta is anteriorly positioned. This is usually performed at 10–12 weeks' gestation. Amniocentesis, the method for obtaining amniotic fluid and its cells, is conducted later, at about 16 weeks' gestation, and therefore CVS is the favoured method for DNA-based prenatal tests in many units [1].

Prior to the development of DNA-based tests, amniocentesis was performed to obtain amniotic cells for morphological, cytogenetic, and biochemical analysis. A number of diseases can be diagnosed *in utero* in this way. These include those exhibiting abnormalities in DNA synthesis and repair such as xeroderma pigmentosum and Cockayne's syndrome [8], and inherited metabolic disorders, through demonstration of the primary protein defect in cultured amniotic fluid cells, such as Fabry's disease [4], and congenital erythropoietic porphyria [12]. In addition, raised maternal serum and amniotic fluid concentrations of α-fetoprotein have been reported in association with fetuses affected by EB simplex and EB with pyloric atresia [13, 52]. However, amniocentesis for all these disorders can not be undertaken before 16 weeks' gestation, and there is often a further delay of up to 4 weeks for cytogenetic or enzyme studies, which could lead to even later termination of an affected pregnancy. Therefore DNA-based tests are now preferred for the prenatal diagnosis of any monogenic inherited disorder, such as EB, where the causative gene is known.

For DNA-based prenatal tests, fetal DNA is extracted from the chorionic villi (Fig. 1.4.2.3-1C) or amniotic cells and analysed for genetic mutations along with samples from the parents and any previous affected siblings, which have previously been screened for the pathogenic mutations. This initial screening is crucial for accurate genetic counseling and in establishing the reliability of the prenatal test as it determines the pattern of inheritance, tracing the transmission of the mutated gene(s) from generation to generation. The possibility of *de novo* mutations, non-paternity and uniparental disomy (Chapter 1.4.2.1) must be excluded before considering the suitability of the prenatal test. The actual analysis of fetal DNA usually can be accomplished within 48 h after its receipt in the laboratory. For CVS, tissue obtained needs to be cleaned under a dissecting microscope to exclude maternal cells, such as decidua or blood, which could contaminate the sample and affect the accuracy of the results. The risk of fetal loss following these procedures is

~0.5–1%, depending on the expertise of the unit conducting the procedures [1]. Initial reports of molecular prenatal testing for junctional and dystrophic EB were published in 1995 [7, 14, 32, 38, 51], and since then hundreds of severe inherited skin disorders have been diagnosed prenatally in couples at reproductive risk [16].

These DNA-based techniques have largely replaced FSB for the prenatal diagnosis of severe inherited skin disorders. However, there is still a role for FSB in prenatal testing, specifically where the causative gene is unknown but prenatal diagnosis in similar cases has been previously shown to be possible, or where the causative gene is known, but mutations or informative markers are unavailable.

Despite the advancement in prenatal diagnostic techniques, reproductive choice and preventative options, for couples at risk of severe inherited skin disorders, are still limited. With all available prenatal tests, the diagnosis can only be made once pregnancy is established, with the need to terminate an affected pregnancy. This is a fundamental issue for many couples at risk. Moreover, some couples will not consider termination of pregnancy because of religious reasons or personal principles. For them other options, such as the use of gametes from donors, adoption or even remaining childless, are preferred.

Preimplantation genetic diagnosis (Fig. 1.4.2.3-1)

Preimplantation genetic diagnosis or PGD is an alternative to conventional DNA-based prenatal tests, for couples at risk of having children with an inherited disease. It is a highly specialised procedure available in a relatively few centres worldwide, involving the testing of cellular material from oocytes or early human embryos, cultured *in vitro*, for specific genetic abnormalities before pregnancy has begun [5, 40, 44]. As such, PGD obviates the need for termination of an affected pregnancy, which can be associated with significant psychological and physical morbidity for couples undergoing conventional prenatal diagnosis.

PGD involves stimulation of the ovaries with exogenous gonadotrophins. When there are appropriate numbers of adequately sized follicles, oocyte maturation is hormonally triggered. The oocytes are then collected by transvaginal ultrasound guided aspiration of the follicular fluid. The individual oocytes are transferred to a suitable culture medium and are fertilized by intracytoplasmic sperm injection (ICSI), a procedure whereby a single spermatozoon is injected directly into a mature oocyte (Fig. 1.4.2.3-1D) [11]. The following day, the embryos are examined for the presence of two pronuclei (the haploid nuclei of the oocyte and the spermatozoa) which indicates successful fertilization. The embryos can then be sampled at various stages of development. A cleavage stage biopsy is the preferred option for many PGD centres and has been used successfully in numerous clinical procedures worldwide [47]. This is performed

at the 8–12 cell stage (about 72 h after fertilization) when the individual cells of the embryo, referred to as blastomeres, are still totipotent [27]. The biopsy procedure involves breaching the zona pellucida, either by a laser beam or by a jet of acidified Tyrodes solution. Following this, a sampling pipette is introduced into the embryo and a single nucleated blastomere is removed by suction for analysis (Fig. 1.4.2.3-1E). After genetic diagnosis on DNA from the single cell, suitable embryos can be transferred to the uterus on day 4 or day 5 of development.

The first successful clinical applications of PGD were performed in 1990 and involved biopsying embryos from couples who were at risk of transmitting two different X-linked disorders (adrenoleukodystrophy and X-linked mental retardation). The cells removed were sex-typed by polymerase chain reaction (PCR) amplification of a Y-chromosome specific repeat sequence and only female embryos were then selected for implantation [25]. For Mendelian disorders, the first live birth following PGD occurred in 1992 in a couple at reproductive risk of cystic fibrosis [26]. Since then, several thousand cycles have been performed worldwide resulting in the birth of hundreds of healthy children [47]. No significant long-term clinical adverse effects have been reported in individuals born following PGD intervention [24]. Until recently, however, there had been no successful cases of PGD for severe inherited skin disorders. Two cases of PGD for Herlitz junctional EB had been described, although pregnancy (beyond initial biochemical tests) was not established in either case [9].

Nonetheless, in 2006 the first case of successful PGD was reported for a severe inherited skin disease, skin fragility-ectodermal dysplasia syndrome [17]. This is a rare autosomal recessive disorder that results from loss-of-function mutations in plakophilin 1 (*PKP1*), a component of desmosome cell–cell junctions. For PGD in this case, the molecular screening involved a nested PCR protocol using DNA from a single cell with primers specific for the *PKP1* mutations in this family [49]. Pregnancy was established and progressed to term with delivery of an unaffected baby girl. Although clinically successful, the laboratory work was very labor-intensive to set up and took 9 months to optimize. For PGD to be more widely clinically applicable, therefore, simpler and more generic protocols are needed.

To develop a more generic test, i.e. a standardized PGD protocol that could be utilized for most couples at risk for a particular disorder, focusing on mutation detection is clearly inappropriate since most mutations are family-specific. Instead, an alternative approach is to assess linkage markers, either microsatellites (variable polymorphic repeats of DNA) or single nucleotide polymorphisms that are close to or within the disease gene locus. Provided that there is no genetic heterogeneity for a particular disease (as is the case for dystrophic EB and the type VII collagen gene, *COL7A1*) or that the candidate gene harboring the mutations in a genetically heterogeneous disorder is known (e.g. mutations in either *LAMA3*, *LAMB3* or *LAMC2*, the three laminin-332 genes, in junctional EB) then PGD by linkage

analysis is, at least on paper, a means to develop a more widely applicable test. Indeed, a protocol for linkage-based PGD for the *COL7A1* gene has been optimized and licensed for use in the UK, although this has yet to be utilized clinically [18].

In designing PGD protocols, however, there are two factors that make the development of clinical tests technically difficult. First, there is only a small amount of template DNA available from a single cell (just ~6 pg) and secondly, because there are only two copies of each chromosome in a single cell, there may be a failure to amplify one or both alleles of interest: when only one allele is amplified this is known as allele drop out or ADO.

One useful approach, therefore, is to try to increase the amount of template DNA by opting for a technique that amplifies the whole genome before any disease markers are assessed. A recently developed and suitable method is that of multiple displacement amplification (MDA) [30]. This is an isothermal whole genome amplification using the bacteriophage ϕ29 DNA polymerase and results in one million-fold amplification, thus increasing the template DNA from a single cell to ~6 μg. Then to counter the risk of PCR failure or allele drop out, a sensible option is to assess more than one gene marker. As such, utilization of multiple polymorphic linkage markers within and flanking the disease gene represents a more robust strategic approach.

PGD using this new approach is now referred to as preimplantation genetic haplotyping (PGH) [43, 44]. PGH represents a major advance in reproductive technology applied to the prevention of inherited diseases. It will reduce the time taken to develop assays for other genetic disorders and will widen the scope and availability of preimplantation genetic testing, making it a reality for many more couples at risk of a variety of severe inherited disorders. Indeed, PGH protocols for the junctional forms of EB have recently been optimized and licensed for clinical use in the UK [19].

The combination of increasing knowledge about the molecular basis of single gene disorders and technological advances in defining genetic markers within single cells is leading to new possibilities for preimplantation testing for dermatological as well as many other inherited diseases. Laboratory protocols are becoming quicker, more reliable and technically easier and therefore counseling of couples at risk for recurrence of a specific disease should include mention of the significant and clinically relevant advances that are occurring in this field.

Non-invasive prenatal diagnosis

The available methods of prenatal testing for EB - FSB, CVS and PGD/PGH – all involve invasive procedures and therefore strategies are also currently being

developed to assess whether it might be possible to undertake prenatal testing by a less invasive approach, perhaps involving a maternal blood test [50]. In the 1960s, it was established that nucleated fetal cells (erythrocytes, lymphocytes or trophoblasts) could be found in the maternal circulation. However, these cells are infrequently found (about one cell per ml of maternal blood) and they may persist for months or years, thus rendering their detection of dubious value for prenatal testing [3]. In 1997, however, it was established that cell-free circulating fetal DNA was present in the maternal circulation [36]. This fetal DNA constitutes ~5% of maternal free DNA and mostly consists of short fragments (80% is <200-basepairs) and is detectable from ~4 week's gestation. Although not currently applicable to prenatal testing for EB, free fetal DNA analysis has been used to test for Rhesus blood group antigen D (i.e. from a RhD positive fetus in the plasma of a RhD negative mother) [21]. In 1997, fetal RNA was also identified in the maternal plasma and a number of specific placental (fetal) transcripts have been characterized [41]. Nevertheless, analysis of fetal DNA or RNA in the maternal circulation is only useful in analysing paternally transmitted mutations or markers – maternally transmitted alleles cannot be distinguished from the mother's own DNA. Currently, to address this, there is considerable interest in establishing epigenetic signatures of fetal DNA (differential DNA methylation status) which will allow for the detection of both maternal and paternal markers [10]. Overall, however, at present there are no maternal blood testing methods currently in use for prenatal testing for EB.

Prenatal diagnosis: clinical practice

From the first prenatal test for EB in 1979 until the present day, many hundreds of fetal skin biopsies and chorionic villus samples from couples at reproductive risk of the severe forms of EB have been analysed worldwide. Our group, the Genetic Skin Disease Group, at St John's Institute of Dermatology in London, has performed prenatal diagnosis in 281 pregnancies at risk of severe inherited skin diseases, using a variety of approaches. Fetal skin biopsies were examined for morphological and, when relevant or feasible, immunohistochemical abnormalities. The DNA-based tests involved screening by nucleotide sequencing, restriction enzyme digests or, in a few cases, by linkage analysis.

Of the 281 tests, 195 were FSB and 86 were CVS, and the majority of these were conducted in couples at reproductive risk of EB (142 and 85 tests, respectively). Of the 195 FSB performed the major indications were EB (a total of 142 cases including 90 junctional and 49 dystrophic), 37 cases of ichthyosis (including 22 tests for harlequin ichthyosis), and 12 cases of oculocutaneous albinism. With improved understanding of the molecular genetics of inherited skin diseases in the early 1990s, the number of FSB tests declined sharply. From 1994 onwards, 86 DNA based prenatal tests were analysed. Of these, 85 were for EB (46 junctional,

39 dystrophic) and one assessment for EEC syndrome (ectrodactyly, ectodermal dysplasia, clefting). All tests provided accurate diagnoses and the fetal loss rate was ~1% for both FSB and CVS [16].

As yet, there have been no successful reported cases of PGD for severe forms of EB. Currently, the Genetic Skin Disease Group at St John's Institute of Dermatology together with the PGD unit at Guy's and St Thomas' NHS Trust are working up couples at reproductive risk of RDEB-HS and JEB-H for new PGD/PGH tests recently licensed in the UK.

Overall, the development of prenatal testing has proved to be of great benefit for individuals or couples at risk of having children with severe inherited skin disorders and, in the absence of a cure, prenatal testing along with appropriate counseling has become an important translational benefit of basic research and an integral part of clinical management.

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1.4.3 Genetic counseling

Johann Bauer

Assembling information

At the moment of referral for genetic counseling, the tone is set that can determine the success or failure of counseling. With the exception of prenatal diagnosis, genetics services are unfamiliar to most families. It may confirm fears when a family already assumes a specific diagnosis, like epidermolysis bullosa. Furthermore, the referral raises fears not only for the index family affected by EB, but also for more distant family members. Thus the basis for future trustful communication is established at this point.

First, the family's understanding of the reason for referral should be determined. What has their physician shared about their concerns? Was the diagnosis EB already mentioned? Unless there is clear evidence for the diagnosis EB at the time of referral, it may be beneficial to leave some hope that the genetic evaluation may reassure them.

Adequate assessment at the time of referral can facilitate judgement of the case. How anxious is the family? If they are panicked about EB or if an urgent medical problem calls for immediate evaluation, a consultation should be arranged as soon as possible. In other instances, postponing an appointment for counseling until results of antigen mapping or mutation analysis on affected family members are obtained may allow for a more efficient visit. Also, issues becoming apparent at the first telephone contact may suggest that the appointment should be scheduled when specific team members (e.g. EB nurse, child psychologist, social worker) will be able to attend.

The primary telephone interaction can provide an opportunity to engage the members of the index family as active participants in the counseling process. A possible pitfall of telephone conversations is, however, that, unless it is clearly explained that genetic counseling will not be provided until the actual visit, the genetic counselor can be talked into providing more information than is appropriate at this point.

At the first visit, it is important to introduce all the people who will be seeing the family and define what the role of each will be in the evaluation. This should be done in any medical consultation, but it is especially important in genetics. Usually families show up uninformed of what will happen. Because of the very private and potentially stigmatising nature of EB, even people who have had a genetics evaluation may be reluctant to discuss this experience with friends, so families rarely know what to expect.

Initial telephone contacts will often take place with just one index family member. The first personal contact is likely to involve additional family members. Asking each about every family member`s understanding of the reason for the referral is necessary to establish compliance and provides an opportunity to look into family dynamics.

Risk evaluation

One of the major purposes obtaining an exact diagnosis of a genetic disease is to determine the genetic risk for further offspring. Families are usually concerned about why EB has occurred, if and in whom it might occur (healthy siblings), and whether more or less severely. In the case of recessive forms of EB for which there is accurate and reliable carrier detection this exercise is quite straightforward. For example, for RDEB the carrier frequency for mutations in the COL7A1 gene in the U.S. population has been calculated at 1: 350 [3]. Also for dominant variants of EB like EBS and the dominantly inherited forms of dystrophic EB, risk assessment is easy (Fig. 1.4.3-1 for prototype pedigrees). For couples with one affected parent, there is a 50% risk of having another child with dominant EB. However, in rare cases the issue is complicated by confounding issues like somatic or germline mosaicism (Chapter 1.4.2.2), late ages of onset [2], uniparental isodisomy (Fig. 1.4.2.1) [4] or new mutations. Furthermore, carrier risks estimated from the pedigree should be modified by taking into account factors like the number of unaffected individuals and the sensitivity of mutation analysis.

The family's perception of risk (rather than the calculated risk) will determine their course of action. Chances are perceived in a binary fashion: either something will happen or it won't. If a member of a family, in which a

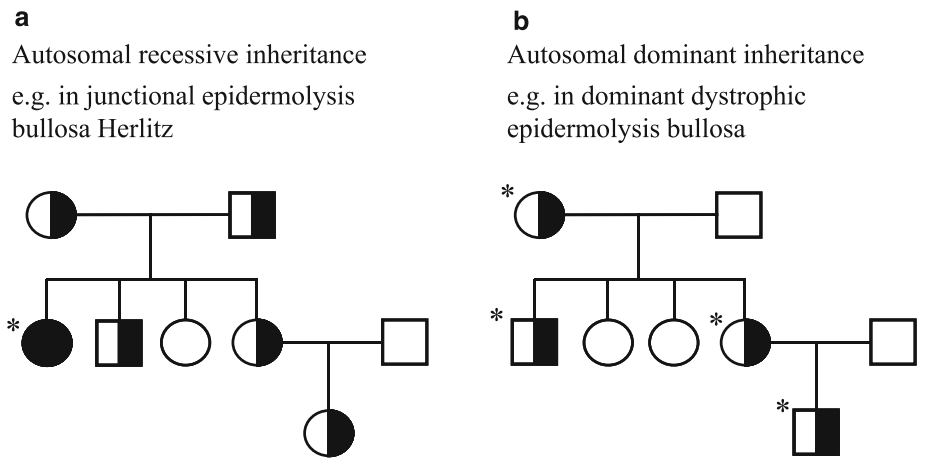

Fig. 1.4.3-1. Prototypic pedigree of (**a**) autosomal recessive and (**b**) autosomal dominant inheritance. *denotes phenotypic disease

child with RDEB, is carrier for the mutation, there is less than 0.1% risk of recurrence (based on US data), if she/he finds a partner outside of the family. However, there is a tendency that these people say "If it happened once, it can happen again." To reassure these patients and to exclude possible fatal counseling errors we perform sequencing of target genes of partners from mutation carriers, who had patients with severe autosomal recessive EB in their family [1].

Risk perceptions are not only influenced by the fact that EB has already occurred in this family, but also by the family's experience with EB and its associated problems. A woman who spent years for caring for a brother finally dying with RDEB after suffering from progressive squamous cell carcinoma will have different feelings about the disease than a woman whose brother was diagnosed having mild DDEB.

Sometimes families come to the appointment with a preformed notion of risk. If the genetic counselor's estimation differs from that, the reactions can be unexpected: "Only 50:50?" or "As high as 0.1%?". In this situation the counselor must not let his own perception of the burden of EB affect how this information is communicated. Therefore, it is prudent to avoid making qualitative statements that the risk is "high" or "low". On the other hand, risk assessment must be made understandable. Percentages and probabilities may be completely incomprehensible to lay people. Sometimes people have a hard time interpreting risk numbers; like 1/400 sounds higher than 1/200 because the denominator is bigger. Also the likelihood of a positive outcome of a pregnancy should be mentioned. For example, "there is a 25% (or a one in four) chance that the

baby will have JEB-Herlitz, but a 75% (or a three in four) chance that the baby will not be affected."

Providing information

Estimating risks and helping our clients to interpret this information is an important task of counseling, but it represents only a small part of the information to be given. Families may be asking about EB that has already occurred in a family member or if EB is dependent on age, ethnicity, a possible teratogen, mutagen exposure, or maternal disease. Usually they inquire about means of diagnosis or carrier detection and the limitations and possible complications of such tests. Furthermore, they will want to understand the genetic basis for EB and to learn who is at risk. They definitely want to know everything about medical management, school progress in childhood, or resources available for financial or social support. Thus the counselor will guide the families by raising issues that are neither medical nor genetic. In many countries these issues are dealt with also by lay support or patient organizations, most notably DEBRA (Dystrophic Epidermolysis Bullosa Research Association), which support clinical services for patients and families, and in some cases may also provide research grants to physicans and scientists.

Explaining genetic aspects on EB (i.e. why EB occurred, its chance to recur, or how a genetic test is done) means that families are exposed to a vast amount of genetic knowledge. Using diagrams and visual aids that utilize common objects can make difficult concepts more understandable. On the other hand, it is important that the "genetics lesson" does not overshadow the actual counseling session.

For the medical terminology used it is advisable to actually write down key words for the clients. Terms that are familiar to the geneticist (like the term "mutation") terrify a patient. Asking clients to summarize what they know about EB from previous sessions is helpful for determining what aspects of the information are helpful.

At the conclusion of the genetic evaluation families receive a letter to summarize data relevant to the diagnosis and to review the information about etiology, pathogenesis, prognosis, or genetic risk that was discussed. This letter gives clients a record of the consultation. Sending a letter also provides another chance to identify possible shortcomings of the interaction. If there is no intention to see the family again, the letter should point out that the family should stay informed of progress relevant to EB via patient groups or online resources. It is important that reliable online sites be suggested to the family, since unfortunately the Internet contains many links, some of which are inaccurate or misleading. The

content of the consultation letters may also provide some medico-legal protection for the geneticist. It confirms in writing what was done and said.

Psychology of counseling

Information about EB is also experienced emotionally and personalized. The client's age and psychological state will determine not only what she/he is ready to hear but how much she/he is willing to accept. Therefore, the psychosocial impact of information must be assessed throughout the counseling process.

Most clients seeking genetic counseling want information about genetic issues, not psychotherapy. On the other hand, many unique psychological challenges are inherent to the process. Clients are asked to make decisions with far- ranging consequences, and have to deal with the thread of EB. It is necessary for the genetic counselor to understand the common emotional reactions to these challenges and sort out normal responses from underlying psychopathology.

Help with decision making

During genetic counseling for EB, clients may need to make decisions about issues as testing options, pregnancy continuation, treatment placement of a severely affected child, reproductive alternatives, and medical or surgical interventions. To do this, they need to understand relevant information also on the emotional level to be able to relate it to their own situation. The counselor describes different alternatives to imagine how each family member would feel with a particular decision. For example, one could discuss all possible alternatives for another pregnancy in a family having had a child suffering from JEB-Herlitz. Here the options range from having preimplantation diagnosis (in certain countries), prenatal diagnosis, adoption or none of all.

A critical situation occurs when different family members, usually the husband and wife, have markedly different opinions, leading to incompatible decisions. For example, one may favors adoption whereas the other one wants prenatal diagnosis. In such a situation the counselor should find out which of the alternatives would be most compatible with what they believe.

Ongoing support

Support from the genetic counselor

Genetic counseling can help clients understand their feelings and what is causing them, providing reassurance for the families. For the counselor it is wise to

prepare the families of responses they are likely to have. This may prevent additional harm that might occur if they were caught unexpectedly. This professional involvement can provide reassurance to the client that he or she is not a "bad person" because of what has occurred. One should remain being involved over time, to support behaviors that increase the clients' self-esteem. This involvement can also help clients discover and cultivate other sources of support that they did not yet know of.

Support groups

DEBRA, the patient organization for EB, is invaluable to the professional who cares for families with EB. DEBRA provides peer support , advocacy, lobbying, public and professional education and support of research. It is of value for families with a child suffering from EB to demonstrate to affected families that they are not alone, and that others have been confronted with the same problems which they are facing. Local groups of DEBRA provide an opportunity for similarly affected individuals or families to become acquainted with others. Activities may range from professionally led group therapy and educational presentations by medical specialists to social or fund-raising events. Some nationally based DEBRA groups also play an important role in providing advice to governments and their health care agencies on the many needs of EB patients and where additional national funding might be best directed. Most DEBRA groups have Internet Web sites that contain useful patient information about the disease spectrum, practical recommendations about basic wound care, calendars of upcoming local events pertinent to patients and their families, summaries of recent clinical and basic research studies, and links to other worthwhile informational resources. Some even provide "chat rooms" for patients and their families. Because families often will have found their way to these sites before their first genetics visit, the counselor should become familiar with the material they contain.

It is helpful to prepare the patient and her/his family for the fact they may encounter other individuals suffering from EB who might have different diagnoses from theirs, or who may have tragically experienced complications that are very atypical or rare. One should also reassure them (for example when a child suffers from EBS) that certain characteristics they may notice in other EB patients (e.g. wheelchair use, skin cancer, finger webbing in DEB patients) are not relevant to their own situation.

Also, it can be overwhelming for parents of a newly diagnosed child with EB to be visited by a support group member whose enthusiasm and positive outlook on EB is not compatible with the shock that the couple was experiencing only recently.

Support groups are valuable resources for patients, and collaboration with them is of great benefit for the professional. Families often view their care provider's involvement as a sign of commitment, and this will increase mutual trust and respect. For the professional, listening to EB-affected individuals and their families talk about challenges and successes with school work, transportation, insurance, interpersonal relationships in daily life will give a wider aspect than the "medical-only" one. The geneticist who has a holistic understanding of EB will be more sensitive to the associated psychological and social issues and, as such, will be better able to assist patients and their families in seeking and receiving the best possible care.

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2. Clinical manifestations and complications

2.1 CUTANEOUS

2.1.1 General cutaneous manifestations

Jo-David Fine

Cutaneous findings in EB

Primary lesions

The hallmark feature of inherited EB is mechanical fragility. Invariably this is associated with the development of erosions. In most forms of EB these erosions are preceded by tense blisters, most of which are filled with clear, colorless exudate. Occasionally these blisters may be hemorrhagic, most notably in the rare Ogna subtype of EBS. In the rather newly described rare EB subtypes characterized by cleavage within the uppermost portion of the epidermis ("suprabasal EBS subtypes"), which include EBS superficialis [3], lethal acantholytic EBS [6], and plakophilin-1 deficiency [7], intact blisters are usually not clinically evident, making diagnosis sometimes confusing on the basis of skin findings alone.

A characteristic feature of the Dowling-Meara subtype of generalized EBS is the presence of vesicles or small blisters in arcuate or "herpetiform" array – hence the name "EB herpetiformis," which was originally used to describe this entity. Of note, this pattern of lesions is best seen when these patients have relatively milder disease activity, and may be less obvious during major clinical flares.

Other *primary* cutaneous findings (Chapter 1.2) that may be seen in inherited EB include milia, dystrophy or absence of nails, alopecia, exuberant granulation tissue, congenital absence of skin, palmoplantar keratoderma, mottled pigmentation, and EB nevi (Chapter 2.1.2).

Milia present as tiny firm white papules, and may arise on normal-appearing skin, in areas preceded by blisters and erosions, or within more

established cutaneous scars. Milia most often accompany DEB and less frequently JEB, but may also occur in EBS, especially in the more severe generalized subtypes.

Nails may become thickened and yellowish, with longitudinal grooving of the nail plate. Abnormal convex curvature may develop in some; in EBS-Ogna, the nails characteristically become markedly curved and deformed, eventually resembling a ram's horns ("onychogryphosis"). With time, many of these dystrophic nails may be shed, especially in JEB and DEB patients, with resultant atrophy, scarring of the nail bed, and anonychia.

Exuberant granulation tissue (EGT), defined as moist, red, friable plaques with a tendency to bleed, are nearly pathognomonic of the Herlitz subtype of JEB. They tend to arise most commonly in bilateral symmetrical periorificial array, at times leading to total occlusion of the nares. Other typically affected body areas include the base of the neck, axillary vaults, proximal nail folds, and the lumbosacral area. Rarely these may also arise within the lumen of the uppermost portion of the throat. Solitary but otherwise similar appearing lesions, however, may instead represent squamous cell carcinomas, suggesting the need for close examination and, when indicated, skin biopsy, especially when lesions like this develop in patients with RDEB.

Alopecia of the scalp, either localized or more diffuse, may be seen in the more generalized EB subtypes, most notably JEB-nHS and RDEB-HS. This may be associated with significant scar formation in some patients.

Congenital absence of skin (CLAS) may be seen in association with inherited EB. This combination has been named Bart's syndrome [1]. Although the original family that was described was later proven to have DDEB, it is now known that Bart's syndrome may also occur in the setting of EBS, JEB, and RDEB. When CLAS arises in association with EB it is characterized by red, angulated or flame-shaped, well demarcated, depressed patches. At birth, some of these areas may appear raw, with superficial erosions overlying them (Fig. 2.1.1-1). These invariably arise on the hands, feet (Fig. 2.1.1-2), wrists, or ankles. Usually unilateral, they rarely may be bilateral or more diffusely distributed.

"Albopapuloid" lesions, which appear as small grouped hypopigmented papules, were once thought to be the hallmark feature of one of the two major subtypes of generalized DDEB, the so-called Pasini subtype. These lesions usually develop over the lower back. This finding is no longer considered to be diagnostically useful, since similar or identical lesions have been seen in other forms of EB. In addition, they are not seen in every affected member of the same family, nor is there evidence that the molecular findings in Pasini DDEB differ from those in DDEB patients lacking albopapuloid lesions (DDEB, Cockayne-

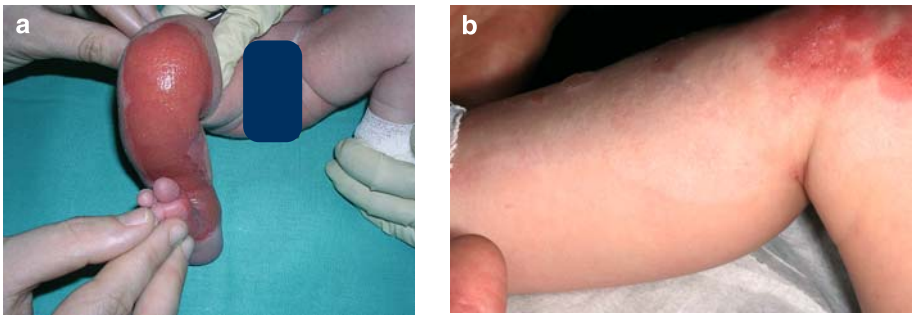

Fig. 2.1.1-1. **a** Congenital absence of skin (CLAS) at birth. **b** Very fine scarring following CLAS; on the knee new lesions of EB

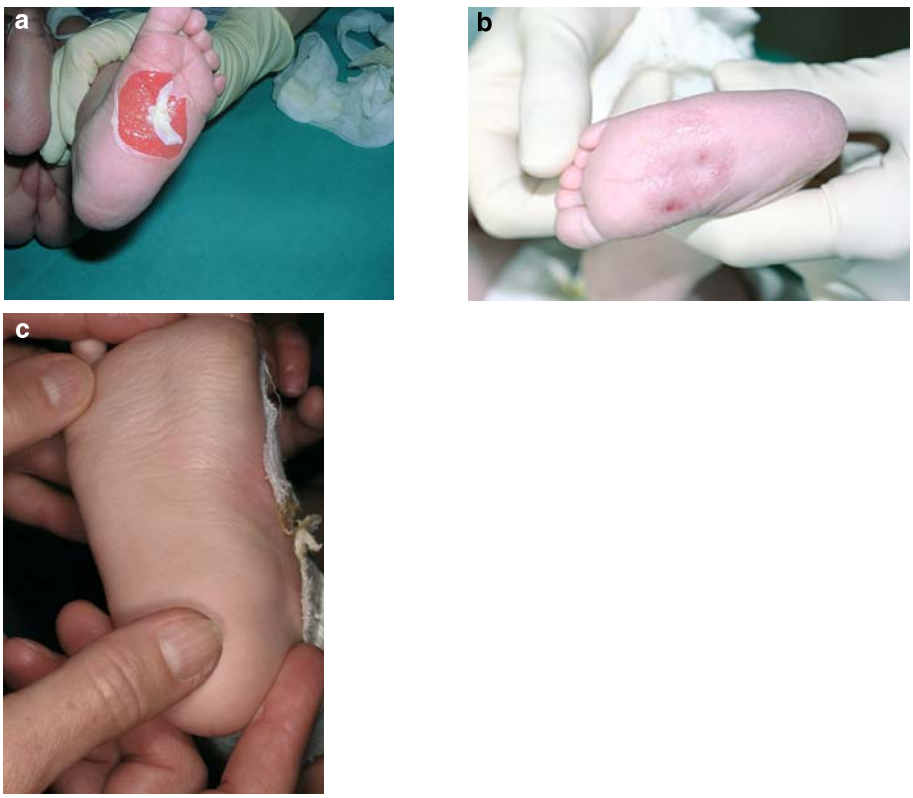

Fig. 2.1.1-2. Planta of patient in Fig 2.1.1-1 at birth **(a)**, after 3 weeks **(b)**, and 1 year **(c)**

Touraine subtype). For these reasons, both Pasini and Cockayne-Touraine eponyms were eliminated from the classification system that is currently being used for the diagnosis of EB.

Keratodermas, characterized as localized or more diffuse callus-like lesions, may develop over time on the palms and soles of some EBS patients. Confluent keratoderma is a typical feature of EBS-DM, but may not be present until late childhood or early adolescence.

Mottled or reticulate hyperpigmentation is the pathognomonic skin feature of a rare EBS subtype descriptively referred to as EBS with mottled pigmentation (EBS-MP). Of note, this particular EBS variant has a very distinctive genotype. These lesions are usually present in early childhood but may become less distinctive or even imperceptible by adult life.

In the originally described Tyrolean kindred of four patients with GABEB (JEB-nH) [5], some also had multiple angiofibromas (especially truncal), extensive sebaceous hyperplasia, and fine stellate scarring (Figs. 2.1.1-3, -4, and -5). In another recently examined patient from Germany who has severe generalized RDEB, multiple angiomas were also observed (Hintner, personal observations, 2008) (Fig. 2.1.1-6).

EB nevi present as oftentimes large patches with irregular borders and variable degrees of brown hyperpigmentation. They are described in much greater detail in Chapter 2.1.2.

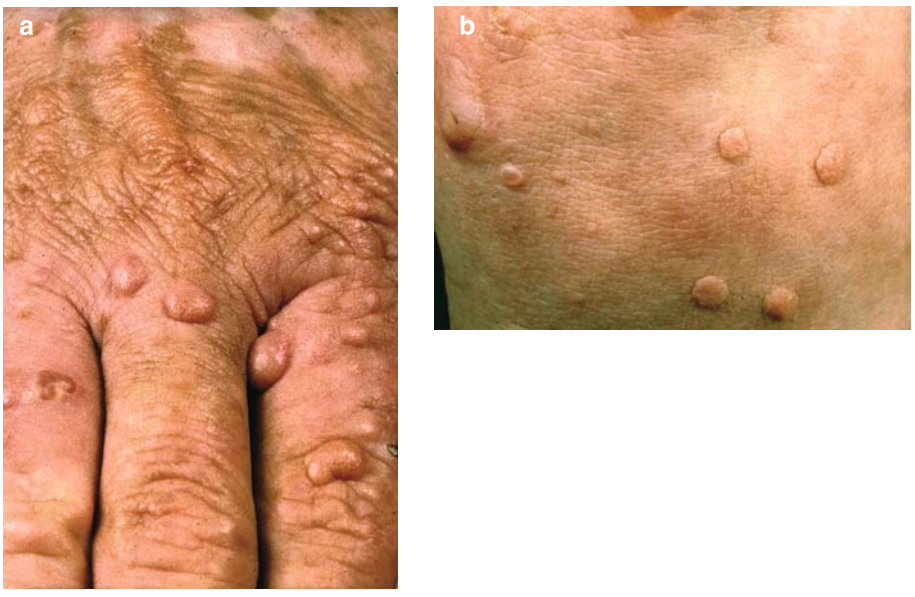

Fig. 2.1.1-3. Multiple angiofibromas in a patient with JEB-nH on the hand **(a)** and leg **(b)**

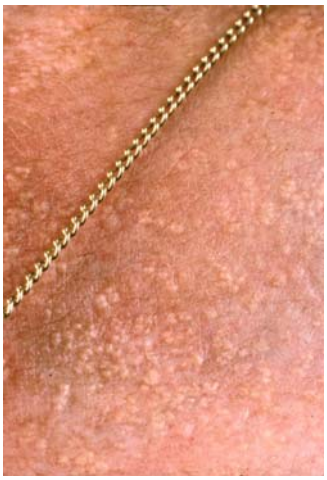

Fig. 2.1.1-4. Sebaceous gland hyperplasia in a patient with JEB-nH

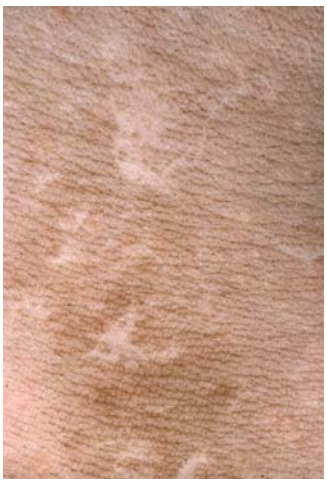

Fig. 2.1.1-5. Stellate scars in a patient with JEB-nH

Secondary lesions

Secondary skin findings in EB include atrophy, scarring, pigmentary abnormalities, webbing and contractures.

Atrophy is seen almost exclusively in JEB and DEB patients. At one time, atrophy was believed to occur in JEB in the absence of preceding scarring, hence the old term “EB atrophicans” for JEB. It is now believed that at least most, if not

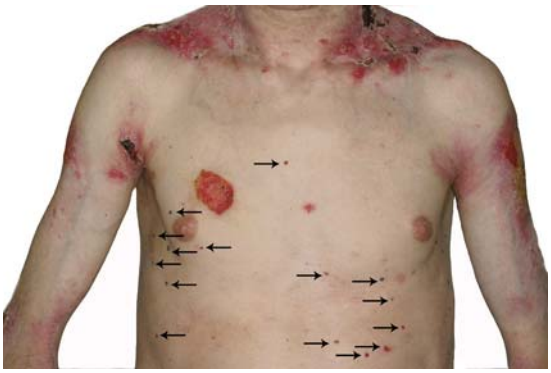

Fig. 2.1.1-6. Multiple angiomas (arrows) in a patient with RDEB-HS

all, of these atrophic areas are preceded by blisters, to include intrauterine ones, and that they are associated with scar formation.

Scarring is a characteristic feature of JEB and DEB, although less frequently, focal scarring may also be observed in the more severe subtypes of EBS. Scarring is most often associated with cutaneous atrophy, although thickened (hypertrophic) scars may also arise in some patients with DEB, especially in those with generalized DDEB.

Dyspigmentation may arise secondary to severe blistering and, at times, scarring, in many EB subtypes. Affected areas may be hyperpigmented, hypopigmented, or depigmented (acquired leukoderma) (Fig. 1.2-7f).

Webbing and contractures are described in Chapter 2.2.5. These are believed to arise secondary to chronic blistering between the fingers and toes, and to reflect intradermal scar formation. It is also possible, though, that microscopic vesiculation, in the absence of overt blisters, may be sufficient to cause early web formation in some patients. Acral webbing is most often seen in DEB, but rarely may also arise in JEB. More central contractures (for example, within the axillary vaults) are commonly seen in JEB-H and RDEB-HS, and develop as a result of repeated blistering, erosions, and scarring of the skin.

Distribution of cutaneous lesions

EB subtypes are most often separated into generalized or localized forms, dependent on the extent of skin involvement. Although a useful prognostic finding by later childhood, it may be difficult to determine during early infancy, when skin fragility is greatest and blistering is usually much more widespread. In

some uncommon or rare EB subtypes, for example RDEB-I and RDEB centripetalis, the more unique distributions of lesions may not become evident until at least one year of life, suggesting instead the presence of a more generalized form of EB. Furthermore, some EB subtypes traditionally considered as localized ones, for example Weber-Cockayne EBS, may instead be associated with severe generalized blistering following major skin trauma.

Diagnostic utility of cutaneous findings in EB

In general, patients lacking scarring, nail dystrophy, atrophy, and milia are most likely to have EBS. Similarly, patients having herpetiform blistering or exuberant granulation tissue most likely represent EBS-DM or JEB-H, respectively. There are several reasons why one must be extremely careful, however, in using these findings for diagnosis in the absence of antigenic, electron microscopic, or molecular confirmation of at least the major type of EB that is present. First, when sensitivity and specificity analyses were performed by the American National EB Registry on 1700 consecutively enrolled patients in this project, no single finding (other than possibly EGT) or any combination of up to three of these findings achieved sensitivity and specificity scores of at least 90% for both parameters, suggesting the fallibility of using even combinations of cutaneous findings as surrogate diagnostic markers for EB [4]. Similar results were noted when a very computer-iterative analytic technique referred to as CART (Classification and Regression Tree analysis) was employed [2]. Second, it is known that some of these cutaneous findings, most notably milia, nail dystrophy, scarring, EGT, and web formation, may not be obvious in earliest infancy when an accurate diagnosis is most often sought [4]. In addition, some findings, such as herpetiform blistering, may be relatively transient or inconstant. Others, such as EGT and keratoderma, may not develop until at least the first year of life or early childhood, respectively. Also, some, to include EGT, may actually spontaneously resolve by mid-adulthood, if these patients survive long enough.

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2.1.2 Epidermolysis bullosa naevi

Christoph M. Lanschuetzer

Epidermolysis bullosa naevi (EB naevi) are large, eruptive, asymmetrical, often irregularly pigmented, highly dynamic melanocytic lesions with sharply demarcated borders that frequently arise in areas of preceding blisters in patients suffering from inherited forms of epidermolysis bullosa (EB) [2, 9] (Fig. 2.1.2-1). Initially, these naevi were considered to be specific for non-Herlitz junctional EB (JEB-nH, previously named generalized atrophic benign epidermolysis bullosa (GABEB) [5, 10]. Today it is well accepted that the appearance of EB naevi is a frequent phenomenon in patients suffering from all forms of recessively inherited EB, and rarely from those transmitted dominantly. These very special pigmented lesions undergo the same fate as most common acquired melanocytic naevi. They gradually appear in the first or second decade of life, begin as flat, dark lesions that grow horizontally, and later while acquiring dermal components and losing pigment, develop papular areas resulting in a shagreen-like appearance of dermal naevi [9] (Fig. 2.1.2-2).

Melanocytic naevi are considered to be benign hamartomatous proliferations of melanocytes. They can be of clinical significance because they are risk markers, simulants, and potential precursors of malignant melanoma [6]. To distinguish benign from malignant lesions, clinical criteria (the ABCD rule) have been established describing asymmetry, border irregularity, color variegation, and diameter larger than 6 mm [8]. EB naevi are highly suspect for cutaneous melanoma if one applies the ABCD rule [2, 13, 14]. Moreover, the continuous (over years!), sometimes explosive growth with the occasional appearance of satellite lesions reinforces the impression of malignancy [13] (Fig. 2.1.2-3). Regular (at least once yearly) controls with repeated biopsies or occasionally complete excisions are often the consequence.

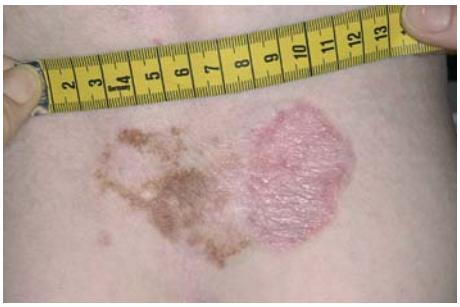

Fig. 2.1.2-1. Typical polycyclic EB naevus with peripherally accentuated pigmentation outlining preceding blisters. The combination with the associated atrophy (also blister shape!) forms the contour of a butterfly, which is coincidently the sign of several DEBRA organizations

Histopathologically, these worrisome moles most often show aspects of common naevus cell naevi or sometimes reveal characteristics mimicking malignancy similar to those seen in persisting naevi/pseudomelanoma, which represent traumatized benign melanocytic lesions (Fig. 2.1.2-4) [2].

With regard to the pathogenesis of EB naevi, it has been shown histopathologically that melanocytes probably deriving from incipient naevi or subclinical nests of naevus cells are found "free-floating" in the blister cavity (produced by the biopsy trauma) in a histological section of an EB naevus of a patient with junctional EB (Fig. 2.1.2-5). In addition, we were able to demonstrate two melanocytic cells in a cytospin specimen prepared of fluid drawn from a blister located on top of a large EB naevus [13]. This has led to the hypothesis that single melanocytes/naevus cells spread "flocking-birds like" within the blister cavity, and after settling down at random, proliferate excessively in the microenvironment

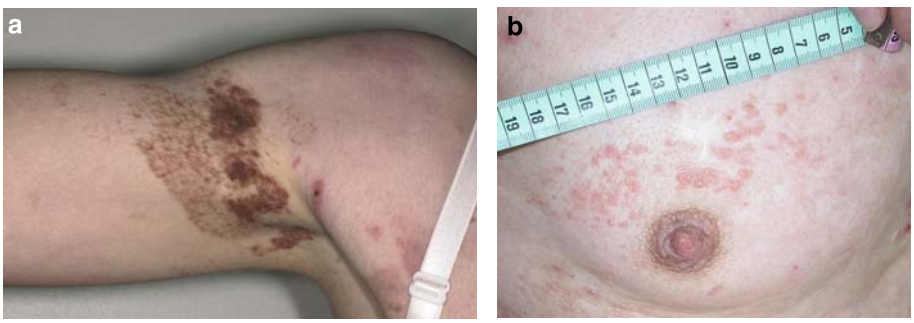

Fig. 2.1.2-2. **a** A prototypical large, polycyclic EB naevus with dark papular and stippled flat portions on the arm of a 14-year old patient with JEB-non-Herlitz. **b** A non-pigmented, "shagreen"-like EB naevus on the chest of a 65-year old patient with JEB non-Herlitz

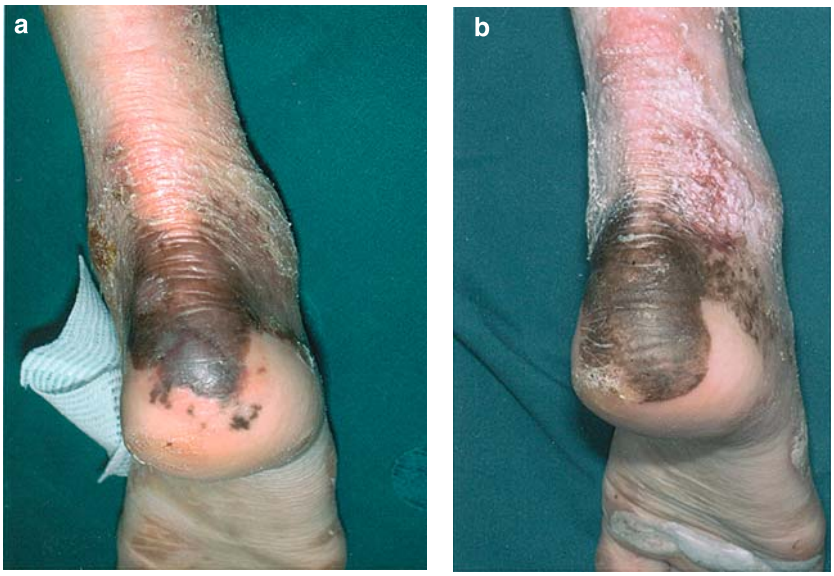

Fig. 2.1.2-3. **a** EB naevus on the heel of a patient with recessive dystrophic EB non-Hallopeau-Siemens shows extensions and satellites reaching to the border of the preceding blister in 2002. Histologically this naevus was diagnosed as a compound acral naevus. **b** The naevus exactly outlines the borders of the preceding blister now homogeneously incorporating the previously seen satellites. A new stippled protrusion is seen extending over the lateral malleolus in 2004

of epidermal regeneration, thus attributing to the malignant aspect with alarming size and satellite lesions [2, 9, 13].

As dermoscopy (epiluminescence microscopy) yields a significant improvement in the differentiation of benign and malignant melanocytic skin lesions [17, 19–21], we investigated 23 EB naevi with a dermoscope to determine if EB naevi could reliably be differentiated from cutaneous melanoma [14]. Clinically, all 23 EB naevi were asymmetrical, most of them in two axes. Many lesions (especially older ones) had random papillomatous portions. On dermoscopic inspection, 21 of 23 pigmented lesions were identified as being of melanocytic origin, thus showing a pigment network or pigmentary dots/globules and streaks. Ten lesions were classified according to the classification of atypical naevi by Hofmann-Wellenhof et al. [11] as reticular (two of 23), reticular-globular (three of 23), reticular-homogeneous (four of 23) and globular-homogeneous (one of 23), while most lesions (13 of 23) were unclassifiable.

With regard to pigmentation, 17 EB naevi showed multifocal hypo- or hyperpigmentation. Regarding the structure, the global patterns of 20 EB naevi were classified as a multicomponent pattern [17] or a three-structure type pattern [3]

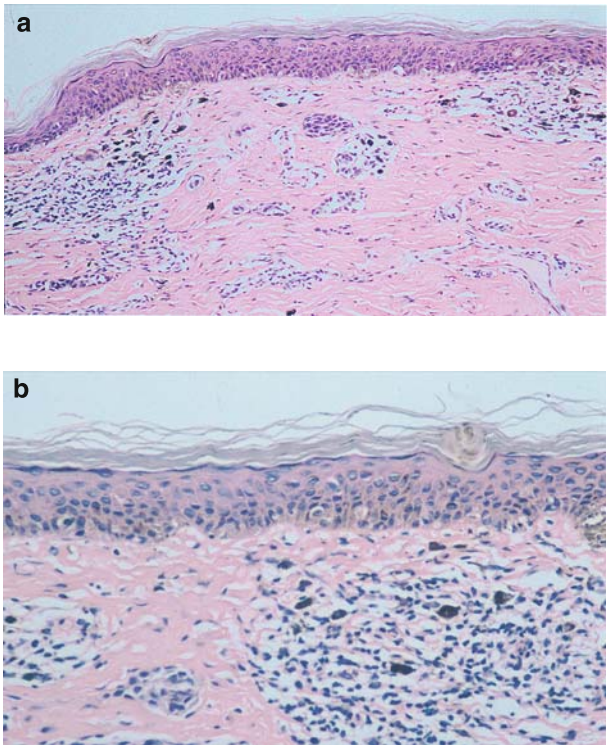

Fig. 2.1.2-4. **a** (200×) and **b** (400×). Typical features of a persisting naevus (pseudomelanoma) with prominent dermal fibrosis, irregular melanocytic nests and single melanocytes with moderate nuclear atypia in the basal layer

reported to be suggestive for malignant melanoma (Fig. 2.1.2-6). An atypical pigment network with irregular meshes and thick lines (17 of 23), irregular dots and globules of different size (16 of 23) haphazardly dispersed, and homogeneous areas (23 of 23) were the predominant structures seen in EB naevi.

Milky red areas, a vascular pattern that is reported to be of special value in the diagnosis of cutaneous melanoma, were present in five lesions. Comma vessels were seen in papillomatous portions of two EB naevi and glomerulus-like clumps of vessels were detected in five lesions. An atypical vascular pattern as defined by the seven-point checklist [1] (i.e. linear, dotted, globular red) was present in only two lesions (Fig. 2.1.2-7).

In addition to these pattern analysis criteria, criteria of the ABCD rule of dermoscopy [16] were applied to all EB naevi. The 23 lesions showed structural asymmetry in two axes; however, an abrupt border cut-off was documented in only three lesions and in one segment, respectively. The colours white (23 of 23),

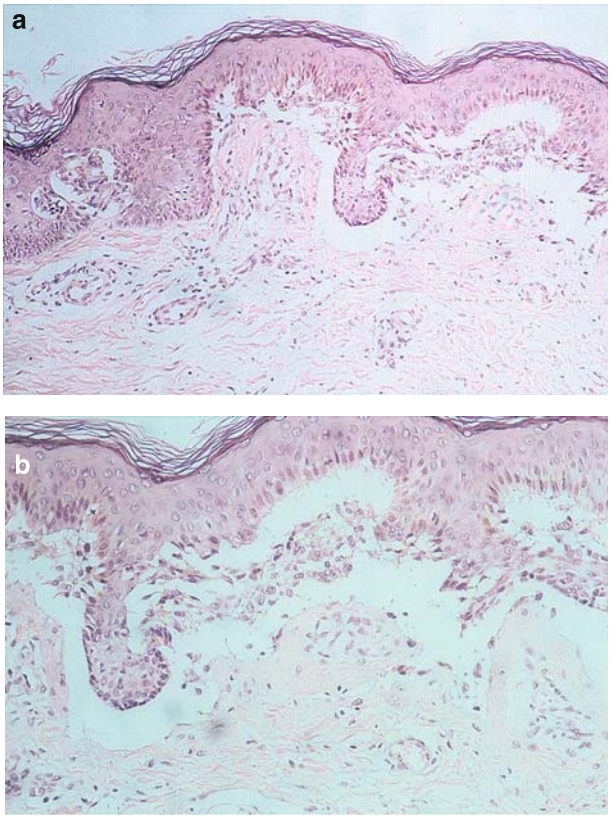

Fig. 2.1.2-5. **a** (200×) and **b** (400×). Dysplastic naevus with unequal nests of melanocytes along the dermoepidermal junction. Nests of melanocytes float free within the subepidermal EB blister

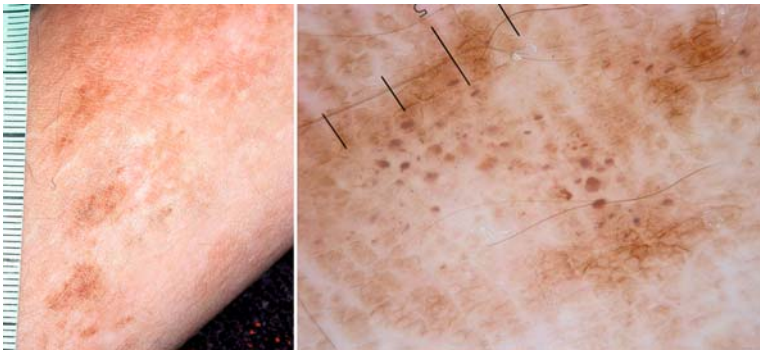

Fig. 2.1.2-6. Dermoscopic evaluation of an EB naevus of a 14 year old patient with JEB non-Herlitz shows a "multicomponent pattern", i.e. irregularly distributed dots, globules, pigment network and structureless areas

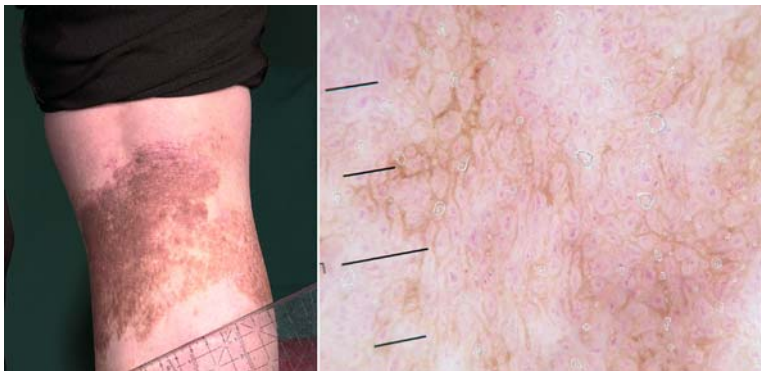

Fig. 2.1.2-7. An atypical vascular pattern is often seen in EB naevi on dermoscopy. For instance, "glomerular clumps" lie within atypical pigment meshes

light brown (23 of 23), dark brown (13 of 23), blue-grey (12 of 23) and red (11 of 23) dominated, while black and blue were almost absent.

The combination of dermoscopic features found in EB naevi resulted in a high total dermoscopy score (TDS) for the ABCD rule of dermoscopy [16] as well as a high score for the seven-point checklist [1]. Thus a TDS >5.45 (ABCD rule of dermoscopy) was achieved in 17 lesions and a score >3 (seven-point checklist) was recognized in 16 lesions, results highly suggestive for melanoma.

We believe that the asymmetry, irregular shape, multifocal hyper- or hypopigmentation and structural variegation of EB naevi revealed by dermoscopy can be attributed to the arbitrary arrangement of proliferating melanocytic clones. Further changes taking place in healing wounds, such as scar formation, disruption of rete ridges and neovascularization enhance the irregular appearance of these moles. Taken together, these repetitive dermoscopic features seen in EB naevi represent a distinctive dermoscopic pattern that cannot, however, always unequivocally differentiate them from cutaneous melanoma.

Like EB naevi, recurrent naevi (pseudomelanoma, persistent naevus), which occur within a few weeks following incomplete surgical excision or trauma of an intradermal naevus, frequently mimic cutaneous melanoma clinically and histologically. These previously traumatized, often stippled moles, which show similarities to EB naevi in their development, also exhibit suspect dermoscopic features. As shown for EB naevi, it was emphasized that they represent an exception to the ABCD rule of dermoscopy [15].

However, a subtle dermoscopic appraisal of EB naevi with the consideration of their pathogenesis will often disclose their benign nature. In the case of an uneven, polymorphous lesion with patchy distribution of pigmentation, i.e. a

lesion classified as a multicomponent pattern or three-structure type *in toto*, assessment of individual nests or clones may help to rule out malignancy, as these areas are often structured and coloured uniformly. Hypopigmented scarred areas can be interpreted as sequelae in traumatized lesions rather than as a consequence of immune-mediated melanoma regression. Multiple papular portions that frequently arise in older EB naevi, and that might clinically be mistaken for the vertical expansion of individual malignant clones in cutaneous melanoma, exhibit a completely benign, globular 'cobblestone pattern' on dermoscopy, which is stereotypical for benign dermal naevi (Unna naevi).

Milky red areas, that are attributed to neovascularization and have been reported to be of exceptional value for the diagnosis of cutaneous melanoma, were also seen in five EB naevi. Exaggerated vascularization following wound healing was also prominent in the form of comma vessels, which are idiosyncratic for benign, dermal naevi, in papillomatous components of EB naevi, further appeasing concerns of malignant transformation. In addition, peculiar, glomerulus-like vascular clumps were located in the centre of the meshes of the pigment network in five EB naevi. Although one is tempted to consider these vascular structures as rampant comma vessels, these vascular clumps were found in papillomatous and flat lesions alike. As this vascular pattern of glomerulus-like clumps has not been observed in any other pigmented lesion so far, we suggest that it is specific for EB naevi, representing exaggerated neovascularization during cutaneous wound repair. The five EB naevi in our series with atypical vascular patterns that were otherwise dermoscopically featureless could not be differentiated from amelanotic melanoma *in vivo*. A histopathological examination is obligatory in these cases, as an atypical vascular pattern is indicative for cutaneous melanoma [4]. Indeed, the diagnostic significance of an atypical vascular pattern in a pigmented lesion was highlighted by choosing it as a major dermoscopic criterion for cutaneous melanoma in the seven-point checklist diagnostic algorithm [1].

Characteristically, strong indicators for invasive cutaneous melanoma (i.e. tumour progression, such as steel-blue areas that are caused by melanin pigment in the papillary dermis, and black dots, which represent melanin in the stratum corneum and which are indicative of a vertical, upward 'pagetoid' spread of melanocytes) were not common features of EB naevi, as melanocytes usually stay in the level of clefting and, unlike melanoma cells, usually do not penetrate into other cutaneous layers. Also, a blue-whitish veil, which correlates with an acanthotic epidermis overlying a melanin containing area, was not found in any of the EB naevi investigated.

Finally, morphological changes of pigmented skin lesions have been identified to be a significant predictor of malignancy [12]. Again, EB naevi are an exception to this rule, as a continuous growth of EB naevi has been documented

over years and cutaneous melanoma was excluded by repeated histological evaluation and serial controls [2].

Although we have not seen malignant transformation of an EB naevus, without complete excison of any lesion in over 25 years of clinical follow-up in several of our patients, the number of EB naevi studied so far is certainly not large enough to completely rule out the possibility that EB naevi might be melanoma precursor lesions. Although there is no evidence for single or persistent traumatic events as causative factors for melanoma formation [15], the state of chronic skin regeneration in patients with EB seems to promote tumour progression, as suggested by the tremendously high incidence of metastasising squamous cell carcinomas in patients with recessive dystrophic EB [18, 22, 23] (Chapter 2.1.3). This must be kept in mind, especially as these patients are reported to be at an elevated risk for the development of malignant melanomas [7]. In particular, three patients with RDEB-HS being followed in the United States by the National EB Registry project did develop malignant melanoma, all arising before age 11, without any family history of malignant melanoma. However, none of these lesions apparently arose within EB naevi, nor did these three patients have evidence of other morphologically atypical pigmented lesions elsewhere.

Therefore, the following regimen seems advisable: regular clinical and dermoscopic follow-up (at least twice a year), and histopathological evaluation if warranted (with punch biopsies of EB naevi showing dermoscopic features of concern as well as dermoscopically featureless lesions). Moreover, dermoscopy is the preferred method for selecting sites for punch biopsies within giant EB naevi. Considering the skin fragility and potentially impaired wound healing in patients with EB, this procedure seems to be favourable as opposed to prophylactic total excision of EB naevi.

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2.1.3 Epidermolysis bullosa and cancer

Jo-David Fine and Christoph M. Lanschuetzer

Cancer is the most severe complication that arises during adulthood in patients with inherited EB. Although extensive literature exists on squamous cell carcinomas, recent epidemiological data have also shed new light on the risk of other tumors, most notably malignant melanoma and basal cell carcinoma, arising in this particular clinical setting.

Squamous cell carcinoma (SCC)

The first report of SCC in association with EB was published in 1913 [36]. Over the next sixty years some 20 additional cases were reported. In 1974 Reed et al. summarized these data, and for the first time clearly demonstrated that RDEB patients were at increased risk for developing skin-derived SCCs, usually during early adulthood [36]. He further provided evidence to suggest that these tumors frequently resulted in death from metastases (having occurred in over two-thirds of all of previously reported patients). Since that seminal paper others have published case reports or small case series confirming this association and suggesting that SCCs also arise in DDEB [8, 40] and JEB [31, 33, 44, 48].

Epidemiology, clinical findings, course and prognosis

When SCCs arise in non-EB patients, they usually present as red scaly nodules, oftentimes with irregular or indistinct borders. They may or not be tender. When SCCs arise in RDEB patients, the morphology is far less obvious (Fig. 2.1.3-1). Oftentimes SCCs appear as non-healing crusted erosions with little

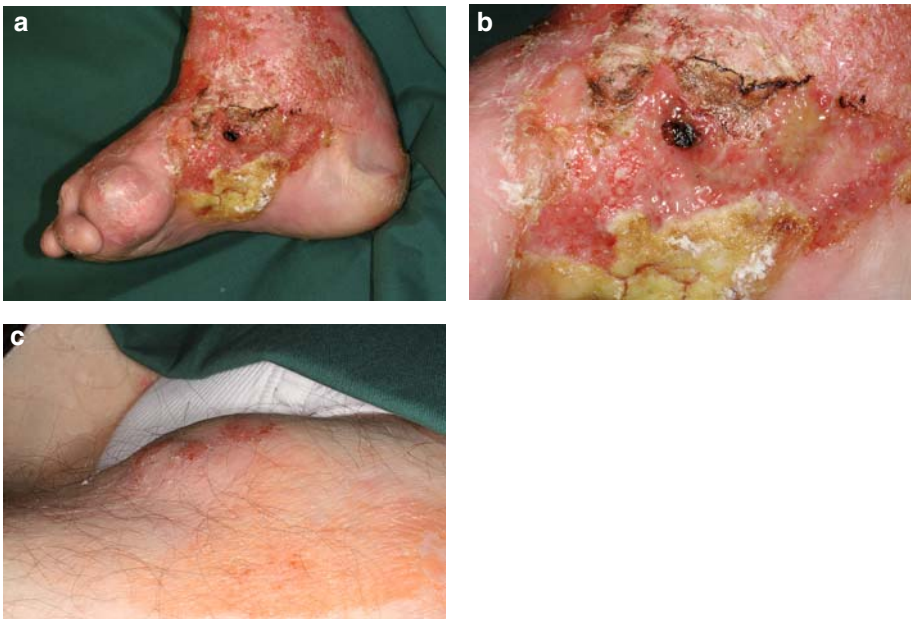

Fig. 2.1.3-1. **a** Squamous cell carcinoma on the edge of a non-healing ulcer on the right foot of a 36 year old patient with recessive dystrophic EB Hallopeau – Siemens. **b** Close up depicting the keratin masses of the tumour. **c** Egg-sized lymph node enlargement on the left thigh probably representing a metastasis of the squamous cell carcinoma on the left foot of the same patient (a biopsy was denied)

or no palpable dermal component, similar to other wounds on their skin. At other times they mimic areas of granulation tissue. Although not seen in every patient, a clinically useful finding is the unsolicited comment by RDEB patients that the lesion has a sensation associated with it that they have not experienced previously within other chronic wounds. Stinging or burning is commonly described by these patients. In such a setting, the diagnosis of SCC must be entertained until histologically disproven.

The most precise estimates of the frequency and risk of SCC in EB have been generated by analyses of the National EB Registry, based on 3280 well characterized patients throughout the United States who were followed from 1986–2006 (Fig. 2.1.3-2) [13]. The overall frequency of SCC was only 2.6%, with the lowest frequencies noted in EBS (range, 0.1–1.0%) and DDEB (0.7%) patients, findings not dissimilar from that expected within the Caucasian American population. In contrast, increased frequencies were noted in JEB-H (4.5%), RDEB-HS (23.0%), RDEB-nHS (9.9%), and RDEB-I (17.7%).

The most common site for SCCs to arise in RDEB was within chronic skin wounds (range, 86.7–100%, dependent on RDEB subtype), with the second

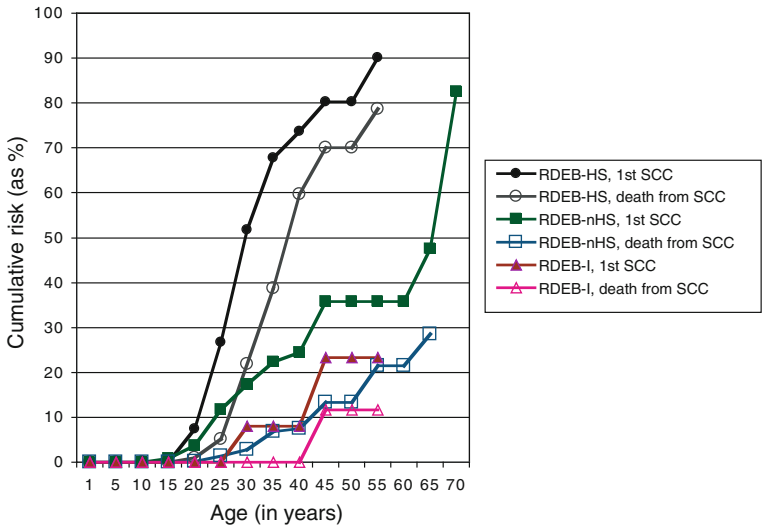

Fig. 2.1.3-2. Cumulative risks of a first SCC and death from any SCC in RDEB patients

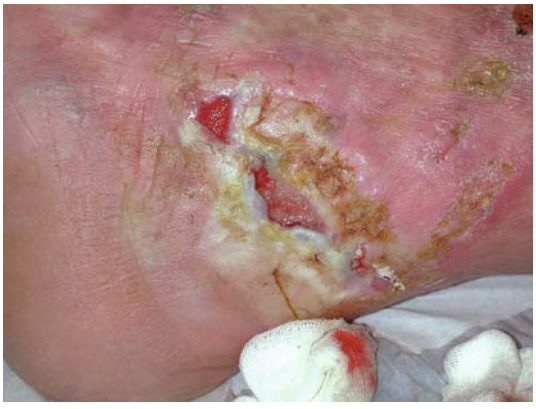

Fig. 2.1.3-3a. Squamous cell carcinoma on the left lateral ankle of a 58 year old patient with junctional EB non Herlitz

being within chronic cutaneous scars (20–26.7%) [13]. With this particular cohort only one SCC (present on the tongue) arose within a non-cutaneous tissue. In contrast, those few SCCs which arose in EBS all were within sun-exposed areas, consistent with where SCCs typically arise in non-EB patients lacking immunosuppression. Although potentially any body site may develop SCC, among RDEB patients these nearly always arose on the extremities, particularly over bony prominences (Fig. 2.1.3-3).

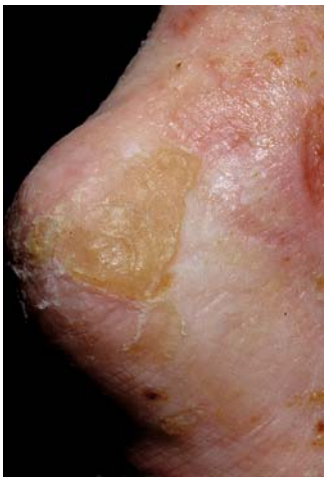

Fig. 2.1.3-3b. Incipient squamous cell carcinoma on the heel of a 26 year old women with recessive dystrophic EB Hallopeau – Siemens. The patient soon thereafter succumbed by the rapidly metastasizing intractable tumour

Another distinctive feature of SCCs, when they arise in RDEB patients, is the number of primary lesions that develop over time. Among NEBR RDEB patients, the median number was about three, but rare patients developed as many as forty [13].

Lifetable analyses have provided estimates of both cumulative and conditional risks for the development of the first SCC among each of the major EB subtypes [13]. No SCCs arose in any EBS subtype, other than EBS-K, before age 65, analogous to that expected within the non-immunosuppressed Caucasian American population. Even among EBS-K, the cumulative risk was only 3.8% by age 40. Similarly, the cumulative risk of SCCs arising in DDEB was low (1.5% by age 45; 4.8% by age 65). Of importance, however, the cumulative risk of SCC in JEB-H patients was 18.2% by age 25, consistent with previous case reports suggesting that SCCs could also arise in higher frequency among those with junctional disease (Fig. 2.1.3-2).

The highest cumulative risk of SCC among EB patients was seen in those with RDEB. Through September 2006 the earliest case among the NEBR population was in a child with RDEB-nHS, resulting in a cumulative risk of 0.8% by age 14 [13]. No SCCs were observed in RDEB-HS before age 15. By age 20, however, the cumulative risk of a first SCC in RDEB-HS was already 7.5%. The risk rapidly increased with increasing age, with 67.8%, 73.4%, 80.2% and 90.1% having experienced at least one SCC by ages 35, 40, 45, and 55. Similar but lower curves were observed with the other two major RDEB subtypes, with the cumulative risks of a first SCC arising by age 25 of 11.8% and 0%, by age 35 of 22.3% and 8.0%,

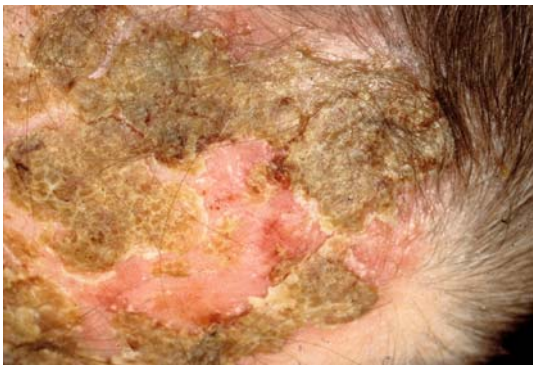

Fig. 2.1.3-4. Thick crusts on the scalp of a 26 year old patient with recessive dystrophic EB Hallopeau – Siemens. The lesions have to be controlled regularly and crusts must be removed to exclude the presence of malignent tumours below. Suspicious epitheliomas have to be biopsied

and by age 50 of 35.8% and 23.3% RDEB-nHS and RDEB-I patients, respectively. The risk of a first SCC in RDEB-nHS rose to 82.5% by age 75.

Conditional risks were also estimated within this cohort [13]. The most common five year interval during which a first SCC arose was 25–30, 40–45, and 40–45 years of life, for patients with RDEB-HS, RDEB-nHS, and RDEB-I. These age interval differences are consistent with the relative extent of chronic skin involvement among the three major RDEB subtypes.

As first suggested by Reed et al. [36], SCCs have a remarkably aggressive course when they arise in EB. When the NEBR cohort was examined, no SCC-related deaths occurred in EBS, DDEB, or JEB [13]. In contrast, however, metastatic SCC was the most common cause of death during adulthood in RDEB patients. The timing of death was earliest in RDEB-HS, followed by RDEB-nHS and RDEB-I. In those RDEB-HS patients who developed at least one SCC, the cumulative risk of death from any SCC was 12.7%, 19.2%, 57.2% and 87.3% by ages 20, 25, 35, and 45, respectively [13]. The cumulative risk of death from any SCC in affected RDEB-nH patients was 11.9%, 30.6%, and 60.0% by ages 25, 35, and 55 and older, respectively, and 50.0% by age 45 in affected patients with RDEB-I. When all patients within each RDEB subtype were considered, not just those known to have had SCC, the cumulative risk of death from SCC at ages 25, 35, 45, and 55 was 5.1%, 38.7%, 70.0%, and 78.7% for RDEB-HS patients, 1.4%, 6.8%, 13.3%, and 21.5% for RDEB-nHS patients, and 0%, 0%, 11.7%, and 11.7% for RDEB-I patients. It is important to emphasize that these conditional risks reflect the risk of death from any skin-derived SCC, despite each preceding primary SCC having been excised with apparently clear surgical margins. This suggests that micrometastases commonly arise early in the course of these

tumors, even though it may take up to 5 years for regional or distant metastases to become clinically evident.

Histology

Given the rather extraordinarily aggressive biological behavior of RDEB-associated SCCs (i.e. tendency to metastasize along the lymphatics [4, 18, 19, 24, 48]), it is surprising that most EB-associated SCCs are histologically very well-differentiated, similar to what is usually found among those SCCs which arise within the skin in non-immunocompromised adults [30]. Similarly, the immunohistochemical status of p53 in stains of tumor sections does not predict tumor aggressiveness in these SCCs [42]. Infrequently, EB-associated SCCs may have the histological patterns attributed to spindle cell and verrucous carcinomas [30], and may even rarely mimic angiosarcomas, later proven instead to be SCCs by the presence of positive anti-keratin immunostaining [29].

Management

Surveillance and prevention

Based on published data from the NEBR [13] and other smaller EB cohorts, the risk of SCC begins during early adolescence. Meticulous surveillance prior to that time appears unnecessary, especially knowing how physically difficult it often is to remove dressings from the entire body of a child. However, once an RDEB or JEB child has reached the age of about 10 or 11, the entire skin surface should be carefully examined, ideally twice yearly. Although it may be very time-consuming, inconvenient, embarrassing, and even physically painful for the patient, it is necessary to remove and then reapply every dressing. This may be made easier on the patient if the dressings are first fully moistened (for example, in a tub filled with water heated to body temperature) and soaked off, and if only small portions of the skin are exposed at any given time, to prevent heat loss or inadvertent bruising or tearing. Exposed areas can then be re-wrapped while others are being carefully examined. Particular attention must be given to any areas about which patients note unusual sensations or grossly atypical wound healing, and deep incisional biopsies should be performed within any suspicious area. Early detection is essential to improving prognosis and may be life saving. As previously noted, though, multiple primary SCCs occur in the majority of RDEB patients and therefore the outcome, despite regularly scheduled examinations, is still variable and often unpredictable.

Assessment is often very difficult, as the appearances of early tumors are similar to the typical chronic ulceration, scarring and crusting widely seen in EB

(Fig. 2.1.3-1). Therefore, there should be a low threshold for biopsing chronic nonhealing ulcers and hyperkeratotic nodules or plaques. Persistent hyperkeratotic crusting should be first removed mechanically so that underlying cutaneous changes can be assessed (Fig. 2.1.3-4). Tumors often start at an ulcer margin and only one portion of the ulcer may undergo malignant change, whilst the rest remains a non-healing inflamed skin defect. Therefore, it is often essential to perform multiple biopsies, even within the same large lesion, to avoid missing an occult malignancy.

In a phase 1 trial, 20 patients with RDEB were treated daily for 8 months with isotretinoin (with a targeted dosage of 0.5 mg/kg/d) to determine whether this drug could be safely administered to patients with RDEB as a possible chemopreventive agent [12]. This drug was chosen since previous literature has suggested that systemic retinoids, even in low doses, may have a chemopreventive effect against tumors of the skin and other organs. Among this cohort, no unusual adverse reactions occurred and it was noted that isotretinoin, at least up to a dosage of 0.5 mg/kg/d, could be safely used in this patient population. Of interest, several patients experienced reduced blistering at lower doses and increased mechanical fragility when they approached the targeted maintenance dosage. Whether systemic isotretinoin has a long-term chemopreventive effect in RDEB patients is yet to be proven. Although there are no published data in EB patients, it is also possible that the chronic use of a systemic COX-2 inhibitor might be chemopreventive, or that the topical application of a retinoid or drugs such as 5-fluorouracil or imiquimod might lead to resolution of hyperkeratotic lesions on RDEB skin, and thus might reduce the risk of those sites progressing into invasive SCC.

Surgical management

At present, experience with surgical management of SCCs in EB is limited to urgent wide local excision down to the fascia and subsequent cover. However, there are no guidelines as to the extent of the excision margins [14]. Most surgical approaches aim for macroscopically clear margins (with subsequent histological confirmation). Because of the difficulty in defining clinical margins on a background of inflammation and scarring, microscopically controlled excision (MOHS technique) has been used in limited numbers of EB patients having SCC to more precisely control resection margins [39, 44], but there are no data confirming that this technique leads to any better outcome, in terms of morbidity or mortality, than conventional wide excision [13]. Laser ablation has also been used and proven to be ineffective [13]. Unfortunately, despite aggressive surgical excision, recurrence and subsequent metastases are commonplace, explaining the high mortality rate predicted by data derived from the NEBR study population [13]. Since most of these SCCs arise on extremities, wide excision oftentimes necessitates the amputation of digits or even entire limbs [3, 4, 45, 52]. Among the

NEBR cohort, surgical amputation of at least a part of a limb was performed, as a result of regional spread of one or more SCCs, in 33.3%, 41.9%, and 21.0% of all patients with JEB, RDEB-HS, and RDEB-nH, respectively [13]. In situations where regional metastases result in large painful non-healing masses, surgical debulkment is recommended, if only for palliation and to improve, even temporarily, quality of life.

Regional lymph node examination should be part of the routine examination of patients at risk for SCC. This is critical because lymphadenopathy may indicate early regional metastasis. If a solitary enlarged lymph node is palpable proximal to a site proven to harbor SCC, then a biopsy (either via fine needle aspiration or complete excision) should be performed [24]. It is important to realize, though, that non-metastatic lymph node enlargement ("dermopathic lymphadenopathy"), due to the presence of chronic skin inflammation or recurrent superficial infections, commonly occurs in EB patients. Although dermopathic lymphadenopathy by itself is a benign process, it may coexist with underlying metastases and obscure their presence.

In many centers, sentinel node biopsy has been a routine part of the evaluation of patients having breast cancer or melanoma. This technique allows selective sampling of those lymph nodes most at risk for harboring early metastases. It is still unproven whether sentinel node biopsy will prove to be of clinical usefulness in the surgical staging of EB patients with SCCs, although detection of early regional metastases might alter the surgical approach to these patients and thereby their longterm prognosis [11]. Use of this technique is also potentially hampered by technical difficulties in getting the injected dye or radioactive probe into draining lymphatics in an RDEB patient with severe cutaneous scarring. There are anecdotal reports, however, to suggest that this approach may be applicable to EB. For example, Yamada et al. [50] reported on a 54-year-old RDEB patient who developed a SCC on the lateral malleolus. Because of palpable lymph nodes in the inguinal as well as in the axillary regions, a sentinel lymph node biopsy was performed in the popliteal area and the groin. Sentinel lymph nodes were tumor-free and the postoperative course was uncomplicated. Although a very promising technique, additional patients will need to be treated in this manner, and much longer follow-up performed, before sentinel node biopsy can be recommended as part of the routine evaluation of SCCs in EB patients.

Diagnostic surgical approaches may need to be individualized, since it is known that some anatomic sites may be more prone to complications than others, especially in an EB patient. Groin dissections were reported in two EB patients with clinically apparent inguinal nodular disease. One of these patients developed a methicillin resistant staphylococcus aureus-positive septic wound infection, while the other intervention was uncomplicated [14]. Complications in groin dissections, such as skin necrosis, infection, seroma formation and

chronic limb edema, occur at rates of between 6% and 65%, even in non-EB individuals [47]. The authors concluded that these potential problems are increased in EB patients due to frequent anemia, poor nutritional status [6], and skin colonising bacteria. For these reasons, they argued that the routine performance of open lymph node biopsies within the groin should be considered contraindicated, and that positive cytology be a prerequisite before total groin dissection is undertaken [14].

Radiotherapy and chemotherapy

It has been suggested that radiotherapy can be beneficial in treating associated lymphadenopathy [30] as well as some primary lesions in patients with EB [36]. Bastin et al. [3] reviewed data on 14 SCCs irradiated in 12 dystrophic EB patients, nine of whom had RDEB (the other three did not have a documented inheritance pattern). Total radiation doses ranged from 12 to 60 Gy (median 45 Gy) delivered over 9 to 47 days. Of those irradiated tumors in which sufficient data on responses were presented, 54% (6/11) showed a partial response with measurable reduction in tumor volume, and 46% (5/11) had no response. However, there was poor wound healing, and skin erosions developed as the total dose approached 45 Gy. Similar experiences have been seen among the NEBR cohort, on whom radiotherapy was implemented in about 17% of all RDEB patients [13]. Based upon these data, it is evident that among dystrophic EB patients there is a narrow therapeutic index between tumor and normal skin radiation tolerance. Unfortunately it is also clear that radiotherapy (to include radium implants) is only partially or temporarily beneficial for the treatment of SCCs, both primary and metastatic, which arise in RDEB patients. Therefore, this technique is not used as primary treatment in this particular disease setting and is now usually employed only for palliation or to reduce pain secondary to the presence of metastases. If the decision is made to use radiotherapy in an EB patient, is is essential that portal fields be carefully designed so as to minimize irradiation of surrounding skin which, by definition, is already extremely fragile, if not also scarred, and therefore prone to acute and chronic radiodermatitis.

Due to its toxicity, systemic chemotherapy is generally avoided in EB patients. There are only three patients with dystrophic EB associated SCCs reported in which the outcome has been documented [22, 49]. Two patients with metastatic SCCs were treated with intravenous cisplatin-based chemotherapy [22]. Both were young female adults with RDEB. One patient was given eight weekly courses of cisplatin (20 mg/m^2) and the other received four courses of cisplatin (85 mg/m^2 on day 1) and fluorouracil (10 mg/kg over 24 h on days 1 to 4) one week every month. There was a partial response in terms of reduction in axillary lymph node enlargement and no significant mucocutaneous,

hematological, gastrointestinal or renal toxicity. One patient chose to discontinue therapy and died 3 1/2 weeks later, whereas the patient who underwent resection of a chest tumor and right axillary lymph nodes was alive 1 year later. No subsequent follow-up data have been reported. Intra-arterial methotrexate has been used in a 47-year-old male with dystrophic EB with metastatic SCC on the right foot [49]. The treatment was effective in reducing tumor and lymph node bulk, but induction of pancytopenia led to sepsis and death. A 24-year-old female with RDEB and metastatic SCC was treated with two cycles of cisplatin and fluorouracil. One cycle involved cisplatin (75 mg/m^2 on day 1) and fluorouracil (187.5 mg/m^2 twice daily on days 1 to 4), followed by a three week intermission. Higher doses were not possible because of diarrhea. A necrotizing left groin SCC in this patient had already been treated with radiotherapy (45 Gy in 20 fractions) that resulted in some reduction in tumor bulk. Although the additional chemotherapy did not produce any significant toxicity, it did not result in any measurable clinical benefit, and the patient died 3 months later [24]. Among all RDEB patients being followed by the NEBR, about 6% underwent chemotherapy during advanced stages of their malignancies; none achieved any lasting benefit [13]. Although evidence is still limited, cisplatin-based chemotherapy may be of some benefit in a palliative setting and does not seem to produce significant myelosuppression or mucocutaneous toxicity, although such an observation will need to be proven by a large randomized clinical trial.

Etiology

Although SCC in RDEB patients is almost invariably associated with rather devastating morbidity and mortality, the cellular pathways that predispose to these SCCs remain undefined, and information on the pathogenesis of RDEB-associated SCCs remains scarce. In particular, it still remains largely unknown whether RDEB-associated tumors represent a distinct entity or share pathways to malignancy with SCCs in the general non-EB population [24, 37].

Clinically the risk of developing SCCs appears to parallel the severity and extent of ulceration and scarring in the skin. Presumably this explains why more patients with RDEB-HS develop SCCs earlier and in greater numbers than do patients with RDEB-nHS and RDEB-I, and why the cumulative risk of death from metastatic SCC is highest in the clinically most severe RDEB subtype. A number of etiological factors has been suggested. For example, it has been proposed that repetitive tissue ulceration leads to a loss of cellular memory and progressively less cellular differentiation, promoting tumor development (Goldberg's tissue stress theory) [15]. EB keratinocytes may be "growth activated" and hence have a premalignant potential [43]. These growth activated keratinocytes could promote tumorigenesis via cytokine stimuli acting through epidermal-dermal signalling. Fibroblast growth factor, which was found to be elevated in the urine of 51% of 39

RDEB patients, has also been implicated to cause increased collagenase activity in RDEB [1] and could be an example of such a cytokine trigger. In addition there may be a diminished immune surveillance of tumor cells in EB. In vitro studies have shown that patients with more severe forms of EB have reduced numbers of non-functional natural killer cells [46] as well as reduced peripheral mononuclear cell cytokine production [7].

Observations made in other diseases might be pertinent to RDEB, as for example, the well known phenomenon of SCCs developing in cicatrizing dermatoses. One study has shown that when comparing 21 burn scar-related SCCs with 50 conventional ultraviolet-induced SCCs, there was a higher incidence of Fas gene mutations in the former (14.3% vs. 0%) [20]. Fas is widely expressed in normal and neoplastic cells, and the Fas–Fas ligand system is a major pathway for the induction of apoptosis [21]. A mutant Fas might protect tumor cells from undergoing apoptosis, allowing malignancy to develop. Although this offers a further possible explanation for scar-associated cutaneous SCCs (with relevance to EB tumors), the Fas gene has not been analysed in EB so far. There may also exist biological markers that reflect an aggressive metastatic potential in SCCs. Shimuzu et al. [41] showed (using immunohistochemistry) that epidermal growth factor receptor (EGFR) was more strongly expressed in lymph node metastases of SCC when compared to primary cutaneous tumors. EGFR was not detectable in normal skin and the assumption was that EGFR might confer metastatic potential in cutaneous SCC. Again this has not been assessed in EB-associated SCC.

RDEB-associated SCCs also express reduced levels of the insulin-like growth factor binding protein IGFBP-3 [25]. In addition, both RDEB associated and sporadic SCCs exhibit enhanced expression of the transmembrane glycoprotein MUC1 [9].

There is currently little information regarding the cell biology of SCC in EB, especially with regard to cancer-related gene expression or mutation analyses. Arbiser et al. [2] observed mutations in p53 and hypermethylation of p16ink4a in 3/8, and 2/8 EB associated SCCs, respectively. The TGC to TAC transition in codon 176 of exon 5 in p53 was observed twice, and one SCC contained an AGG to AAG transition in codon 249 of exon 7. Unlike the p53 mutations observed in ultraviolet light SCCs, these mutations did not affect the codons usually affected by ultraviolet light (“UV signatures”); these mutations are rather signature mutations for oxidative damage to guanine bases in DNA and suggest the possibility that forms of mutagenesis other than direct ultraviolet-induced DNA damage are operative in RDEB-associated SCCs [10].

Hypermethylation of p16ink4a is also a hallmark of reactive oxygen-induced carcinogenesis [16]. Chronic growth factor stimulation is known to cause

prolonged production of reactive oxygen, and cancers associated with reactive oxygen induced carcinogenesis tend to be highly aggressive and exhibit p16ink4a hypermethylation [1, 23, 26].

Recently, Ortiz-Urda et al. [32] proposed a link between certain mutations within the collagen VII gene, COL7A1, that underlie RDEB and the development of SCCs. They reported that RDEB patients are predisposed to developing invasive SCCs only it they express a crucial N-terminal non-collagenous domain (NC1) of the truncated collagen VII molecule which forms the bundles of anchoring fibrils and provides stability to the cutaneous basement membrane zone. Cultured human keratinocytes from RDEB patients xenotransplanted to immunodeficient mice developed invasive SCCs only if the COL7A1 mutation left intact the amino-terminal noncollagenous domain (NC1) of collagen VII, and more specifically the fibronectin III-like repeats within the NC1 domain (FNC1) that bind to laminin-332. In contrast, RDEB keratinocytes carrying mutations that totally abrogated the expression of the NC1 domain did not become cancerous in this specific experimental model. Furthermore, introduction of either the NC1 or FNC1 domains into patient keratinocytes deficient in collagen VII restored a predisposition to tumorigenesis, whereas introduction on NC1 without the fibronectin repeats did not. Also, invasion studies *in vitro* confirmed the *in vivo* findings and further revealed that interaction of FNC1 with laminin-332 was required for the invasive phenotype to develop [53]. Interestingly, antibodies that specifically recognized the FNC1 domain of collagen VII either prevented tumor development or suppressed tumor invasion when administered to mice with SCC tumors caused by Ras/NF-kB transformed keratinocytes from normal individuals [32]. Thus, it is possible that the convergence of two mechanisms contributes to the clinical aggressiveness of SCCs in RDEB patients expressing NC1. Whereas loss of collagen VII dependent cell anchorage enables keratinocyte dissemination in the metastatic process, retention of the NC1 domain enables signal transduction (via PI-3- kinase activity) in support of cell survival during transit to metastatic sites [37].

The hypothesis that NC1 expression is required for SCC development in RDEB patients was recently challenged by the isolation of keratinocytes from two RDEB patients with SCC that lacked procollagen VII expression [35]. Importantly, NC1-dependent tumor-formation has as yet only been described in keratinocytes that were immortalized by the coexpression of Ha-Ras V12 and mutant IκBα to inhibit NF-κB activity. Ha-Ras mutations are relatively infrequent in sporadic SCC [5, 34, 38] and, at least in fibroblasts, Ha-Ras itself has significant effects on the production of extracellular matrix components, including fibronectin and collagen I [28]. This complicates the interpretation of results in Ha-Ras-V12 expressing keratinocytes, as they relate to extracellular matrix composition and tumor formation. Thus, it will be

important to investigate whether NC1 expression is required for tumorigenesis in models of RDEB SCC other than the Ha-Ras V12/IκBα model (discussed in Ref. [37]).

Malignant melanoma

Within the NEBR study population, three children with RDEB-HS, aged 2.8, 6.5, and 12 years old, developed malignant melanoma [13]. This accounted for 2.1% of all RDEB-HS patients, with a cumulative risk for this tumor of 2.5% by age 12. This cumulative risk is higher than that seen in any other genodermatosis other than xeroderma pigmentosum. Of importance, each of these melanomas arose in intact skin rather than in areas of chronically scarred non-healing wounds or within "EB nevi", and each was successfully excised without recurrence.

Basal cell carcinoma (BCC)

Among non-immunocompromised individuals, BCCs are the most common malignancy, and they usually arise in areas of chronic sun exposure. No increased frequency of BCCs was seen within the NEBR cohort, with the exception of EBS-DM, where there was a cumulative risk of 43.6% by age 55 [13], a risk far higher than that observed within the normal aging Caucasian population. None of these BCCs arose within non-healing or chronically scarred skin sites, in contrast to the skin sites most commonly associated with SCCs in RDEB patients, suggesting different underlying pathogenic mechanisms. It is possible that BCCs arise in EBS-DM, the most severe subtype of EBS, as a result of chronic recurrent injury to the basal layer of the epidermis, and that repeated blistering within basal keratinocytes might predispose to pre-malignant transformation of these specific cells, leading to the eventual development of BCCs.

Extracutaneous malignancies

There are only a few published case reports of extracutaneous malignancies arising in EB, such as the occurrence of a squamous cell carcinoma on the hard palate of one patient [27] and in the esophagus of a 57-year-old woman with RDEB-I [17], as well as an osteogenic sarcoma arising within the tibia of another [51]. Altough oral cavity SCCs have been seen infrequently among RDEB patients enrolled in the NEBR, no increased frequency of any other extracutaneous cancers was observed among any EB type or subtype, when compared to the general American population [13].

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2.2.1 Ophthalmologic aspects of epidermolysis bullosa

Josef Stoiber

Epidermolysis bullosa (EB) can involve both the skin of the eyelids and the ocular surface [6]. The latter consists of the cornea, which represents the clear and most anterior part of the eyeball, and the conjunctiva. The cornea acts more or less as our "window" to the world. Marked visual impairment may result from repeated injury to this structure, especially if scarring develops. In the periphery the cornea converts to the conjunctiva, which is a transparent membrane covering the anterior part of the eye and the inner surface of the lids. Both cornea and conjunctiva come with a stratified squamous epithelium and basement membranes similar to that of the skin. Because of this, both structures – in the case of epidermolysis bullosa – are prone to friction-induced injuries resulting in erosions and blisters. The density of sensory nerve fiber endings is exceptionally high within the superficial cornea, exceeding even the tooth cavity. This feature makes the cornea the most sensitive tissue in the human body, acting as protective mechanism against foreign bodies which could threaten the integrity of the ocular surface. As a correlate, EB-associated corneal lesions are usually extremely painful, adding considerably to the overall morbidity of these patients.

Ocular problems in epidermolysis bullosa

Table 2.2.1-1 summarizes the most common ocular findings in EB patients. Both acute and chronic lesions may occur, especially in those having more severe generalized EB subtypes.

Ocular surface and lid abnormalities have been reported for all types of EB. The incidence of ocular involvement in general as well as the rate of the different

Table 2.2.1-1. Ophthalmologic findings in EB

Corneal blisters and erosions
Corneal scarring
Pannus formation
Limbal broadening
Conjunctival blisters and erosions
Symblepharon
Eyelid blisters and scars
Ectropion
Lacrimal duct obstruction

clinical features varies enormously between the different subtypes of EB, as summarized by Fine et al. [5].

Epidermolysis bullosa simplex

Ocular problems are least often seen in patients with EB simplex. If present, the course is usually mild. Corneal involvement, however, to include blisters, erosions, and scarring, has been described in several EB patient cohorts [1, 5, 8]. Within the large patient population of the American National EB Registry (NEBR), the frequencies of blisters and erosions ranged from 0.9 to 6.2%; for corneal scarring, the frequencies were lower (range, 0.3–3.2%). In general, both types of corneal lesions occurred most frequently in generalized EBS. Bullous lesions can be detected in the deep corneal epithelium, associated with an irregular, multilaminar basement membrane. Tong et al. [11] found peripheral corneal vascularisation in 12% of their patients, all of whom suffered from the Dowling-Meara subtype.

Junctional epidermolysis bullosa

About 40% of all JEB patients present with ocular findings. Blistering and scar formation of the lids, with subsequent ectropion formation is frequent in these patients. Tong et al. [11] found subsequent exposure keratitis in 33% and corneal scarring in 20% of the patients. Corneal erosions and blisters are particularly common [10]. Among 230 JEB patients being followed by the NEBR, blisters or erosions occurred in 48% of those having the Herlitz subtype and in 25% of the non-Herlitz JEB patients, with relatively little conjunctival involvement [5].

Dystrophic epidermolysis bullosa

Just a small number of patients with the DDEB develop lesions of the ocular and eylid surfaces, whereas ophthalmic disease is both prevalent and severe in patients with RDEB. Ocular involvement occurs in approximately 50%, with an

increased frequency in the Hallopeau-Siemens subtype. Fine et al. [5] found recurrent corneal erosions in 74%, corneal scarring in 50%, symblepharon formation in 10%, ectropion in 7%, lacrimal duct obstruction in 6% of RDEB patients, and 38% suffered from impaired vision.

Epidermolysis bullosa aquisita

Although ophthalmic manifestations are well described for hereditary EB, very few reports have been published on ocular involvement in EB acquisita [3, 4, 12]. Severe findings, including ectropion, symblepharon formation and severe keratitis leading even to blindness, have been described.

Clinical features

Corneal blisters and erosions

Blisters and erosions of the cornea are the most common ophthalmic manifestations of EB [5]. This is mainly due to the similarities between the epithelium of the skin and that of the cornea. The density of sensible nerve endings is very high in the corneal epithelium; therefore an alteration of its integrity usually results in a very painful condition. Other symptoms are photophobia, tearing, redness of the eye and swelling of the lids. As soon as the roof of the blister ruptures (which mostly happens sooner or later, induced by normal blinking), an area of cornea remains without epithelial coverage (i.e. an erosion). Children are more prone to corneal erosions, the frequency of which usually gradually diminishes until adulthood. Blisters and erosions can occur spontaneously or after minor trauma, as minimal as rubbing of the eye. Even a disruption of the tear film on windy days can trigger the development of corneal blisters. Blistering most often occurs during the morning hours, shortly after waking up. Tear production is significantly reduced during sleep, therefore the inner lid might stick to the epithelial surface of the cornea. If the eyes are abruptly opened in the morning, the epithelial layer might break up in that case, leading to epithelial defects.

Epithelial defects can easily be detected by slit lamp examination. After staining with fluorescein and illumination with blue light, defects of the epithelium appear in green color (Fig. 2.2.1-1). Anaesthetic eye drops can be helpful for making the diagnosis (especially in children). However, they should never be handed out to patients or parents, as their frequent application leads to delayed healing and an increased risk for the development of a corneal ulceration. Eyelids should never be forced open for diagnostic purposes, as this might result in further damage of the lid skin.

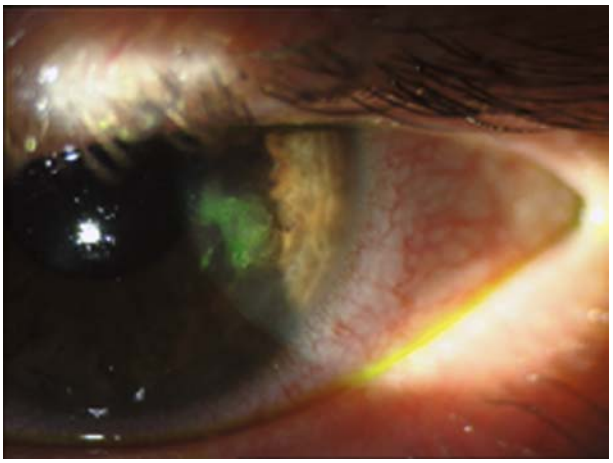

Fig. 2.2.1-1. Corneal erosion, epithelial defect stained with fluorescein

Spontaneous healing of erosions usually occurs within two to three days without scarring. As the intact epithelium also acts as an effective barrier against microorganisms, antibiotic ointment is advisable to prevent infection until the epithelium is completely healed. Blinking results in further friction on the epithelial defect, leading to further pain. Immobilisation of the lid by patching the eye can be helpful for patients. However, tapes for fixation should strictly be avoided in EB patients, as they result in mechanical damage of the skin. However, "pirate-like" bandages could be applied as an alternative. In small children the right position of the pad should be checked regularly, as the pad itself can cause further damage by friction. For pain therapy, oral analgesics may be of great help. Sunglasses can be used to reduce symptoms of photophobia, especially under bright sunlight.

During daytime hours, lubricants, gels or artificial tears will reduce friction of the lids over the eye, especially in patients with tear film problems or an irregular ocular surface. Routine prophylactic application can reduce the incidence of corneal erosions. Most products that are available on the market are effective in lubricating the ocular surface and most contain chemicals to reduce the risk for contamination. Preservatives do not become a problem if drops are administered just a few times a day or for just a short period of time. However, when given more frequently, accumulation of preservatives has negative effects on the tear film and the corneal epithelium. Some patients may also develop sensitivities to preservatives. For that reason, a considerable number of preservative-free artificial tears are now available. The latter come in special containers or single dose units to keep the sterility during time of usage. Artificial tears containing hyaluronic acid (a substance that can be found naturally in the human body, e.g. in the synovial fluid) has been shown to be beneficial and somewhat superior to other ingredients. Besides its anti-inflammatory and excellent lubricating properties, hyaluronic acid has been shown to improve epithelial healing. Vitamin A-containing ointment might

also effectively reduce the incidence for blisters and erosions, by preventing nightly adhesions between inner lid and corneal epithelium. The ointment should be administered just before bedtime. However, during the daytime, ointments should be rather avoided, as they cause blurring of vision.

Corneal scarring

In case of an infection following a corneal erosion, scarring of the corneal tissue can occur. Scars in the corneal periphery usually do not have a negative impact on vision (Fig. 2.2.1-2). However, if the central cornea is affected (Fig. 2.2.1-3), a reduction of visual acuity might be the consequence.

Pannus

"Pannus" is defined as a superficial vascularization of the cornea with infiltration of inflammatory-connective-granulation tissue. A pannus usually results secondary to an infection of the cornea.

Limbal broadening

The corneal periphery next to the limbus (i.e. the junction between cornea and conjunctiva) can show marked clouding. This is called "broad limbus" and occurs mainly in patients with dystrophic EB. It has no impact on the visual acuity of the patient (Fig. 2.2.1-4).

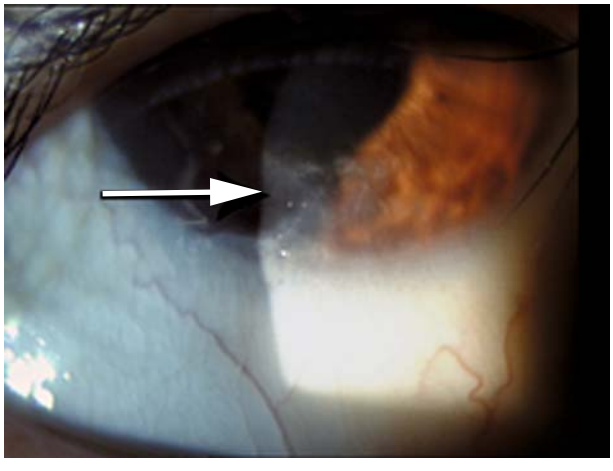

Fig. 2.2.1-2. Scarring (arrow) of corneal periphery in 28 year old woman with RDEB-HS

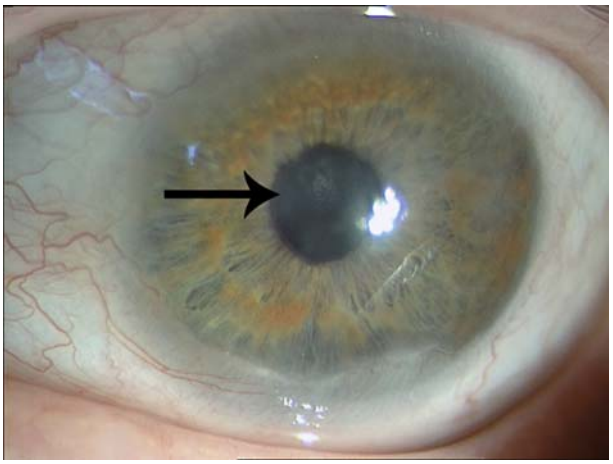

Fig. 2.2.1-3. Central cornea scarring (arrow) with vision impairment in a 34 year old woman with RDEB-HS

Conjunctival blisters and erosions

Blisters and erosions can also develop within the conjunctiva. Compared to the cornea, this condition is usually less painful and the symptoms are milder. The treatment and prophylaxis is basically the same as for corneal erosions.

Symblepharon

Frequent conjunctival injury may result in scarring and development of a symblepharon, where the inside of the lid fuses to the adjacent conjunctiva

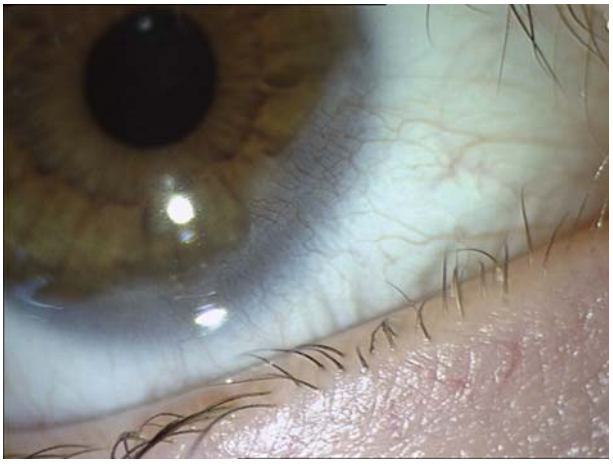

Fig. 2.2.1-4. "Broad limbus" in 4 year old boy with RDEB-nHS

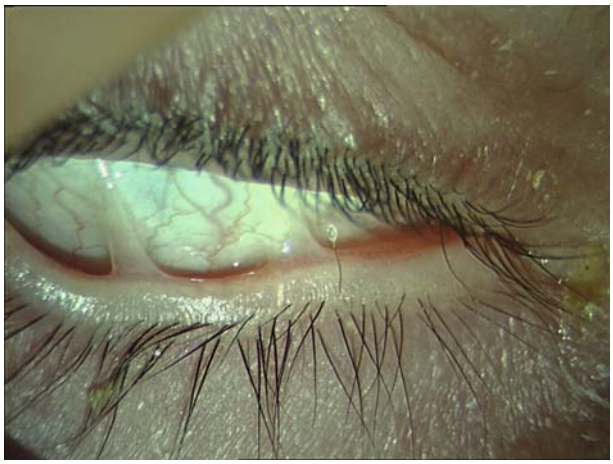

Fig. 2.2.1-5. Symblepharon, 10 year old girl with RDEB-HS

(Fig. 2.2.1-5), or even the cornea (Fig. 2.2.1-6). This most often occurs in patients affected by RDEB (approximately in 10%) and JEB (∼5%) [5]. The condition can be treated conservatively with artificial tears in the beginning. However, adhesions tend to progress in the long term. If the bulbus becomes immobile or visual acuity decreases due to involvement of the cornea, surgical treatment should be considered and offered to the patients or parents. Treatment of choice is symblepharon lysis with/without a lamellar keratectomy (in case of corneal opacities). To reduce the risk for recurrence, amniotic membrane

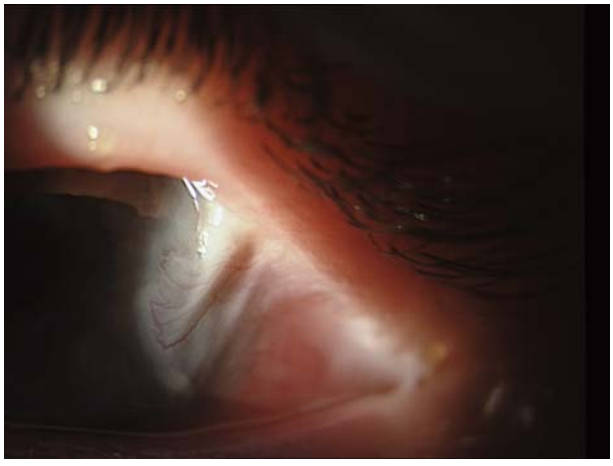

Fig. 2.2.1-6. Massive symblepharon in 7 year old girl with RDEB-HS

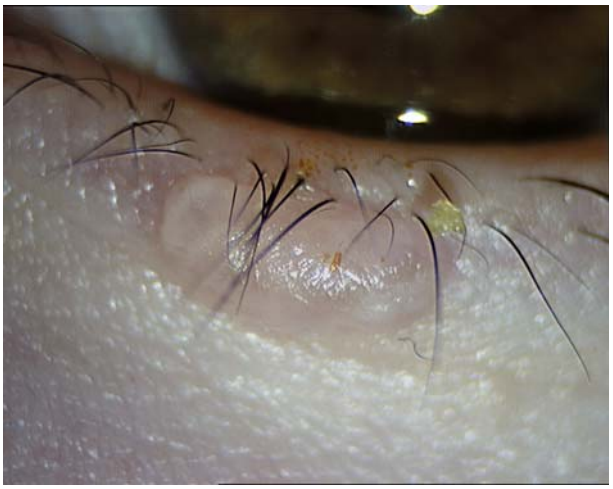

Fig. 2.2.1-7. Blistering of the lower lid in a 12 year old girl with JEB

can be used to replace the damaged surfaces and for reconstruction of the conjunctival sac [2, 7].

Eyelid blisters

As on any other area of the body in an EB patient, the skin of the eyelid can be affected by blistering (Fig. 2.2.1-7). Especially in children, this is often a result of rubbing the eyes (mostly when they are tired). Blisters are treated in the same way as elsewhere. However, due to the difficult location, dressing of these lesions remains a problem.

Ectropion

Recurrent lid blistering can eventually result in scarring. This can lead to a distortion and eversion of the lid edges, leading to an incomplete closure (Fig. 2.2.1-8). The eyelids can remain open in such a way while sleeping ("lagophthalmos"). Consequently, abnormal lid function often exacerbates the already existing ocular surface problems. Ointment should be applied for the night to prevent drying of the eyes. In severe cases, corrective eyelid surgery can be considered.

Lacrimal duct obstruction

Scarring of the eyelid can also affect the upper and lower lacrimal punctum (Fig. 2.2.1-9), leading to obstruction of the lacrimal ducts. As a consequence, tears cannot be drained through the nasolacrimal duct. Patients complain of excessive

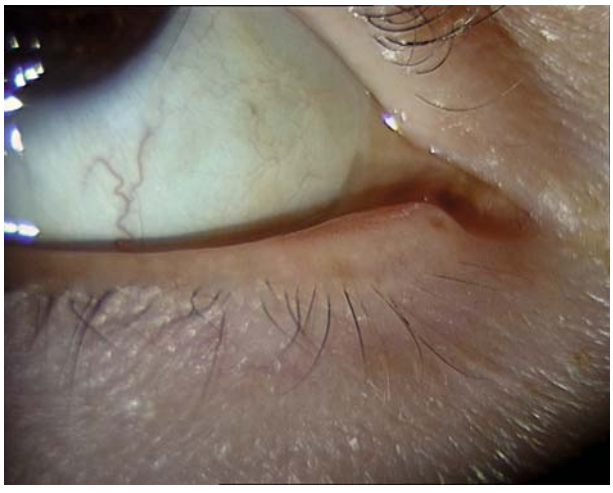

Fig. 2.2.1-8. Mild ectropion formation of lower lid and eversion of the lacrimal punctum in a 28 year old woman with RDEB-HS

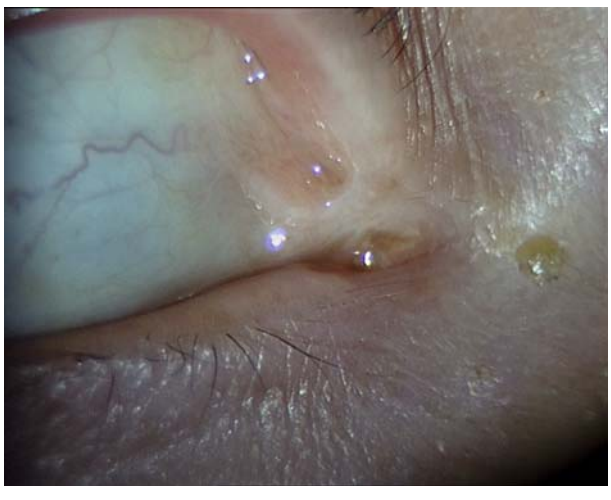

Fig. 2.2.1-9. Symblepharon and lacrimal duct obstruction in a 28 year old woman with RDEB-HS

tearing ("epiphora"). Surgical treatment is difficult, as there is a high risk for recurrence.

Tear film

Tear film quality was found to be partly reduced in some EB patients, especially in the Hallopeau-Siemens subtype [9]. This further aggravates the problems of the

ocular surface. Application of preservative-free lubricants, vitamin A ointment or autologous serum eye drops were found to be beneficial.

Refractive errors

Refractive errors, such as myopia (near sightedness), hyperopia (far sightedness) or astigmatism are more likely coincidental rather than truly caused by EB. However, wearing glasses can cause skin problems by friction behind the ears and on the bridge of the nose. Both frame and lenses should be as light as possible, to reduce the pressure on the nose bridge. A smaller diameter of the lenses should always be favored, as this reduces the weight of the spectacles significantly. Padding the frame over the ears and nose reduces the risk for blistering in those areas. For small children, saddle-shaped spectacle bridges made of silicone appear superior to conventional nose pads, as they spread the pressure on a larger area (Fig. 2.2.1-10).

Soft contact lenses may be used by EB patients. Besides the refractive effect, soft contacts may also serve as protective covering and can be used as an alternative to a patch in case of an epithelial defect. If contact lenses are considered, they should always be fitted and supervised by an ophthalmologist.

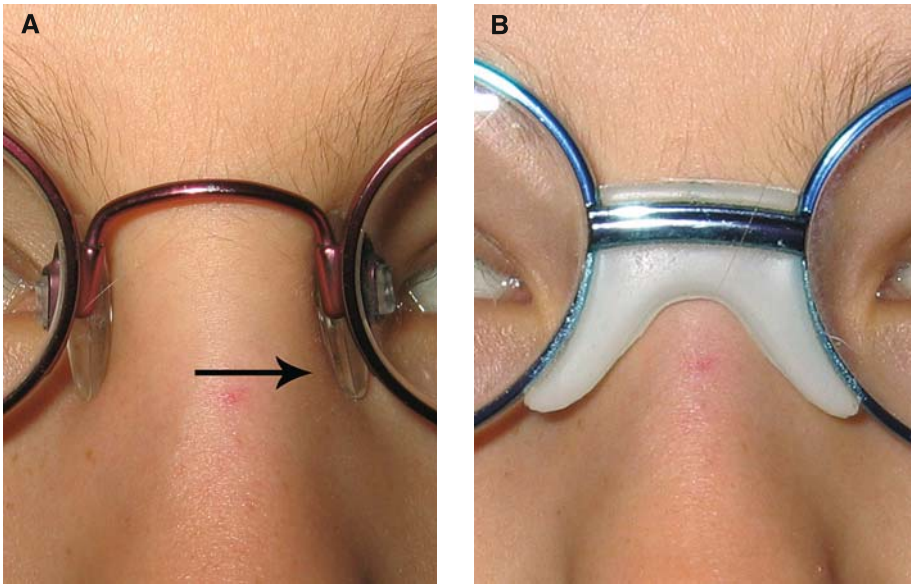

Fig. 2.2.1-10. A and **B.** Rubbing on the bridge of the nose should be avoided. Saddle-shaped spectacle bridges made of silicone appear superior to conventional nose pads, as they spread the pressure over a larger area

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2.2.2 Ear, nose and throat complications

Martin Laimer

Ear, nose and throat (ENT) complications, including upper airway involvement even with potentially life-threatening outcome, have repeatedly been documented in the literature for most EB subtypes [3]. Unfortunately, these data are based on limited case reports and case series, as well as anecdotal experiences of a small number of experts, thus on a type of information that may be subject to both under- and overreporting. It may therefore partly reflect a recall bias, as a select group of patients was studied primarily because of the presence of one or more clinical manifestations of particular interest to the authors. In addition, the small number of patients involved provides little if any confidence in the clinical relevance or statistical significance of the observations noted. Thus, the relative frequency of occurrence of many of the currently reported ENT findings cannot be reliably extrapolated to the general EB population. Furthermore, data are also lacking on the frequency with which each of the ENT symptoms occurs in different subtypes of EB as well as on quantification of lifetime risk.

In an attempt to address these issues, Fine [3] and Hore [6] recently published their cumulative experience on ENT problems in EB patients. Fine's observations are based on 16 years of cross-sectional and longitudinal studies of the U.S. National Epidermolysis Bullosa Registry (NEBR), the world's largest cohort of well-characterized EB patients, whose demographics have been shown to closely mirror that of the entire American population, as well that of EB patient cohorts elsewhere in the world. Hore et al. performed a retrospective case note review of EB patients referred to the otolaryngology department at Great Ormond Street Hospital for Children in London (UK), a tertiary paediatric hospital that is linked to a national EB service dedicated to managing children with this genodermatosis. The intention of both groups of authors was to provide epidemiological

data on the risk of selected extracutaneous ENT complications, that would greatly influence the prediction of which patients are at highest risk for distinctive outcomes, the intensity of surveillance needed, and the timing of medical and surgical interventions if indicated [3, 6].

Their analyes now make an epidemiological approach possible to correlate and stratify disease specific ENT problems in patients with EB for the first time.

Upper respiratory tract

Inspection of the upper respiratory tract of affected patients via endoscopy or indirect mirror technique or also during post-mortem examination has revealed a variety of EB manifestations during the last decades. Signs and symptoms include weak or hoarse cry, dysphonia, inspiratory stridor, soft tissue edema, vesiculation or blistering including all tracheolaryngeal structures, ulcerations, and thickening and scarring of the true and false vocal cords. In addition, lumenal web formation, false membranes, distortion of other soft tissue structures, exuberant granulation tissue, submucosal cyst formation secondary to obstruction of seromucinous glands by squamous metaplasia, and most importantly, clinically significant upper airway stenosis or stricture have been found (as summarized in [3]).

According to the studies by Fine and Hore [3, 6] the laryngeal complications can be predicted according to the type of EB. EB simplex Weber-Cockayne (EBS-WC) and EBS-Köbner (EBS-K) do not specifically affect the larynx/pharynx. EBS-Dowling-Meara (EBS-DM), in which there is more severe, generalised herpetiform blistering, was associated with hoarseness and weak cry in about 7% of all patients [3]. In this variant, inspiratory stridor occurred at a frequency of 3.51% [3]. In EBS with muscular dystrophy (EBS-MD), stridor due to supraglottic scarring with consecutive requirement of tracheostomy was reported [6]. Notably, plectin has a wide tissue distribution and also localizes to airway epithelia. Patients with this rare subtype of EB should therefore be referred to an ENT specialist early at the onset of first respiratory symptoms and managed jointly by regular visits [6].

Dystrophic EB occasionally involves the larynx but complications appear to be unusual. Chronic hoarseness was reported only in 1.6%, 5.8%, and 3.8% of DDEB, RDEB-HS, and RDEB-nHS patients, respectively [3]. However, sporadic instances of stridor caused by glottis and supraglottic stenosis leading to tracheostomy were reported [6].

In contrast, upper respiratory tract involvement is a frequent phenomenon in both subtypes of junctional EB. The most common complication are chronic

hoarseness or a weak cry, seen in nearly 50% and one-third of all patients with JEB-Herlitz (JEB-H) and JEB-non Herlitz (JEB-nH), respectively [3]. Slightly lower frequencies have been reported for inspiratory stridor (up to 44.19% in JEB-H) [3]. Known end-stage sequelae of severe junctional EB (JEB) like laryngeal webs, stenosis or airway obstruction occur in 12.37% and 26.83% of all JEB-nH and JEB-H patients, respectively [3].

In order to provide insight into either the most likely time of occurrence or the cumulative risk of the potentially fatal laryngeal complications over time, Fine et al. performed lifetable analyses on the entire study population of the NEBR [3]. Based on their results, the predicted cumulative risk of laryngeal stenosis, stricture, or complete airway obstruction in JEB-H is about 13% by as early as age 1, plateauing at a risk of nearly 40% by age 6. Similarly, the predicted risk is 8.3% by age 1 and 12.75% at or after the age of 9 in JEB-nH children. Laryngeal stenosis or stricture may also very rarely occur by mid-childhood in unclassified EBS and RDEB-HS patients (0.43% and 0.81%, respectively, by age 8). By age 30 nearly 5% of all RDEB-HS patients will have also developed this complication. However, the latter coincides with the age period during which RDEB-HS patients commonly have developed metastatic cutaneous squamous cell carcinoma, making it difficult to differentiate whether this reported airway narrowing reflects blister formation within the larynx and its sequelae or compression by metastatic tumor masses [3]. Patients with DDEB (0.89% on or after age 40) are at only very minimal risk for this complication, and no such risk occurs in EBS-WC or EBS-K [3]. Although upper respiratory tree stenosis has been reported in EBS-DM patients by others [1, 7, 9, 11], this phenomenon has not yet been observed among the NEBR population, presumably a reflection of the rarity of this EBS subtype and the infrequency of this complication arising in those particular patients.

Considering these results, clinically significant tracheolaryngeal disease activity does indeed have its onset at a very early age, particularly in patients with JEB, being present in a significant minority of patients as early as age one. Early surveillance is thus important since tracheolaryngeal disease activity in EB has been associated with death, the result of sudden and complete airway obstruction. The risk of laryngeal stenosis or stricture plateaus for both major JEB subtypes by mid-childhood, obviating the need for continued close surveillance after about the age from 6 to 9 years [3]. It is unlikely that disease activity within the upper airway ceases at this point in time. Rather, it is more likely that airway obstructive signs and symptoms become clinically undetectable beyond that point in time, as a result of age related increase in lumenal diameter of the airways.

Therapeutic interventions in the case of upper airway involvement must be appropriate, efficient and, at a relevant risk level, also preventive. Mild stridor

can be managed with dexamethasone, adrenalin nebulisers and humidified oxygen (as summarized in [3]). If symptoms worsen, tracheostomy is indicated. Microlaryngoscopy and bronchoscopy including the use of laser and sharp dissection (e.g. in case of additional anatomic defects such as interarytenoid scars, anterior commissure and glottic webs) may represent additive approaches to re-establish airway. The supplemental use of topical mitomycin C, an alkylating agent that inhibits DNA and protein synthesis, applied at microlaryngoscopy to granulation tissue at new developing glottic stenoses, is a promising development [6].

Retrospective analyses of the study population by Fine et al. [3] revealed that within the two EB subtypes known to be at highest risk for laryngeal stenosis or obstruction, 24.44% and 6.74% of all JEB-H and JEB-nH patients, respectively, underwent tracheostomy. Only small numbers of EB patients also had a tonsillectomy and adenoidectomy. No obvious correlation was seen between the frequency with which these latter two procedures were performed and the relative severity of EB present. On the basis of limited reports regarding severe upper airway affection, some experts have recommended early elective tracheostomy placement in those infants with JEB who are documented to have involvement of the tracheolaryngeal region, especially since sudden, fatal occlusion of the airways has repeatedly occurred in the setting of JEB, even in some children who previously had only minimal symptomatology. Such lethal complications are presumably due to acute obstruction of the uppermost airway, via sudden lumenal occlusion by blisters or diffuse edema of usually supraglottic tissues, or as a result of progressive pulmonary compromise due to the development of severe stricture or progressive scar formation around and above the level of the true vocal cords [3]. Other reported occlusive causes include cyst formation in the aryepiglottic folds or the presence of large, intralumenal accumulations of exuberant granulation tissue following blistering on the edges of the laryngeal cords [3, 6]. Early decannulation might thus be avoided, given the potential for chronic disease activity and the possible need for re-tracheostomy at a later time [3].

Others have argued against tracheostomy, given the limited overall number of published cases, concerns over technical issues related to performing such a procedure in small infants, and the absence of published data documenting the longterm survival of more than only a few EB patients who were treated in this manner and were later successfully decannulated (as summarized in [3]). Moreover, some affected EB patients have still died, despite successful tracheostomy, most probably due to progressive compromise of other portions of the upper airway.

However, since it is now possible to prevent death of at least a substantial subset of JEB infants and children from other causes, most notably septicemia, several experts argue that the possibility of preventing airway obstruction in

these patients seems to outweigh any concerns over surgical risks from the performance of elective tracheostomy [3]. Further advances in pre-, peri- and postinterventional anaesthetic care additionally favour the perspective that tracheostomy may prove to be a sustainable preventative intervention to save lives [6, 10].

Ear and nose diseases

Otitis media is a common phenomenon in all EB subtypes (range, 9.33–23.53%) [3], however without an obvious correlation between relative disease severity and the frequency with which this particular disorder occurs. Thus, it is comprehensible that this ENT pathology is not considered at present as being specifically related to EB. In contrast, a higher frequency of chronic otitis externa with up to 9.5% occurs in the three EB subtypes, JEB-H, RDEB-HS, and RDEB inversa [3], of which all are known to have the highest risk of severe involvement within other epithelial-lined tissues directly adjacent to the skin. Thereby, the colonization and infection of a barrier-impaired cutis may represent the source for bacteria.

According to the experiences of Fine and Hore, stenosis of the external auditory canal involving both the cartilaginous and bony parts is an infrequent phenomenon, ranging among NEBR participants from 0.2% in EBS to 2.1% in RDEB [5, 8]. Among RDEB patients, the frequency is 10.0% for RDEB inversa and 2.0% and 1.8% for RDEB-HS and RDEB-nHS, respectively.

Hearing loss (of unspecified etiology) was reported in only a minority of patients within all but one major EB subtype, suggesting that this latter complaint is unrelated to the underlying blistering disease, although one previously case report had conductive hearing loss attributed to stricture of the external auditory canal [4]. In contrast, Fine et al. [3]reported that nearly one-quarter of all RDEB inversa patients had experienced some type of hearing impairment. However, the authors noted that this frequency may have been artificially high, as there were so few patients within this rare patient sub-population included [3]. Therefore, this ENT pathology is not considered at present as being specifically related to EB [6].

No clinically significant nasal complications were reported for any EB subtype by Fine and Hore. Of note, there was also no increased frequency of atopy (asthma, hayfever, eczema) found in any of the major EB types [3].

Therapeutic options regarding ear involvement in EB are largely symptomatic [8]. In otitis externa regular suction does not prevent short term recurrences [6]. In otitis media, it may be necessary to apply grommets [6]. Symptomatic

treatment includes cleaning with sterile normal saline solution and for repeated episodes eardrops with antiseptic and antibacterial agents. An overall aim should be a limited use of antibiotics (either topical or systemically given) to avoid resistance as that may pose a lifelong problem in EB [6]. Systemic drugs that have been used with variable results include phenytoin, vitamin E, minocycline, cyclosporine, and retinoic acid. Scar excision, bony canal enlargement, and split-thickness skin graftings have been reported in patients with strictures of the external auditory meatus. Moreover, allogeneic keratinocytes and cultured keratinocytes have been used with variable results [2, 12].

In case of profound sensorineural hearing loss, cochlear implantation is indicated as an approach with clear benefit lacking considerable intraoperative or postsurgical complications [6].

Nasal crusting and intrinsic rhinitis (REBD) – the latter is not considered as being specifically related to EB – can be efficiently treated with topical steroids and a salt-water based nasal spray [6].

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2.2.3 Intraoral disease

Martin Laimer and Elke Nischler

Depending on the specific EB subtypes, variable involvement of soft and hard tissues of the oral cavity may be seen. The kinds and severity of intraoral clinical manifestations vary considerably across the EB spectrum, although oral tissue fragility and blistering are seen in all forms of EB, even those subtypes, like EBS-WC, which have been traditionally viewed as extremely localized ones lacking any significant extracutaneous involvement. Some intraoral findings are limited primarily to distinctive subtypes, such as the severe scarring of soft tissues which occurs typically in RDEB-HS, RDEB inversa, and to a lesser extent also JEB-H, or enamel hypoplasia, a characteristic feature of all subtypes of JEB. These complications cause considerable challenges for both prophylactic and therapeutic approaches, as well as psychological strain. One major problem in assessing the spectrum of EB-associated intraoral disease is the limited information provided by older literature. The rarity of this disease makes it difficult to perform large-scale studies and to evaluate the benefits and risks of certain treatment or management procedures, when data on such potentially promising interventions are based solely on observations from isolated case reports or small case series. In an attempt to more scientifically address these concerns, beginning in the mid-1980s Wright et al. performed clinical and laboratory examinations on a large number of consecutively enrolled patients in the American National EB Registry (NEBR), using methodical, standardized techniques. As a result, his and his collaborators' collective published work has helped greatly in better defining the true clinical spectrum of oral disease in inherited EB [33, 35, 36] (Table 2.2.3-1). This chapter will therefore rely heavily on Wright's clinical observations, placed into context with more recent findings on the impact of EB-specific molecular alterations on the formation and integrity of teeth, dental support structures and oral mucosa.

Table 2.2.3-1. Frequency in % of selected intraoral finding or symptoms, by major type of EB, at the time of first presentation to the NEBR [36]

Sign or symptom	EBS	JEB	DDEB	RDEB	DEB of unknown subtype
Microstomia	0.2	1.2	1.5	44.6	12.9
Ankyloglossia	0.1	2.4	0.7	51.1	14.6
Gingival blisters or erosions	14.6	58.3	37.3	77.5	55.3
Abnormal appearing teeth (abnormal enamel, dysplastic teeth)	3.0	38.7	5.1	22.0	9.0
Excessive caries	12.2	28.3	20.1	37.3	18.8
Premature loss of teeth	8.1	21.6	14.9	34.2	13.8

Dental disease

Determination of dental EB phenotypes commences with the rather complex sequence of tooth development (Fig. 2.2.3-1). During tooth histomorphogenesis and cytodifferentiation, the basement membrane is thought to be important for coordinated, sequential, reciprocal and inductive epithelio-mesenchymal interactions of cell-matrix and cell-cell components within the dental primordium. The latter are expressed in a temporospatially orchestrated manner resulting in the highly complex amelodentinal junction [10]. If in EB basement membrane zone (BMZ) defects obviously result in skin and oral mucosa fragility, it may be expected that the basement membrane involved in tooth development and function (containing identical structure proteins and in consequence the respective defect of the individual component) is also affected. This would provide a base to explain disease-typical dental hard tissue manifestations.

Intact cell-cell and cell-matrix interactions are important for the differentiation process in dental histomorphogenesis

Remodeling of dental tissue during development is accompanied by differential mitotic activity and spatially specified apoptosis, as well as differential cell adhesion and cell migration. These procedures are believed to depend on the temporospatially coordinated, specific expression patterns of matrix structure proteins, cell adhesion receptors, and proteolytic enzymes that degrade the extracellular matrix (ECM). This affects the organization of the cytoskeleton, and the segregation, migration and cytological differentiation of cells. This evolving process influences to a variable extent cohesion of dental epithelial cells and plasticity during tooth morphogenesis, as well as stablization at later stages of epithelial histogenesis [5, 12, 13, 27, 37]. A comprehensive registry covering the chronological, dynamic expression profile of BMZ components during human odontogenesis is accessible via the internet operated by the University of Helsinki, Developmental Biology Research Program [http://bite-it.Helsinki.fi].

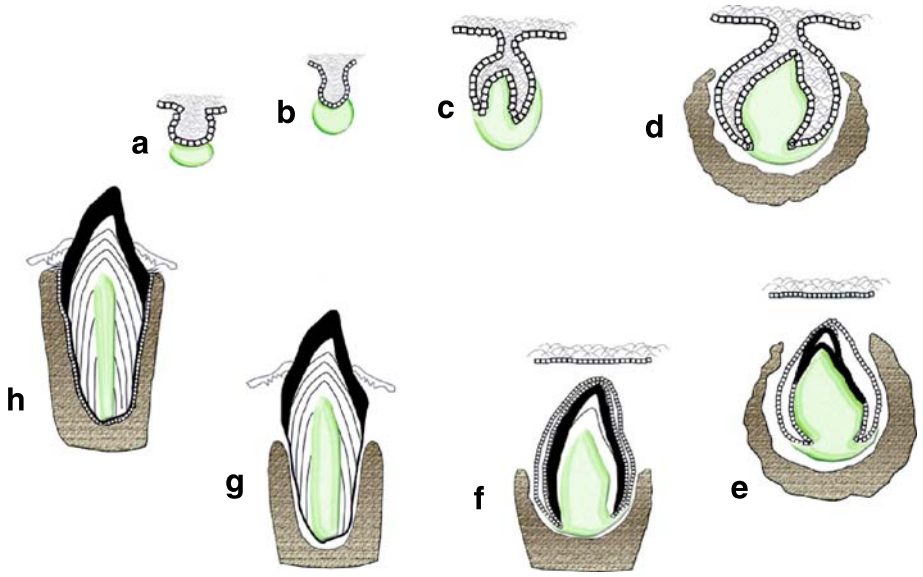

Fig. 2.2.3-1. Tooth development. Ondontogenesis comprises tooth initiation, morphogenesis, epithelial histogenesis and cytodifferention [29]. Development starts as a local thickening ("placode") of the oral epithelium (i.e. dental lamina), that subsequently forms a bud around which mesenchymal cells condense (**a**). Classic tissue recombination experiments using mouse and chick embryonic skin from different body parts revealed that initial signals arising in the mesenchyme (dermis) causes formation of placodes. Molecularly, a complex network of integrative signaling pathways is thereby mediating regulatory processes at the distinctive stages of odontogenesis. During morphogenesis, it develops into a cap-like structure enclosing the mesenchymal dental papilla cells which later give rise to odontoblasts and the dental pulp (**b**). The morphology of the tooth crown is established during the bell stage (**c**), when the cusps of the tooth develop as a result of folding of the epithelial-mesenchymal interface. In the course of odontogenesis, the rather homogeneous dental bud gives rise to the more complex enamel forming organ. This complex process is characterized by growth, histogenesis of the stellate reticulum, stratum intermedium, inner and outer dental epithelium, as well as by the terminal differentiation of ameloblasts (**d**). Ameloblasts differentiate from the inner dental epithelium and start secretion of the enamel matrix after a layer of predentin has been deposited by the underlying odontoblasts (**e**). After having secreted the full thickness of enamel, the ameloblasts change in morphology and function and become so-called maturation-stage ameloblasts regulating the mineral composition of the enamel matrix as a mineralized epithelial tissue (**f**–**h**). The enamel producing epithelium is unique in that the epithelial cells reverse their polarity when they differentiate into secretory ameloblasts: the pole of the cells that was originally basal, i.e. orientated towards the basal basement membrane (BM), becomes functionally apical and is further on a site of secretory processes involved in enamel deposition. Concomitant with or preceding this reorganization at the preameloblast stage, the original BM is degraded and a BM-like extracellular matrix (ECM) at the opposite side of the cells between ameloblasts and stratum intermedium (apical BM) appears, which becomes functionally basal. This newly formed ECM present along secretory phase ameloblasts may be required for sustaining reversed polarity of secretory ameloblasts, and the BM could function to anchor the reversed organization of cell structure at this stage. Later, during enamel maturation, the BM disappears and ameloblasts reform a BM on the enamel surface, and the secretory polarity of the cells once again reverses (modified from Piesco NP, Avery JK (2001) Development of teeth: crown formation. In: Avery JK (ed) Oral development and histology. Georg Thieme, Stuttgart [21])

Increases in mitotic activity seem to correlate with the decrease in adherens junctions at the same developmental stages which influence adhesive, segregative, motile or proliferative tissue-properties associated with histological rearrangements of specific cell populations. Molecules responsible for cell adhesion are also thought to play a significant role in the signaling and promotion of intercellular communication via gap junctions that might integrate cells in synchronized colonies and coordinate cell migration (analogous to what has been observed during extraoral wound healing). Apart from their abilities to bind and store growth factors, the BMZ and the ECM may further represent an informative network of developmental and homeostatic regulation.

It is thus logical that mutations of key structure proteins pathogenetically involved in EB may impair proper odontogenesis, thus accounting for common symptoms such as abnormal dysplastic teeth or enamel hypoplasia.

Enamel hypoplasia and JEB

Quantified by the results of NEBR-based investigation [36], enamel defects are unique within the spectrum of EB-associated dental manifestations in being significantly more frequent as compared to normal controls. This stringent correlation applies, however, only to one major group of EB, i.e. JEB, as enamel composition and structure appears normal in EBS and DEB teeth (Table 2.2.3-2;

Table 2.2.3-2. Enamel hypoplasia in EB [10, 38]

Subtype	Incidence of enamel hypoplasia in % (control 10%)	Commentary
EBS	33%	Rarely displaying generalized enamel hypoplasia
JEB	100%	Pitting, furrowing, thin enamel, defects in prism structure and orientation JEB-Herlitz with evidence of marked enamel deficiencies (up to thin covering of prism-less enamel), non-Herlitz variants display thicker enamel that is pitted
DDEB	11%	Some DDEB patients may display generalized enamel hypoplasia due to expression of allelic EB-related gene mutations, nonallelic mutations in basement membrane related genes or the unique expression of modifying or interacting genes
RDEB	24%	Teeth generally well formed but exhibit an increased frequency of localized enamel hypoplasia and have marked neonatal line (demarcation between prenatal and postnatally formed enamel) secondary to systemic stress

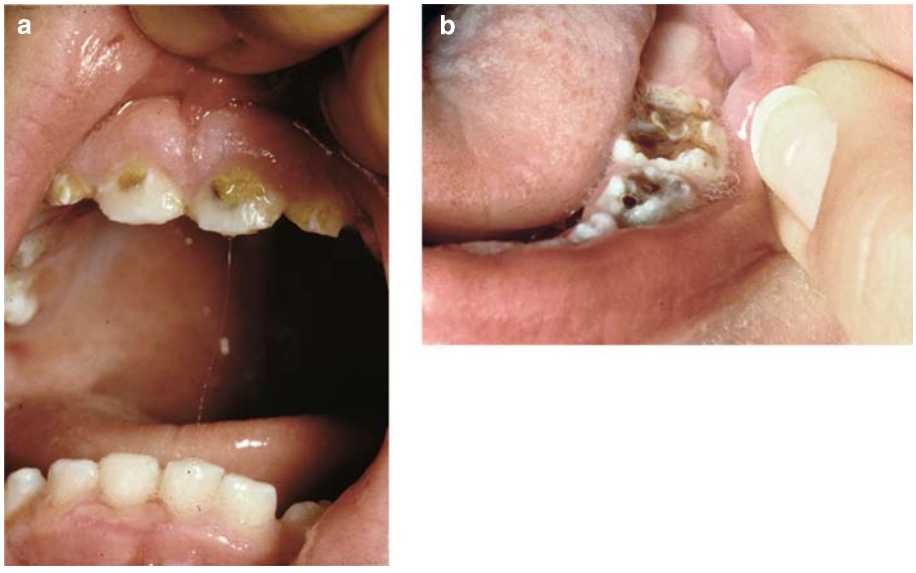

Fig. 2.2.3-2. Enamel defects in a girl with junctional EB non-Herlitz, **a**) incisivi **b**) molars

Figs. 2.2.3-2 to -5). Since primary enamel hypoplasia is not a feature of even the most severe RDEB subtypes, this argues against the notion that other secondary factors (e.g. severe anemia, systemic infection, abnormal nutrition), which typically coexist with all of severe types of EB, contribute importantly to the genesis of enamel defects in EB by alteration of somatic growth or the delicate homeostasis of tooth-forming cells [36]. It further indicates that specific EB-related mutations, such as for example in the genes encoding keratins 5 and 14 as well as type VII collagen, which are all expressed in the developing odontogenic system,

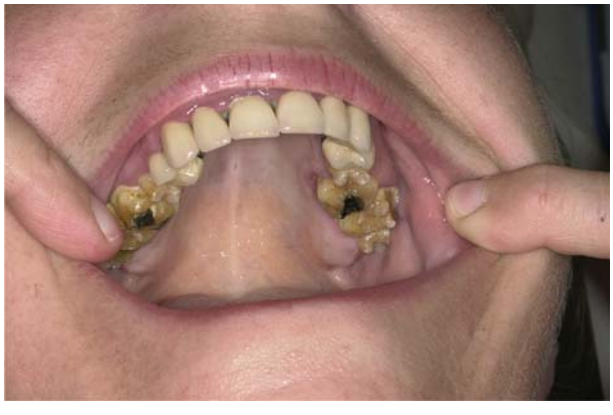

Fig. 2.2.3-3. Enamel defects of the molars of a 40 year old patient with junctional EB non-Herlitz. Front teeth are covered with stainless steel crowns

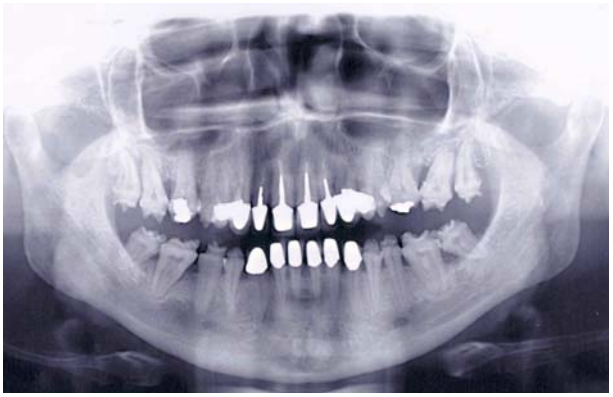

Fig. 2.2.3-4. Panoramic x-ray of the upper and lower jaw of patient in Fig. 2.2.3-3: crowns (teeth 11–14, 21–24, 31–33 and 41–43), fillings (teeth 16 and 26) and enamel and dentin lesions (teeth 16–18, 26–28, 34–37 and 44–47)

do not lead to pronounced disruption of ameloblast function and enamel formation [33, 36].

The genetic basis for JEB has been linked to mutations with the genes encoding for three major BMZ components – laminin-332 (in JEB-H and most cases of JEB-nH), α6β4-integrin (in JEB-PA), and type XVII collagen (in a minority of cases of JEB-nH) – all of which have the potential to alter the relationship of ameloblasts to the developing enamel ECM [26].

During dental morphogenesis, the basal basement membrane separates mitotic pre-ameloblasts and pre-odontoblasts and disappears from the interface shortly after early ECM secretion by odontoblasts. Postmitotic secretory ameloblasts are separated from enamel by a basal lamina-like structure during the maturation stage of development. Mutations in the gene of one of the

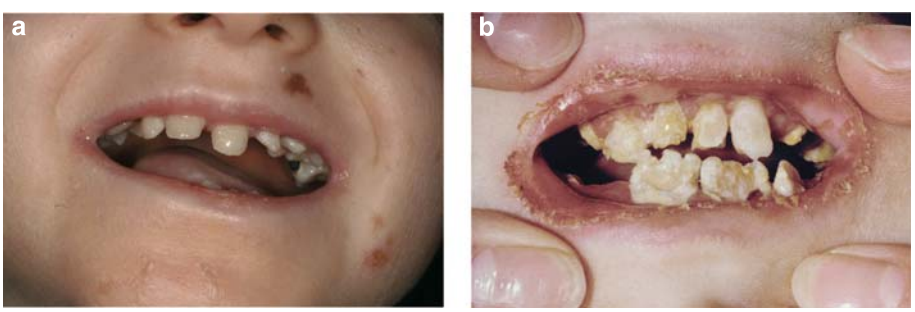

Fig. 2.2.3-5. a and **b** Tooth deformities, caries and periodontitis in two patients with recessive dystrophic EB. Note the presence of microstomia

principal anchoring filament proteins accompanied by the loss of mechanical stability might therefore be expected either to seriously compromise ameloblast differentiation and/or its interaction with the underlying matrix. A disability in maintaining dental cell populations in a particular relation to one another would have a considerable influence on the final morphology of the tissue.

Enamel defects in JEB and their relationship to mutated laminin-332

Laminin-332 (formerly known as laminin-5), whose molecular disruption is prototypical for JEB, is a major BMZ component. It mediates cell adhesion, growth, migration, differentiation, and apoptosis through signaling pathways that are induced by ligation of integrin $\alpha 6\beta 4$ and $\alpha 3\beta 1$. In mice, the spatial and temporal regulation of laminin-332 in dental epithelia was found to correlate with the histogenesis of the dental organ, ameloblast differentiation, and enamel formation [37]. At the interface between the enamel matrix, mesenchyme and ameloblasts, laminin-332 thus plays an important role in the formation of adhesions between ameloblasts and the underlying enamel matrix, and co-regulates enamel-matrix formation and mineralization.

Laminin-332 co-mediated junctions between ameloblasts themselves and ameloblasts and the cells of the stratum intermedium of the enamel organ are important in maintaining channels for both mineral ions and cell nutrients [10]. Deficiencies in these bonds could thus result in a defective mineral transport or compromised ameloblast metabolism. Indeed, data revealed that while the carbonate content is similar in JEB patients and unaffected controls, JEB enamel seems to be altered in its chemical composition, with a significantly reduced mineral per volume content [10].

Moreover, laminin-332 is highly expressed in Tomes' processes, which are considered to control the growth in length and width of enamel crystals, thus affecting their supramolecular aggregation, orientation, and function [37]. Defects in the attachment of ameloblasts to their underlying matrix caused by JEB-specific mutations might therefore lead to the ultrastructurally atypical prismatic appearance with pitting or thin enamel seen so characteristically in enamel of JEB.

Histological investigations of developing teeth in infants with JEB-H have shown small blisters between the plasma membrane of ameloblasts and the basement membrane, causing disorientation of the ameloblast layer and the excessive disruption of ameloblast function and life cycle with metaplasia [10]. An early separation or degeneration and consequent dysfunction of ameloblasts due to mutated laminin-332 could result in thin or absent, irregularly mineralized enamel, either localized or generalized, as the ability of these cells to develop a

normal thickness and/or a structured ECM is impaired. Late degeneration of ameloblasts would produce thicker enamel with pitted areas.

In addition, secretory ameloblasts have been reported to be shorter and reduced in size in relation to wild-type teeth in homozygous LAMA3 knockout mice (LAMA3 is the gene encoding the α3 subunit of laminin-332) [24]. In this animal model, enamel edges appeared frayed and in the region where the ameloblasts and the adjacent stratum intermedium usually form, the so-called reduced enamel epithelium (i.e. where the stratum intermedium no longer forms a discrete boundary), tissue organization was completely disrupted and the otherwise regular formation of a stratified wild-type epithelium was lost.

Zhou et al. [38] indirectly supported the assumption that a genetically determined deficiency of ECM molecules, such as of laminin-332, interferes with proper enamel formation/dental development. The authors described enamel hypoplasia in a transgenic mouse model that stage-specifically expressed urokinase-type plasminogen activator (uPA) under the control of the keratin-5 promoter during odontogenesis. The experimentally induced (dysregulated) proteolysis of ECM molecules by uPA, beyond its permissive role in cellular mobility and tissue plasticity, altered the murine tooth development significantly: focal disinteractions of apical and basal basement membranes within the enamel-producing epithelium, absent or barely visible enamel layer overlying the dentin, large crystals, disorganized stratum intermedium, fragmented or absent amelogenin layer at the enamel-dentin junction, extracellular amelogenin spots in the apical area of ameloblasts and a stratum intermedium indicating altered polarity of amelogenin transport and/or secretion. Enamel hypoplasia was attributed to a defect in enamel formation resulting from structural and functional alterations of the transgenic ameloblasts. Disorganization of the ameloblast layer was associated with the proteolytic degradation (i.e. loss) of laminin-332 and the subsequent failure to establish or maintain the appropriate BMZ-type structures required for correct organogenesis. Interestingly, the enzyme was also produced by the enamel epithelium which normally does not express uPA. This indicates that the K5-promoter (harboured by transgene transfer devices) is active in this tissue and could explain why enamel development may be occasionally affected in patients with EBS.

In summary, all these observations indicate that the compromised interactions between ameloblasts themselves as well as between ameloblasts and the stratum intermedium or the enamel matrix, due to the dysfunction of laminin-332 in JEB, interfere with the intercellularly orchestrated regulation of enamel formation, signal transduction, cell polarity, mechanical stabilization and nutritional supply of ameloblast layers, enamel mineralization, orientation and deposition at the enamel/dentin junction, as well as amelogenin transport and secretion. Since enamel serves as a functional substratum to anchor ameloblasts, failure to

establish an enamel layer can in turn aggravate the disorganization and dysfunction of ameloblasts. Finally, this would result in the typical EB-associated dental phenotype.

It is very important to emphasize that enamel hypoplasia has now also been described in patients suffering from JEB caused by mutations not only of laminin-332 but also of α6β4-integrin and type XVII collagen [2, 9, 15, 19]. Although stringent data on the specific (pathogenic) role(s) of these latter two hemidesmosomal adhesion proteins during enamel formation and tooth development are as yet lacking, their given close (structural as well as functional) association with laminin-332 indicates that similar pathogenetic pathways are operative due to molecular defects of both of these proteins [15].

Enamelogenesis and the perspective of research

Being the only mineralized, ectodermally derived epithelial tissue in the human body, enamel is produced by ameloblasts whose existence is restricted to a distinctive period during dental odontogenesis. Mature human tooth is lacking those cells. Accordingly, there are no comparable alterations in the enamel of patients suffering from autoimmune bullous diseases, such as bullous pemphigoid and EB acquista, in which the very same basement membrane proteins as those affected in hereditary EB are being targeted by pathogenic autoantibodies.

Model systems to visualize and further characterize (human) odontogenesis to investigate effects of mutated BMZ anchorage components on the genesis, structure and function of ameloblasts, are thus a technical challenge. Established animal models include mice, whose incisors display the unique feature of a persistent postpartal ameloblast differentiation gradient to keep the cutting edges of the incisors sharp [31]. The mouse incisor therefore provides a powerful model for studying the spatial and temporal regulation of ameloblast differentiation and, more generally, the mechanisms of patterning during organogenesis.

Unfortunately, murine knock-out models for recessively inherited variants of EB are mostly lethal and pups die perinatally. Therefore, this approach to *in vivo* visualize effects of an (inducible) alteration of BMZ components on ameloblast differentiation for research purpose is highly problematic.

Using a specialized technique, murine embryonic incisors can be extracted and cultured for about 14 days until trophic limitations become relevant. Applying such a tissue-specific cell culture system, effects on integrative networks of signal transduction during development and differentiation have been studied by manipulative application of antibodies or pathway intermediates [31].

However, the transitory stability of the cell culture system is hitherto considered to be too short for efficient induction and evaluation of EB specific mutations. In this respect, although progress has been made, continuing research will be necessary to provide more promising (therapeutic) perspectives.

Mineral content

In NEBR-associated studies, Wright found that the enamel mineral content (calcium/phosphorus ratios, magnesium, fluoride) is similar in all EB types and is on average not significantly different from that of normal enamel, suggesting that, despite marked structural abnormalities, the mineralization process itself progresses relatively unimpeded in EB. Moreover, the composition and amount of protein in EB teeth showed no statistical difference to that observed in normal enamel.

According to the NEBR database [36], abnormalities of other dental mineralized tissues are also insignificant. Irregular dentin formation was reported only in severe forms of EB associated with perinatal death. Although as yet unproven, it was postulated that poorly organized or mineralized cementum exhibiting an irregular surface might be due to EB-associated gene mutations or secondary to multifactorial epigenetic factors, such as anemia, malnutrition, and drug therapy.

Caries and EB

Only the most severe forms of EB, RDEB-HS and JEB, are at a significantly increased risk of developing dental caries, when compared with controls [3]. Other EB variants have decayed-missing-filled-surfaces (DMFS) caries scores similar to controls.

Developmentally compromised enamel of JEB patients with subsequently higher enamel porosity could lead to increased surface area and stagnation sites for the effects of cariogenic acids. NEBR-analyses, however, showed no obvious correlation between caries and enamel hypoplasia or intraoral soft tissue lesions, neither between DMFS scores and ankyloglossia nor vestibular obliteration, which are strong indicators of severe soft tissue involvement. This probably reflects the known multifactorial etiology of caries. External factors like a deficient oral hygiene (due to peri- and intraoral blistering, contractures by scars, limited tongue mobility and food clearance, and/or deficient manual dexterity) certainly play important roles in dental cariogenesis associated with EB. An extremely cariogenic diet, with small quantities of pureed food throughout the day supplemented with drinks that are high in carbohydrates, to increase caloric intake, undoubtedly further adds to this overall problem.

Moreover, the presence of remineralization/enamel crystal inhibitory proteins, like serum albumin in enamel of mature JEB teeth, has also been directly linked to dental caries risk and plaque accumulation in JEB [10]. As salivary flow rates are normal, it is supposed that an increase in salivary albumin levels in those patients having oral lesions probably alters caries and remineralization processes [36]. The role, if any, of additional defects in salivary secretory immunity and secondary changes in oral microflora, remains controversial [7, 32].

From a practical prophylactic and therapeutic perspective, regular follow-ups by a dentist, starting as soon as tooth eruption begins, are mandatory to detect and treat dental involvement. This is of particular importance as more severely affected patients commonly face EB-specific limitations to restorative dentistry in later life, as a result of microstomia. Unfortunately it has been often observed that, given the severity of other manifestations in EB, oro-dental examination of the child is not always considered to be a major need by either the patients' parents or their pediatricians until significant damage has arisen, resulting in major delays in implementing aggressive dental care [4]. Therefore, preventive counseling is indicated and more public awareness is necessary.

Dental care in EB patients, beginning in earliest childhood, includes several strategies [25] . These include (1) preventive measures via aggressive oral hygiene (including fluorides, non-alcohol-based rinses) and the reduction of cariogenic nutrition so as to minimize caries development; (2) early usage of stainless steel crowns on baby teeth, when indicated, to minimize further enamel destruction during childhood and to maintain normal spacing for the later eruption of permanent teeth; (3) restoration of enamel and dentin defects with fillings so as to guarantee structure and continued function of teeth; (4) application in some cases of nerve blocks to avoid critical mucosal tension by depositing a fluid-bolus near the epithelial surface; (5) lubrication of oral tissues to reduce shear forces; and (6) extractions of most severely affected teeth with osteolytic foci in order to remove continuous sources of oral infections.

Successful restoration of masticatory function and esthetics, ensuring appropriate nutritional intake and improved quality of life, has been reported by using metal-resin or ceramic implant fixed complete dentures [11]. Rehabilitation has been possible even in completely edentulous patients due to rampant caries. However, the prognosis of a conventional removable prosthesis and the comfort of an implant-tissue-supported overdenture could be compromised in the EB patient by the presence of frequent blisters resulting from mechanical friction. Therefore, fixed prostheses are likely to be more comfortable to the patient with limited non-load-bearing contact with mucosa. Moreover, implant osseointegration should not be compromised, since EB affects only the skin and mucosa. Long-term data on applicability and durability, however, remain to be evaluated.

Supporting structures

The role of adhesion proteins in retention of teeth

As EB-specific target structures are also expressed within the ligamentous apparatus fixing the tooth in the alveolar bone, it is plausible that disease-specific mutations effect its integrity, thereby accounting for various clinical manifestations. Data referring to this assumption are rare, although histological and functional characteristics of the peridental soft tissue give some clues to support the thesis.

Periodontal ligament, connective tissue and junctional epithelium

A dense network of collagen fiber bundles make up the connective tissue located between the cementum and the alveolar bone (i.e. periodontal ligament) whose functions are formative, supportive, protective, sensory, and nutritive (Fig. 2.2.3-1) [1, 14]. ECM structural proteins, like integrin α2, α5, β1, and α(v) β3 as well as fibronectin or tenascin, have also been reported to be expressed in the periodontal ligament [30].

Moreover, integrin α6β4 and laminin-332 colocalize unequivocally in the tooth facing cells of the dental junctional epithelium, which attaches the gingiva around the neck of each tooth. The basal keratin 14 and the α(v) integrin subunit are additionally expressed [8]. All hemidesmosomal components, such as type XVII collagen and plectin, are likewise present. On the other hand, type IV collagen, laminin-1/10, type VII collagen, and the basement membrane proteoglycan perlecan, expressed in all gingival BMs, are all absent from the dentoepithelial junction, indicating its functional and structural specialization [8].

As the dental junctional epithelium contacts both the hard tooth surface and the connective tissue, it forms an epithelial barrier against mechanical and microbial damage (Fig. 2.2.3-1). Its low density of *inter*cellular junctions (desmosomes and gap junctions) additionally allows transmigration of inflammatory cells and components of the immunological host defense system from the gingival vascular plexus to the gingival margin. This provides the internal protection against gingival and periodontal infections.

The major structural protein laminin-332 promotes adhesion of dental junctional epithelium cells onto the tooth surface by interacting with a cementoblast marker protein (bone sialoprotein-like molecule) and integrin α(v)β3 on the periodontal ligament cell surface [28]. The binding of specific laminin receptors on gingival cell surface to cementum can be disrupted by bacterial lipopolysaccharides, which accounts for the loss of gingival attachment in the pathogenesis of periodontal disease [6]. Consequently, periodontal disease like periodontitis,

characterized by the degeneration and detachment of the dental junctional epithelium from the tooth surface and the conversion of a gingival sulcus into an infected periodontal pocket, is predicted to be most severe in JEB, where defects of laminin-332, preventing a proper seal of the dento-epithelial junction, allow bacteria to infect the connective tissue. Thereby they would not require special virulence factors or a host-immune defect to get through or past the epithelial attachment. This correlation, however, has not been reported so far for laminin-332 and JEB, whereas in Kindler syndrome, an inherited defect in the actin-ECM linker protein kindlin-1 of keratinocytes, there is detachment of skin and gingival epithelium and early onset periodontitis [23].

Besides stabilizing cell anchorage to the matrix, laminin-332 has also been proposed to support keratinocyte migration during wound healing as well as to transduce signals from the ECM to the cell interior participating in regulation of gene expression, proliferation, differentiation, migration, and apoptosis (see also above) [18]. Thus, inborn alterations of key ECM molecules in EB may mediate a significant negative impact on development and structural, as well as functional, maintenance of the periodontium, favouring mechanical instability, crowded dentition, malocclusion, bacterial invasion/periodontitis and, in the end, premature loss of teeth. Standardized study data or pathogenetic disease models to test this hypothesis remain to be established.

Mucosal disease

In analogy to the EB-typical skin vulnerability, oral soft tissue manifestations (Table 2.2.3-3) are characterized by an overall increased mechanical fragilty of

Table 2.2.3-3. EB associated soft tissue manifestations ([36], correspondence)

Sub-type	Incidence of oral soft tissue lesions in % (control 0%)	Commentary
EBS	39%	Blister formation in 34% of localized and 53% of generalized variants; most often during perinatal period, less often persistent
JEB	81%	*JEB-Non-Herlitz*: vesicles and erosions; no vestibular obliteration or ankyloglossia and only rarely severe intraoral scarring *JEB-Herlitz subtype* tend to develop site-specific perioral lesions that may produce significant scar tissue and microstomia
DDEB	72%	
RDEB	95%	Microstomia most pronounced in patients with generalized RDEB; squamous cell carcinomas in perioral sites; no milia (presumably reflecting severity and extent of mucosal disruption)

tissue that causes painful blistering and chronic erosions or ulcerations. These oral findings are associated with a higher risk of infections (especially candidiasis). Phagodynia in conjunction with a restrictive diet, increased protein turnover, and gastrointestinal tract involvement, promote malnutrition and in consequence, growth retardation, anemia, and impaired wound healing. Long-term effects, such as soft tissue scarring or stenoses (ankyloglossia, microstomia and vestibular obliteration), dental crowding and deep bite, are due to progressive perioral or intraoral scarring following initially the presence of granulation tissue.

All these pathologies have a serious impact on EB-associated morbidity. The precocious onset of tissue distortions like microstomia and tooth malpositions, as well as the high caries index, further secondarily lower the patient's compliance with oral hygiene procedures and the dental surgeon's ability to perform accurate treatment, which easily evokes painful erosions [4]. In cases of severe crowding inaccessible to other orthodontic therapy, invasive approaches like serial extractions (i.e. selectively removing several primary teeth followed by removal of four permanent teeth) to ensure proper dental alignment were reported to lead to symptomatic amelioration [36]. However, the burdens of surgery and advanced manipulation of highly fragile tissues should be noted (Fig. 2.2.3-6). Thus, as therapy options are *a priori* limited, oral prophylaxis commencing in early childhood remains the most effective approach.

Oral cancers

Individuals with RDEB, especially those with RDEB-HS, are at a particularly increased risk for recurrent, highly aggressive and early metastasizing squamous skin cell carcinomas which are unfortunately unresponsive to radio- and chemotherapy (Chapter 2.1.3). These malignancies can also emerge intraorally, arising mostly in tissue sites that are subjected to chronic, non-healing ulceration and repeated epithelialization [34]. It is believed that defects of structural components mediating cellular adhesion play an important contributory role in malignant transformation. Haas et al. [6] described a reduction of laminin-332 and its cellular receptor integrin $\alpha 6\beta 4$ in oral squamous cell carcinomas associated with the discontinuity of the basement membrane at sites of tumor invasion. Nevertheless, laminin-332 was abundantly synthesized by invading cells, leading to its deposition within the local stroma, probably interacting with fibronectin and tenascin-C. The authors speculated that laminin-332 thereby might provide a provisional equivalent of the basement membrane stimulating migration. Structurally and/or functionally deficient adhesion molecules in EB might thus not only promote blister formation and impede wound healing and tissue recovery but also support the invasive potential of tumors. Nutritional deficiencies and an altered immune surveillance (for example, *in vitro* data have shown that patients with more severe types of EB have nonfunctional natural

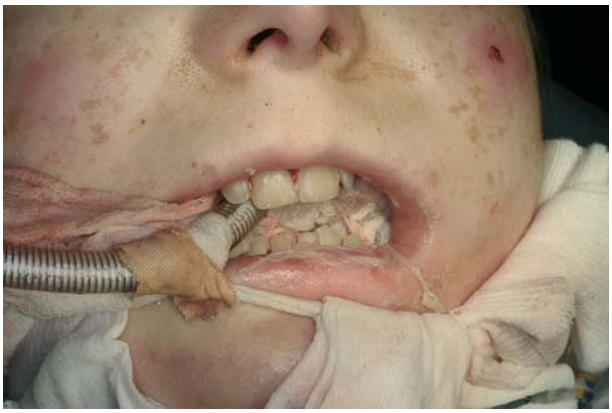

Fig. 2.2.3-6. Sloughing of the lip epithelium due to manipulation during dental treatment

killer cells and a reduced production of cytokines by peripheral blood mononuclear cells) may additionally favor the accumulation of promoters of transformation. Periodic oral examination throughout life is thus mandatory in EB patients at risk, and all clinically suspicious intra- or perioral lesions should be immediately biopsied.

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2.2.4 Gastrointestinal complications

Elke Nischler

The gastrointestinal tract (GI) is one of the most common sites of extracutaneous injury in inherited epidermolysis bullosa (EB). Mucosal trauma results in formation of blisters and erosions and in severe EB subtypes leads to scarring and the development of stenoses and strictures.

The frequent involvement of the oral mucosa and the esophagus often impairs adequate oral food intake. Intestinal involvement on the other hand frequently results in secondary malabsorption or loss of nutrients and vitamins. This worsens the nutritional status of an already debilitated patient whose nutritional status is compromised anyway by the high caloric demands of wound healing, infection, and growth [10].

Oral cavity

Lesions within the oral mucosae are painful blisters and erosions that may be found in all three major types of EB (simplex, junctional and dystrophic), with the most severe involvement seen in RDEB-HS and RDEB inversa. In these subtypes, serous and/or hemorrhagic blisters are oftentimes large and may show a peripheral inflammatory reaction. Upon rupturing, the resultant erosions tend to heal with fibrosis and scarring, sequelae of which include decreased oral aperture (microstomia), a bound down tongue (ankyloglossia) which cannot be extended beyond the teeth, altered architecture of the oral cavity, lingual depapillation, and atrophy of the palatal folds [34, 35]. Additionally, in RDEB milium cysts may be found particularly in the region of the hard palate.

Areas of leukoplakia and an increased risk to develop oral squamous cell carcinomas have also been reported in RDEB-HS patients, the consequence of repeated ulceration and re-epithelization mainly within the lingual mucosa [7]. Moreover, both JEB and RDEB are associated with an increased risk of dental caries (Chapter 2.2.3), caused and maintained by external factors such as difficulties in oral hygiene (because of pain while cleansing the teeth and prolonged exposure of teeth to food) and, in JEB patients, the presence of enamel hypoplasia [30, 36] . This underlines the importance of periodic follow-up examinations of the oral cavity and close interdisciplinary cooperation.

Dysphagia and esophageal strictures

In the oropharynx and esophagus, repeated trauma caused by eating and swallowing of solid food often leads to recurrent blister formation, ulceration, and ultimately esophageal stenoses and strictures (Fig. 2.2.4-1), which involve

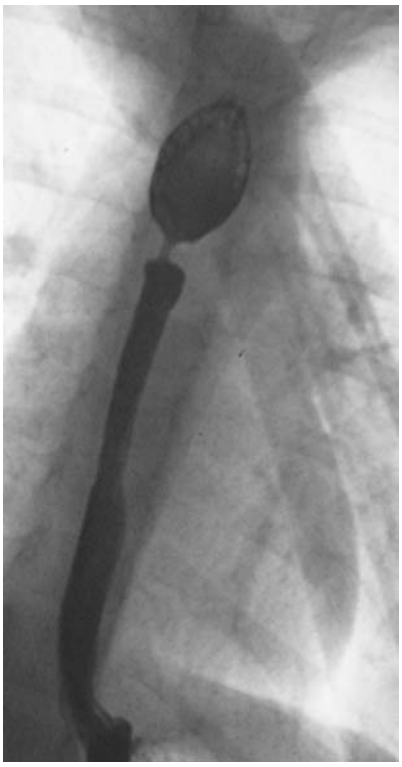

Fig. 2.2.4-1. 8 year old patient with eb dystrophicans Hallopeau – Siemens with a severe stricture of the esophagus (Barium swallow)

only the mucosa and muscularis mucosa [6, 8, 13, 32]. EB-associated strictures may arise anywhere in the esophagus. Fifty percent of them are located in the upper third of the esophagus (usually at the pharyngoesophageal junction at vertebrae C6–C7). This proximal location may predispose the patient to additional complications, most notably aspiration pneumonitis [6, 13]. Of the remaining esophagus, the middle third is affected more often than the distal one [4, 8, 13, 23, 25]. Since the vast majority of strictures arise within the upper two-thirds of the esophagus in EB patients, gastro-esophageal reflux seems to play a significant role in their formation only in distal strictures. In some small series of publications multiple strictures or webs were found with a frequency of 11.1 to 40% [4, 8, 22, 33]. Radiological studies of esophageal strictures showed that they vary in length and shape, from thin webs to lesions up to 8 cm and more [2, 5, 22, 33]. Over time, intraluminal bullae, web formation and strictures result in progressive dysphagia with all its consequences, including severe malnutrition, growth disturbances, and risk of aspiration and pneumonia [6, 13].

Previous studies have reported the mean age of onset of dysphagia in EB to be between 11.25 and 17 years [22, 25, 32, 33]. However, dysphagia has also been reported in early childhood and in a more recent study of 22 REBD patients, the mean age of onset of dysphagia was 48 ± 34 months, with the earliest occurring by 10 months of age [6, 22]. This underlines the need for careful monitoring for symptoms and signs of esophageal strictures even in early childhood to prevent further complications.

Recent cross-sectional and longitudinal data analysis of the National EB Registry on 3280 and 450 EB patients, respectively, confirmed that dysphagia is indeed very common, with frequencies ranging from 1.37% in patients with localized EB simplex (EBS) to 100% in patients with RDEB inversa [10]. Esophageal stenoses or strictures were also found very often, ranging from 0% in EBS Dowling-Meara (EBS-DM) to 86.67% in RDEB inversa patients [10]. Lifetable analysis of these patients showed the significant influence of age on the risk of these complications, in RDEB-HS being present in 94.72% of patients by age 45, in RDEB-nHS in 70% by age 50, and in RDEB inversa in 90% by age 30 [10]. Esophageal dilatations were performed rarely in patients with EBS, junctional EB (JEB), and dominant dystrophic EB (DDEB). In contrast, 36.03%, 28.29%, and 70.59% or all patients with RDEB-HS, RDEB-nHS, and RDEB inversa underwent at least one dilatation [10].

Other esophageal problems reported in EB patients include pre-stenotic dilatation, esophagitis, Barrett's esophagus, uncoordinated and decreased peristalsis, intramural pseudodiverticulosis, esophageal atony, esophageal spasms, Mallory Weiss tears, esophageal perforation, and shortening of the esophagus in conjunction with a traction-type hiatal hernia.

Treatment of esophageal strictures and stenoses

A wide variety of surgical treatment strategies, to include but not be limited to colonic interposition, resection, gastrostomy, and bougienage, have been used to treat or offset the presence of esophageal stenoses and strictures. Non-surgical drug therapy has included collagenase inhibitors (phenytoin) and corticosteroids, but has not been proven effective in the prevention or treatment of esophageal strictures [9, 13, 14, 16, 19, 31]. Because application of shearing stress to the esophageal wall has been shown to produce severe injury (epithelial detachment or perforation), bougienage of stenoses is only infrequently still performed in most EB centers [1, 9, 12, 14, 31]. Some authors have recommended early esophageal replacement with colonic interposition as the treatment of choice, but this approach should be reserved for patients with frequent severe esophageal obstruction, mucosal dysplasia, or perforation [13, 16]. During the last two decades endoscopically guided balloon dilatation has become the most widely used treatment and replaced the more invasive approaches [3, 4, 6, 21, 29]. It applies only radial stretching forces to the involved area of the esophagus and therefore theoretically avoids potential bougienage-induced morbidity secondary to longitudinal shearing forces [3, 29]. A further development is fluoroscopically guided balloon dilatation, which can be performed without the use of an endoscope, minimizing the inherent risk of iatrogenic oropharyngeal trauma. This technique has been shown to be a safe, gentle, effective, repeatable, and minimally invasive procedure. Additionally, it allows the use of larger balloons that are capable of achieving a greater dilatation diameter and increases the likelihood of more prolonged intervals between repeated dilatations [3, 4, 6, 16]. Other advantages of fluoroscopy are that it allows the evaluation of the calibre of the balloon in relation to the calibre of the esophagus, and direct visualization of balloon inflation (if contrast media is used to fill the balloon) and ablation of the stricture, facilitating the assessment of the completeness of stricture effacement and duration of inflation [6]. Patients have reportedly experienced immediate relief of symptoms after this procedure, allowing some to swallow soft solid food as early as 2–4 h after treatment. Moreover, rapid recovery and dramatic improvement in the quality of life have been observed. Azizkhan et al. who performed 92 fluoroscopically guided balloon dilatations in 25 RDEB patients, achieved with this method a mean interval between treatments of 1 year, which exceeds treatment intervals previously reported in the literature [3, 4, 6, 16].

Gastric complications

Based on published population based data from the United States, gastroesophageal reflux disease (GERD) would appear to be an uncommon problem in EB patients, with the highest frequency (13.95%) reported among JEB-H patients. Despite these data, many pediatric dermatologists still routinely use proton

pump inhibitors in these children, to control for possibly clinically silent reflux. Hopefully a properly designed study will eventually determine whether the latter hypothesis is correct. Only rare EB patients experience hiatal hernia, gastritis or peptic ulcer disease [10].

Pyloric atresia arises in a small subgroup of JEB patients (and even rarer, within a few with generalized EBS) [10]. Those infants presenting with neonatal blistering associated with pyloric atresia are now referred to as EB with pyloric atresia (EB-PA) subtype. EB-PA has been known for several years to be caused by mutations in the genes ITGA6 and ITGB4, which encode for the α6 and β4 hemidesmosomal integrin polypeptides, respectively [27, 28]. However, recent studies have identified a few families with EB-PA in which genetic analysis instead revealed homozygous mutations in the plectin gene (PLEC1), which encodes for another hemidesmosomal protein previously linked to patients having both EBS and muscular dystrophy [26]. These results provide further evidence for the molecular heterogeneity in EB and emphasize the importance of screening of EB-PA patients by immunofluorescence not only for α6β4 integrin but also for plectin deficiency. The clinical diagnosis of EB-PA is invariably made within the first 24 hours of life via simple radiogram of the upper abdomen. If present, this gastric anomaly must be surgically corrected.

Lower gastrointestinal tract complications

The most common complaint regarding the lower gastrointestinal tract in EB patients is chronic constipation (at times associated with fecal impaction), which is reported most commonly among the more severe EB subtypes (Table 2.2.4-1).

Constipation may partially be the result of conscious or reflex refusal to defecate because of painful anal erosions, fissures or strictures. Additional risk factors for developing constipation are limited oral food intake, low fiber intake, and excessive loss of fluid through the skin. Treatment with stool softeners and laxatives alone is usually unsuccessful when not supported by sufficient fluid and high fiber intake [17]. In severely affected children, oral lactulose may prove beneficial. In very rare cases chronic constipation may even result in the development of a megacolon and its related risk for perforation, as reported in one patient with dystrophic EB [17].

Severely painful anal fissures develop in some patients with RDEB (range, 3.55% in RDEB-HS to 11.76% in REBD inversa) as do anal strictures (in up to 25% of all patients with either of these two RDEB subtypes). Diarrhea is a rare complaint in all major EB types, as are rectal tears, rectal prolapse and strictures, perianal fistulas, encopresis, inflammatory bowel disease, irritable bowel disease, diverticulosis, hemorrhoids, or intussusception [10].

Table 2.2.4-1. Frequency of gastrointestinal complications in EB, stratified by major EB subtype (modified from Fine et al., ref. [10])

	Frequency of gastrointestinal complications (expressed in percentages)									
	EB simplex				Junctional EB		Dystrophic EB			
	Weber-Cockayne	Dowling-Meara	Koebner	EBS-other	Herlitz	Non-Herlitz	DDEB	RDEB-HS	RDEB-nHS	RDEB-inversa
Enamel hypoplasia	0	0	0	0	100	100	0	0	0	0
Dysphagia	1.37	7.96	13.54	5.53	32.56	23.40	16.98	93.62	60.90	100
GERD	1.83	5.31	3.12	2.89	13.95	4.19	1.18	2.84	3.03	0
Esophageal stenosis or strictures	0.09	0	3.12	1.58	14.29	3.24	3.78	79.10	37.16	86.67
Peptic ulcer disease	1.74	0.88	2.08	1.05	2.32	1.05	1.18	1.42	1.89	5.88
Pyloric stenosis or atresia	0	0	1.04	0	0	6.81	0	1.44	1.14	0
Chronic diarrhea	0.37	3.51	2.08	1.58	0	1.57	0.94	5.67	2.27	0
Constipation	6.78	27.19	12.50	11.05	18.60	19.37	21.41	76.98	50.00	76.47
Rectal bleeding or blood with defecation	0	0.87	0	0.26	2.32	1.05	0	0	1.89	11.76
Rectal tears or fissures	0.09	0	0	0.26	4.54	1.05	0	1.42	0.76	0
Rectal strictures	0	0	0	0	0	0	0	0.71	0	0
Anal fissures	0.09	0	1.04	0.53	2.32	3.66	1.88	3.55	7.20	11.76
Anal strictures	0.27	0.89	2.08	1.32	4.76	5.82	2.60	23.36	10.31	25.00
Encopresis	0	0	0	0	0	0	0.24	0.71	0	5.88
Megacolon	0	0	0	0	0	0	0	0	0.38	5.88

Malnutrition

Failure to thrive and growth retardation result in part from restricted intake of nutrients, malabsorption due to widespread and persistent intestinal erosions, and from the remarkably high protein and calorie requirements that accompany the most severe EB subtypes [18]. The highest cumulative risk of growth retardation was found in JEB-H and RDEB-HS, with risks of 52.59% by age 2 in JEB-H and 79.41% by age 20 in RDEB-HS [10]. Additional reports showed that patients with severe forms of EB such as RDEB are often deficient in key vitamins and trace elements [11, 18]. Deficiencies in carnitine and/or selenium may be associated with dilated cardiomyopathy, as was proposed in two unrelated children with severe REBD [24]. The latter complication is discussed in greater detail in Chapter 2.2.6. Moreover, low intake of vitamin D, lack of sunlight and lack of physical exercise may collectively contribute theoretically to the development of osteoporosis in some EB patients [20]. Nutrition aspects of EB are discussed in Chapter 3.4.

One measure used to treat growth retardation is parenteral nutrition (TPN) such as by a nasogastral tube (Fig. 2.2.4-2). Poor oral intake caused by painful

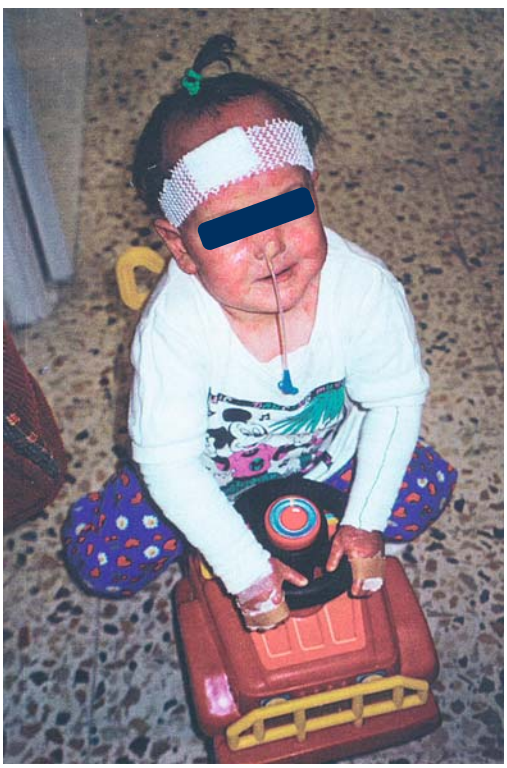

Fig. 2.2.4-2. Nasogastral tube

mouth lesions or poor wound healing thought to be secondary to malnutrition are common reasons for starting TPN. Fine et al. recently reported that in a large study population of EB patients only rare patients with localized EBS and DDEB underwent TPN, but about 10% of all EBS-DM and RDEB inversa patients, 15% of RDEB-nHS patients, 25% of JEB-nH patients, 30% of RDEB-HS patients, and 33% of all JEB-H patients were treated at some point in time with TPN [10].

Another measure now frequently employed in an effort to try to increase the weight and height of severely affected EB patients, as well as to reduce morbidity, is gastrostomy feeding supplemented by oral intake (Chapter 3.3). The obvious clinical value of gastrostomy in EB and the introduction of smaller button appliances have led to a broader use of feeding gastrostomy in EB infants and small children [15].

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2.2.5 Musculoskeletal deformities

Jo-David Fine

Clinical features

Acral deformities

The most visible extracutaneous complication of inherited epidermolysis bullosa is pseudosyndactyly, the endstage of which is commonly referred to as mitten or claw deformities. Primarily seen in recessive dystrophic EB (RDEB), this debilitating complication results from repeated blistering on the hands and feet. Beginning as partial fusion of or synechiae formation between the proximal portions of the web spaces of one or more digits, in the most severe cases this typically leads to complete fusion of all of the digits, with subsequent encasement of the extremity within a keratinaceous cocoon-like structure [1] composed of a hyperplastic epidermis containing a stratum corneum which is five times as thick as the remaining epidermis underneath [12]. These usually progressive acral deformities cause marked functional disability, to include reduced fine manipulative skill and loss of digital prehension [5]. Unless these mitten deformities are surgically corrected early, the underlying digital muscles eventually undergo atrophy from disuse and the bones may become at least partially resorbed (Figs. 2.2.5-1 and -2).

Among the study population of the National (American) EB Registry (NEBR), those most often afflicted with mitten deformities of the hands had some form of RDEB, to include 95.0% and 41.2% of those with Hallopeau-Siemens (RDEB-HS) and inverse (RDEB-I) subtypes, respectively. It also occurred less frequently in dominant dystrophic (DDEB), junctional EB (JEB), and generalized EB simplex

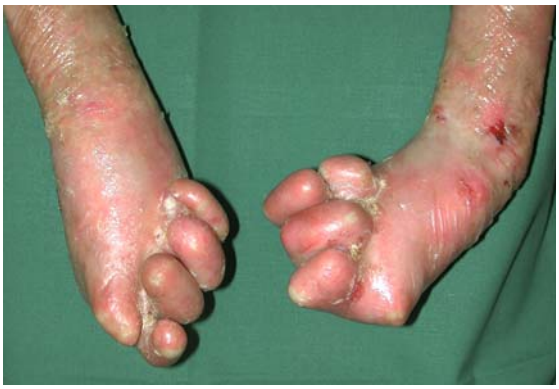

Fig. 2.2.5-1. Mitten formation and severe contractures in a 23-year old patient with RDEB – Hallopeau – Siemens

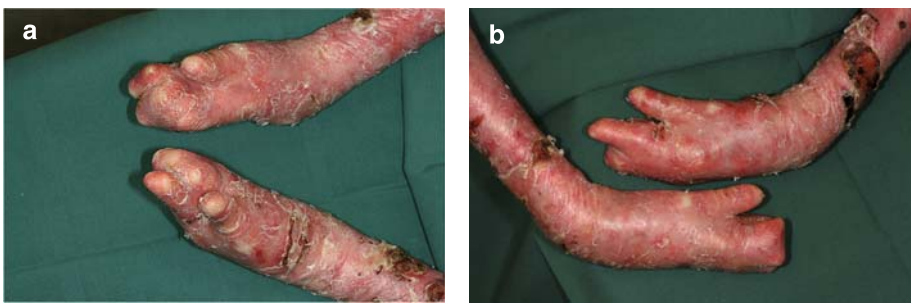

Fig. 2.2.5-2 a) and b). Mitten formation and severe contractures in a 28 year old patient with RDEB Hallopeau – Siemens

(EBS). This led to a small subset of JEB patients being given the name "cicatricial JEB" in past [6], to reflect the presence of hand findings in JEB which had in the past been routinely attributed to RDEB.

Life table analyses on data generated from 1986–2002 by the NEBR study population have demonstrated that the highest cumulative risk for pseudosyndactyly occurs among RDEB-HS patients, reaching a plateau level of nearly 100% by age 20 (Fig. 2.2.5-3). Among those with RDEB-nHS, the cumulative risks are 54.3% and 77.1% by ages 35 and 70, respectively, and in RDEB-I, the cumulative risks are 7.7% and 25.3% by ages 8 and 15, respectively. Of considerable clinical importance, these mitten deformities may occur early, with 16.2% and 13.0% of RDEB-HS and RDEB-nHS children so affected within the first year of life.

Lower cumulative risks of pseuosyndactyly occur in JEB, with a maximum of 17.2% by age 15 in Herlitz JEB (JEB-H) and 9.1% by age 45 in non-Herlitz JEB (JEB-nH) patients.

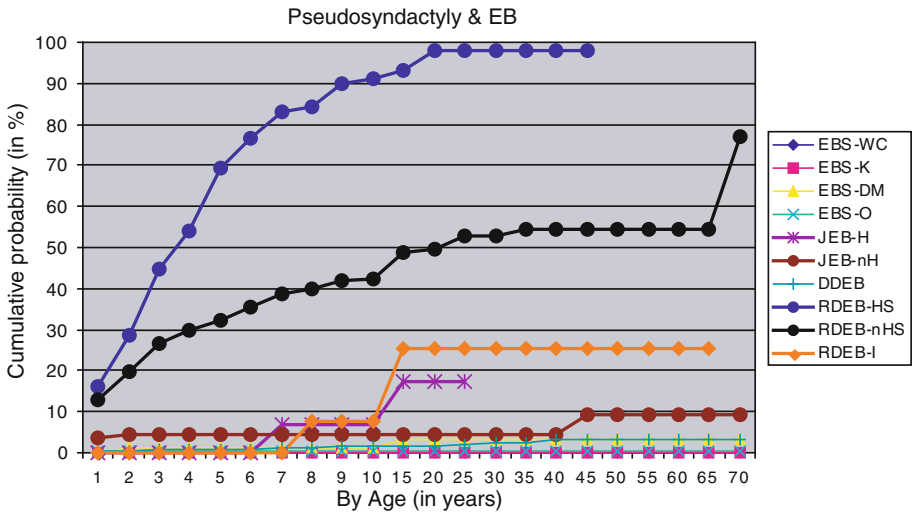

Fig. 2.2.5-3

The lowest cumulative risks for pseudosyndactyly of the hands occur in DDEB (maximum level of 3.4% on or after the age of 40) and EBS. Among EBS subtypes, the subtype at highest risk is Dowling-Meara (EBS-DM), and this is only 3% on or after age 3.

Other musculoskeletal deformities

Musculoskeletal contractures also arise in non-acral sites. In general, they occur with higher frequency than those which arise within the hands and feet. These deformities add to the impairments in ambulation, dressing, and the performance of other basic activities of daily living that are experienced by EB patients. In the most severe EB subtypes, Hallopeau-Siemens RDEB and JEB-H, they may be worsened by confinement in a wheelchair or a bed.

When life table analyses were performed on NEBR data (Fig. 2.2.5-4), the highest risks were seen in RDEB-HS (98.9% by the age of 20), RDEB-nHS (48.7% and 77.8% by the ages of 25 and 60, respectively), and RDEB-I (24.5% and 59.5% by ages 15 and 50, respectively). Maximum levels of 8.1%, 72.9%, and 22.5% were seen in dominant dystrophic epidermolysis bullosa (by the age of 55), JEB-H (by the age of 20), and JEB-nH (by the age of 40), respectively. Among EBS subtypes, cumulative risks ranged from 1.3 to 12.9%, with the highest risk occurring in Dowling-Meara EBS (EBS-DM).

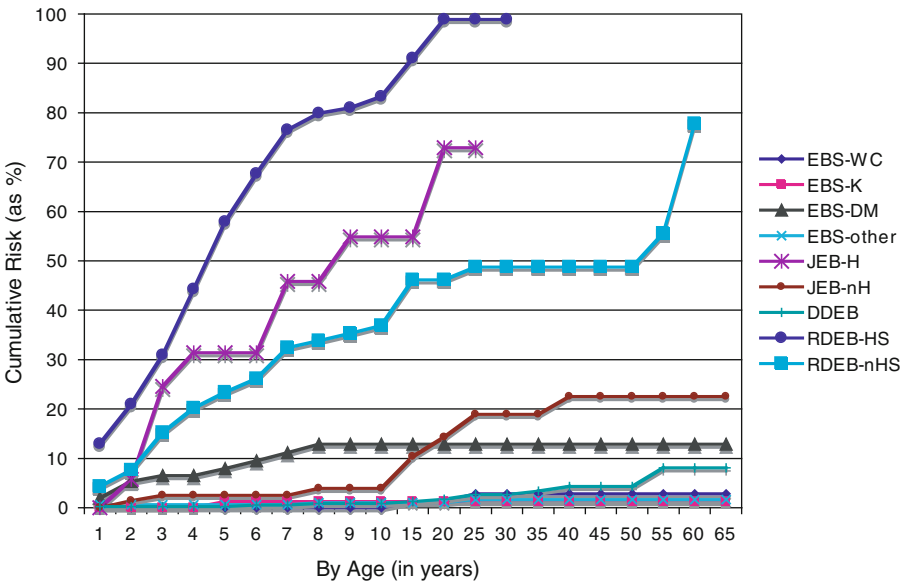

Fig. 2.2.5-4. Cumulative risk of non-acral contractures, stratified by major EB subtype

In JEB-H, those contractures which arise within the axillary and inguinal vaults, and at the base of the neck area, are commonly seen in association with large areas of non-healing exuberant granulation tissue.

Radiographic findings

A variety of bony findings have been described in EB [1, 11, 17]. These include generalized osteoporosis, wedge-shaped thinning and hooking of the distal phalanges, slender and overconstricted bones, acro-osteolysis, flexion contractures, metatarsalphalangeal and metacarpalphalangeal joint subluxation, distal trophic changes, webbing or epithelial bridging or fusion of the digits, adduction contracture of the thumb as the result of obliteration of the first web space, encasement of the hands and feet in a skin-covered pouch or mitten, soft tissue calcification, retarded skeletal maturity, bony ankylosis of the proximal interphalangeal joints, resorption of the metatarsal and metacarpal heads, shortened metatarsal bones, carpal and tarsal fusion and destruction, and cystic changes of the distal radius and ulna.

Radiological findings in non-acral sites include hip dysplasia with premature osteoarthritis, knee joint bony ankylosis, and thoracic and thoraco-lumbar scoliosis [17].

Treatment of musculoskeletal complications

Non-surgical interventions

Although not as yet rigorously proven to significantly alter the natural history of EB-associated pseudosyndactyly, it is still a common practice to mechanically separate the digits, particularly the fingers, most often using sterile gauze strips followed by the wearing of open ended gloves or digital sleeves. Both passive and active range of motion exercises are encouraged. These wraps are usually applied on a daily basis, with the hope that they will prevent, minimize, or at least delay the development of clinically significant acral deformities. This is not usually done on the feet unless webbing is accompanied by other pedal deformities that might further impair ambulation. Some clinicians have proposed instead the use of specially constructed padded wrist splints, worn at bedtime and individualized to each patient's specific anatomy, to maintain the functionality of the wrists and hands by physically preventing further contractures from occurring during sleep.

Surgical interventions

Repair of hand deformities (see also Chapter 3.3)

A number of surgical approaches have been described for EB-associated pseudosyndactyly of the hands, so as to improve functionality, as characterized by improvement in grasp and gross pinch [15]. Postoperative recurrence, however, of pseudosyndactyly is the norm (53% in one series [16] and 50% in another [8]), with repeated procedures needed about every 2 years, if optimal functionality is to be maintained [2, 5, 15]. Within the American based NEBR, 154 of 2742 patients underwent at least one hand repair procedure, with a median number of procedures of 3, 2, 5, and 3 for patients with RDEB-HS, non-RDEB-HS, dominant dystrophic EB, and RDEB-I, respectively. One of these RDEB-HS patients underwent as many as 22 serially performed procedures.

In some of the published series, recurrence occurred as early as only a few months postoperatively [7]. Recurrences also may be more frequent in the dominant than non-dominant hand [5], although no differences have been noted in clinical improvement when left and right hands were compared [15].

Data have been published on several series of EB patients undergoing hand reconstruction. One of the earliest, involving 23 dystrophic cases, was reported in 1971 from the University of Colorado [7]. In 1993 Vozdvizhensky and Albanova reported on 19 children aged 2 to 14 who were referred for hand surgery [16]. Of these, all had some degree of pseudosyndactyly and contracture, and 12 of 19 had

advanced cocoon formation. The largest published cohort outside of the NEBR represents the experience at St. Thomas' Hospital in London over a ten year period (1981–1990) in 45 patients, and involved 122 operations on 80 hands [15].

Several different surgical approaches for the correction of these acral deformities have been reported. Response is best if intervention is begun before severe webbing has arisen. Most of the earlier studies described degloving of the hands and immobilization of the digits (to include placement of rigid wires along the longitudinal axis of the digits), followed by grafting with either split-thickness grafts [4, 5, 7, 10] or full-thickness skin [9]. Since the extremity is usually immobilized for many weeks, usually only one hand is repaired at a time, to allow use of the untreated hand. For children, this procedure is usually scheduled during a prolonged school break, so as not to interfere with their attendance in school, with the remaining hand repaired the following year. A variety of variations on this technique have been described since then, to include the use of artificially prepared autologous skin graft sheets [3] and no grafts [16].

Repair of foot deformities

Although performed far less frequently on severely affected feet, mitten release can improve walking ability and decrease pain. The usual reasons for such intervention have included pain or difficulty in standing and walking, and the inability to wear shoes due to hyperextension contractures of the toes [13]. Only six of 25 (24%) patients in the New York cohort, and six of 50 (12%) patients in the St. Thomas' Hospital cohort, underwent such intervention. Among NEBR participants, 7.9% of RDEB-HS patients had foot deformities surgically treated. Overall, the number of repeat procedures is much less than that seen with hand deformities. Among NEBR patients, for example, mean and median number of foot repairs was 1.3 and 1.5, respectively.

Other rarely performed pedal surgical procedures have included tendon release [2], distal amputation of a phalanx [2], Fowler's arthroplasty for correction of metatarsal head prominence [14], and astragalectomy for correction of an equinovarus deformity of the foot [14].

Summary

Cross-sectional and longitudinal data from cohorts of EB patients in several different countries confirm that the risk of mitten deformities is extremely common in RDEB, especially in those with the Hallopeau-Siemens subtype, in whom essentially every patient will develop this debilitating complication by age 20. Lower cumulative risks for pseudosyndactyly are seen in nearly every other EB subtype. Although surgical release of hand deformities may temporarily

enhance functionality, recurrence is the norm, necessitating repeated repairs if improved functioning of the fingers is to be maintained. Furthermore, surgical repair, if it is to be pursued, must be done early in the course of disease, prior to the development of secondary muscular atrophy and bony resorption of the digits. These collective data, therefore, raise the question of how practical it is to pursue hand surgery, unless the patient is willing to undergo repeated procedures throughout life.

Since these deformities arise as early as within the first year of life, this argues compellingly for surveillance for this complication during early infancy, and for consideration those non-surgical interventions, to include physical therapy (Chapter 3.5) and digital wraps and/or hand splinting, which might reduce the risk or at least severity of a child developing severe mitten deformities.

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2.2.6 Other internal complications

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As discussed in several other chapters, there are a variety of extracutaneous complications of inherited EB. Major organ systems that may be affected by EB include the gastrointestinal tract (especially the esophagus), upper respiratory tree, external eye, hands and feet, and oral cavity. In the present chapter, other internal complications of EB will be discussed, and the pertinent literature reviewed.

Cardiac complications

Cardiomyopathy

Cardiomyopathy encompasses three main subtypes – dilated, hypertrophic (idiopathic hypertrophic subaortic stenosis), and restrictive cardiomyopathies [42]. A characteristic feature of dilated cardiomyopathy is congestive heart failure. Dilated cardiomyopathy may be caused by many different injurious processes, to include micronutrient deficiencies (carnitine [32, 43, 45]; selenium [1, 19, 22]; possibly thiamine), iron overload (due to chronic transfusion therapy) [33], chronic anemia [25], and viral infections (presenting as myocarditis) [12, 28, 31]. Of clinical pertinence to EB, the causal association of cardiomyopathy with selenium deficiency has recently been challenged [1, 13].

In 1989 dilated cardiomyopathy was reported in a 17 year old patient with recessive dystrophic EB (RDEB) [4]. This was attributed to iron overload and secondary hemosiderosis, given the number of transfusions that the patient had received since age 9. In 1996, Melville et al. [29] reported the presence of fatal

dilated cardiomyopathy in two unrelated RDEB children, one of whom was found to have low selenium levels, suggesting that this micronutrient deficiency might be the possible cause. Low selenium levels were also found in 14 of 25 other RDEB children lacking evidence of cardiac disease, however, raising a question as to the clinical relevance of this finding in only one of these two children with cardiomyopathy. Echocardiographic examination of an additional 18 children failed to reveal additional cases, raising a question about the true frequency with which cardiomyopathy might occur in the setting of RDEB. In a follow-up study with seven years of observation, dilated cardiomyopathy was detected in 6/61 (9.8%) RDEB children who were evaluated within the same institution, with a mean age of confirmation of the diagnosis by echocardiography of 8.7 yrs (range, 5.8–12.5 yrs) [40]. Three of six died of their cardiac disease within 0.2 to 0.4 years. When data on each of these 61 patients were reviewed, statistically significant reductions in blood levels of free and total carnitine, but not selenium, were noted prior to nutritional supplementation, and none of these children was found to have any coexistent viral infection that might have played a causative role. These collective findings suggested the possibility that carnitine supplementation in RDEB children might be preventative against the development of dilated cardiomyopathy. In support of the latter, L-carnitine therapy has reversed cardiomyopathy in some patients having other underlying diseases associated with carnitine deficiency [43, 47].

In a recent study Fine et al. reviewed the medical records of 3280 EB patients enrolled in the American National EB Registry (NEBR) from 1986–2002 for possible cardiac complications [17]. Data were stratified by EB subtype and strength of evidence of congestive heart disease (CHF) or dilated cardiomyopathy. In an effort to determine the risk of CHF or cardiomyopathy most likely resulting from EB, patients with other causes of CHF (atherosclerotic heart disease; congenital heart disease; rheumatic heart disease; chronic renal failure (in five patients, secondary to glomerulonephritis (n=1); renal amyloidosis (n=1); unknown etiology (n=3)) were excluded from these analyses. Similarly, those with CHF with onset on or after the age of 55 were excluded.

Congestive heart failure or dilated cardiomyopathy was first reported by ages 3, 2, and 1 year of life in JEB-nH, RDEB-HS, and RDEB-nHS patients respectively. Among JEB-nH patients, the cumulative risk was 1.14% by age 3, rising to 4.94% on or after age 40, with the highest conditional risk, 3.85%, occurring between ages 35 and 40. In RDEB-HS patients, the cumulative risks were 0.72%, 1.45%, 2.28%, and 5.52% by ages 2, 3, 9, and 20. The highest conditional risk, 1.29%, occurred between ages 15 and 20. In our RDEB-nHS subpopulation, the cumulative risks were 0.40% and 1.81% by ages 1 and 30, respectively.

When only those patients carrying the diagnosis of dilated cardiomyopathy were considered, the cumulative risks among RDEB patients were 0.72%, 1.45%,

2.28%, 3.26%, and 4.51% by ages 2, 3, 9, 13, and 20, respectively, with the highest conditional risk, 1.29%, arising between ages 15 and 20. In contrast, the maximum cumulative risk in JEB-nH was 1.14% by age 3 and 0.40% in RDEB-nHS by age 1, respectively.

Among patients having CHF or cardiomyopathy, 3/10 (30%) patients, all with RDEB-HS, died as a result of their cardiac disease at ages 2.9, 8, and 21. When the study population was restricted to those diagnosed with cardiomyopathy, two deaths (2/7, 28.6%) were directly attributed to this complication. An additional patient with JEB-nH who was also included within Groups 2 and 3 died at age 3 of respiratory insufficiency following anesthesia; dilated cardiomyopathy was noted at autopsy but was not listed as a cause of death. Of note, two other RDEB-HS patients who had concurrent CRF reportedly died of CHF or cardiomegaly at ages 21 and 31. The older of these two also had numerous transfusions, which may have contributed to iron overload, whereas the younger patient with CRF had her renal disease for 5 years prior to the onset of CHF, and then died 3 months later, reportedly as a result of the latter complication.

On the basis of the NEBR population-based database, it is clear that dilated cardiomyopathy is a real complication, primarily among those with RDEB-HS, with about one in twenty patients developing otherwise unexplained CHF by age 20. Furthermore, cardiomyopathy may prove to be a lethal complication in a substantial subset of EB patients. Although it is currently believed that this develops primarily as a result of micronutrient deficiency, there are definitely other pathological processes associated with EB, to include iron overload, glomerulonephritis, and renal amyloidosis which may also lead to CHF in these patients. In particular, within the NEBR cohort the cumulative risk of CHF among RDEB patients having concurrent chronic renal failure was nearly 19% by age 35.

Based on these collective data, any patient with EB, especially those with RDEB-HS, should be monitored for early signs and symptoms of CHF. In these patients, echocardiographic and radiologic studies should be done, and both carnitine and selenium levels evaluated, with replacement therapy in any having low levels. It is less clear, though, whether all EB patients at risk for cardiomyopathy should routinely receive carnitine or selenium supplementation, or undergo echocardiograms in the absence of clinical evidence of early cardiac involvement. Similarly, the presence of CHF should be monitored for in any EB patient who develops chronic renal failure.

Renal complications

Renal involvement is known to occur in EB, most notably RDEB-HS and the JEB-pyloric atresia syndrome (JEB-PA) [10, 39]. Renal injury may be caused by a

variety of different mechanisms. First, chronic ureteral blister formation and strictures may lead to hydronephrosis [24], although this usually does not lead to clinically significant renal impairment. A second and very important cause is glomerulonephritis, most commonly secondary to skin-derived streptococcal infections [27], although apparently it rarely may result from IgA mesangial disease. Third, secondary amyloidosis may develop within the kidneys [2, 3, 5, 6, 8, 21, 26, 27, 30, 35, 37, 41]. Finally, there is also one report of hereditary nephritis in a patient with a localized subtype ("pretibial") of dystrophic EB [20].

There are only a few longitudinal series from which frequency estimates on the risk of renal disease in EB have been extrapolated. In 1988, Mann et al. reported their experience with 100 consecutive patients with RDEB-HS who underwent diagnostic electron microscopy at the University of Heidelberg from 1973–1988 [27]. Two cases were found to have renal disease. One of these, in a 10 year old boy, was associated with significant azotemia (creatinine, 6.9 mg/dL (526 μmol/L)) which resolved without dialysis in two weeks. Biopsy demonstrated the presence of crescentric glomerulonephritis, and was associated with an elevated serum anti-streptolysin-O titer. A second patient, a 17 year old girl, had nephrotic syndrome (14.3 g/24 hr) and azotemia (creatinine 6.2 mg/dL (473 μmol/L); blood urea nitrogen 300 mg/dL). Dialysis was refused by the parents and the patient died within four days. Autopsy revealed amyloid deposits within the kidneys, liver, and spleen, and bilateral renal vein thrombosis was also present. The findings in this series, if representative of all RDEB-HS patients, would suggest that the frequency of glomerulonephritis and secondary amyloidosis in this EB subset is roughly 1% each. However, these estimates may not adequately measure these risks, since they do not represent an unbiased sample of the EB population but instead reflect data only on those patients who were referred for diagnostic confirmation via electron microscopy.

The presence of nephropathy was sought in a second smaller series of RDEB patients, prompted by an electron microscopy-proven case of renal amyloidosis in a 17 year old male [21]. Eleven patients were studied (7 males, 4 females; mean age 17.7, with a range of 5–28 years). Nephropathy was defined as the presence of both proteinuria and hematuria (with erythrocyte casts) on three or more occurrences; although creatinine levels were obtained, results were not reported. Seven of 9 patients with generalized RDEB met these criteria; three developed end-stage renal failure, two of whom died within two years of the onset of renal impairment. In contrast, neither of two patients with localized RDEB developed renal disease. Elevated serum amyloid A (SAA) protein levels were detected in all 11 patients, regardless of their renal status. The highest SAA levels were noted in those patients who had nephropathy, with an average level being 51 times above normal limits (range, 24.3–67.7×). This was contrasted with levels 38.2 times above normal limits (range, 22.9–53.6×) in those generalized RDEB patients lacking apparent nephropathy and with levels 6.1 times above normal limits

(3.1–9.0×) in those two patients with localized RDEB, neither of whom had measurable nephropathy. The authors argued that secondary amyloidosis was present in each case, based on the SAA levels, although amyloid deposition was proven by biopsy in only one patient, the 17 year old proband. Such a conclusion is unwarranted, however, based on the evidence presented, for several reasons. First, while they have demonstrated that microscopic hematuria and proteinuria are common in RDEB, they failed to exclude other sources of urinary blood and protein (i.e. from tears and ruptured bullae present within the uroepithelium which lines other portions of the genitourinary tract). Whether the frequency of urinary erythrocyte casts is as prevalent as they have suggested has yet to be confirmed by others. Second, other types of renal disease (to include mesangial proliferative glomerulonephritis) cannot be excluded in the absence of renal biopsy. Third, it is well known that any number of otherwise unrelated conditions, to include chronic infections, may be associated with oftentimes markedly elevated SAA levels, and that not all of these patients then develop biopsy-confirmed secondary systemic amyloidosis [11]. Instead, SAA acts as an acute phase reactant, increasing in response to tissue damage or inflammation. As such, elevated SAA levels are probably necessary but not sufficient to cause amyloid deposition within tissues.

There is similarly a paucity of published data on the risk of death from renal disease in the setting of inherited EB. Woo et al. for example, reported a fatal case of chronic renal failure in a patient with a generalized form of JEB who lacked concurrent pyloric atresia [46]. Of note, death was the result of intracranial hemorrhage and sepsis, not end-stage renal failure, and a renal biopsy was not obtained to confirm the underlying cause of renal disease, although hydronephrosis was excluded by ultrasound. Another case of systemic amyloidosis, confirmed by autopsy to be present within the kidneys, spleen, liver, and lungs, was reported in a 25 year old female with RDEB-HS [8]. She, however, also had metastatic squamous cell carcinoma, which is the usual cause of death in adults with RDEB-HS [18]. Cuesta-Estelles et al. described a 20 year old male with RDEB-HS with a history of recurrent urinary tract infections and macroscopic hematuria who developed nephrotic syndrome, azotemia, and hypertension [9]. This patient eventually died secondary to uremic coma after vascular access for dialysis could not be achieved. Although renal disease was attributed to mesangial IgA glomerulonephritis, renal biopsy was not obtained, preventing exclusion of amyloidosis or poststreptococcal glomerulonephritis as the underlying cause of renal failure.

Using data collected by the NEBR over a 16 year period, estimates were made on the risk of death from renal failure in the setting of EB [15]. Seven deaths occurred within this cohort. In none of these patients was there any evidence of chronic mechanical obstruction within the genitourinary tract, nor did any have concurrent diabetes mellitus or pre-existing hypertension. Two of these patients were treated by dialysis (hemodialysis 1; peritoneal dialysis 1). 3.55% and 0.52%

of patients with RDEB-HS and JEB-nH died of renal failure. When biostatistical modeling was performed, the cumulative risk of death from renal failure was 12.33% in RDEB-HS by age 35. None of these deaths occurred before age 14, with the highest conditional risk (6.0%) occurring during ages 20–25. In contrast, only one death each was seen in RDEB-nHS and JEB-nH, with cumulative risks of 1.16% and 0.67% by ages 25 and 1, respectively.

In contrast, renal impairment in JEB-PA has usually been attributed to hydronephrosis [10, 34, 36, 44], presumably the end result of recurrent bullae within the uroepithelium, especially at level of the ureterovesicular junction, leading to progressive scar formation and compromise of the ureteral lumen. When this literature was critically reviewed, 70 JEB-PA patients were identified, 51 of whom underwent surgical repair. Of these 51, hydronephrosis, ureterovesicular junction stenosis or obstruction, and renal failure (cause undefined) were noted in 7, 3, and 2 patients, respectively.

Based on the above collective literature, is it clear that about one in eight patients with RDEB-HS will likely die of chronic renal failure. As such, it would be prudent to routinely screen these patients for subclinical renal impairment, knowing the potential for fatality. It is less clear, however, to what extent aggressive therapy, most notably dialysis, can reduce the risk of death once renal failure has occurred. It is also unclear whether different underlying renal pathologies influences the risk of eventual fatality from renal injury in the setting of EB. In contrast, it is well known that mechanical injury to the ureters and other portions of the genitourinary tract, rather than injury to the glomeruli, occur in the setting of JEB-PA.

Genitourinary tract complications

In general, genitourinary tract complications are uncommon in EB, with the first report published in 1973 [24], most likely in a child with RDEB-nHS. It is now known that a number of urologic complications may occur in EB. Within the urethra, meatal stenosis or stricture and diverticuli may develop. Penile complications include scarring of the glans penis, hypospadias, and epispadias. Within the female external genitalia, partial fusion of the labia, narrowing of the vaginal vestibule, and urinary reflux into the vagina and uterine cavity have been reported. Bladder abnormalities include microscopic cleft formation, macroscopic blisters, bladder edema, cystitis, bladder infections, reduced bladder capacity, thickening of the bladder wall, and bladder extrophy. Ureteral complications that have been reported include stenosis of the ureter or ureterovesicular junction, ureteral fibrosis, ureteral reflux in conjunction with posterior urethral valves, microscopic cleft formation within the ureter, and hydroureter. Kidney findings include renal pelvis stenosis and/or microscopic cleft formation, hydronephrosis, pyelonephritis, recurrent urosepsis, and renal insufficiency.

Fine et al. used the NEBR database to estimate the frequency of urinary tract complications within the American EB population [16]. In general, urologic abnormalities occurred in only a minority (17–31%) of patients across all major EB types, with the highest frequency seen in JEB-H. Urethral meatus stenosis was the most common complication, occurring in 11.6% and 8.0% of patients with JEB-H and RDEB-HS respectively, and presumably the result of recurrent blisters and erosions. Urinary retention, hydronephrosis, and bladder hypertrophy most often occurred in JEB-H, with frequencies of 9.3%, 7.0%, and 4.6%, respectively. Less than 3% of RDEB-HS patients experienced urinary retention. In contrast, pyelonephritis and cystitis were most often seen in the setting of generalized EB simplex (Koebner variant) and inversa RDEB.

A wide variety of urologic procedures have been performed in an effort to correct these complications. These have included cytoscopy, suprapubic cystostomy, ureterostomy, ureteral dilatation and stent placement, ureteral resection and reimplantation, urethral catheterization, urethral meatal dilatation, meatomy, lysis of the hymen and fused labia, electroresection of the bladder neck and valve, vesicostomy, ureterosigmoidostomy, nephrolithotomy secondary to urinary concretions, nephrostomy tube placement, and continent cutaneous urinary diversion. Although oftentimes successful, the literature also describes postoperative complications, presumably a reflection of the mechanical instability of some EB tissues. However, based on NEBR data, there does not appear to be any increased risk of complications from the performance of circumcision on infants with EB and, at least in this particular cohort, several uncircumcised adult males developed painful phimosis, leading to acute urinary retention and necessitating immediate surgical intervention. Therefore, there is no contraindication to circumcision in EB, regardless of the EB subtype present, and at least limited data suggest the potential medical value of pursuing this in infancy, to prevent phimosis during later life.

Given the overall relative infrequency of genitourinary tract complications, it does not seem cost-effective to pursue routine surveillance for these complications in the absence of obvious clinical findings or symptoms. One exception to this, however, is JEB-PA, since that particular EB subtype is well known to be associated with multiple genitourinary tract complications.

Osteoporosis and osteopenia

As discussed elsewhere (Chapter 2.2.5), acral mitten deformities are a well known complication of EB, primarily RDEB-HS. Until recently, however, little was quantitatively known about the risk of osteoporosis and osteopenia in these patients. In a recent publication [14], osteoporosis was documented in 39 children with EB, 32 of whom had RDEB. A correlation was noted between low bone mass

and reduced physical mobility. In another cohort of 42 adult RDEB patients (mean age 29; range 15–66), dual energy X-ray absorptiometry (DEXA) scans were performed in an effort to ascertain the extent to which osteoporosis and osteopenia occurred. Among all RDEB patients, the frequencies were 43% and 41%, respectively. When stratified by RDEB subtype, the highest frequencies were observed in RDEB-HS patients, with 60% and 32% having osteoporosis and osteopenia, respectively. Whereas no gender effect was noted, higher frequencies were noted among younger patients. The latter, however, may simply reflect the limited number of patients evaluated and means by which they were recruited for this study.

On the basis of these studies, it is clear that osteoporosis and osteopenia are significant issues among RDEB patients, even in those lacking growth retardation or evidence of acral deformities. It is also likely that bony abnormalities occur in other severe EB subtypes, most notably JEB-H. The occurrence of osteoporosis in EB patients is of clinical importance, since it is well known that osteoporosis is a major risk factor for bony fractures. There are many known causes of osteoporosis which might be particularly relevant to the development of this complication in severely affected EB patients, to include chronic malnutrition, concurrent renal insufficiency, low calcium intake, immobilization or impaired physical activity, increasing age, and if previous data on EB are incorrect, female gender [7, 38]. In the setting of severe RDEB, impaired nutritional intake may be a reflection of the severity of mucosal involvement within the oral cavity, esophagus, and small intestine, further complicated by decreased intake due to oral pain and chronic constipation. In support of the role of diet, one adult patient with RDEB who had developed multiple vertebral fractures was found to have low serum levels of 1,25 hydroxy-vitamin D [23]. Of interest, though, these findings also occur in lower frequencies than in RDEB-HS in other RDEB subtypes which lack these other risk factors, implying that there are other as yet undetermined factors that may be contributing to the development of bony abnormalities in less severely affected patients.

Although as yet unproven in a randomized double-blinded clinical trial in RDEB patients, these data raise questions as to whether calcium and vitamin D supplementation should become part of the routine care of severely affected EB patients. It is also possible that oral bisphosphonate therapy might play a worthwhile role (Chapter 3.4), although the severity of esophageal disease in many patients with RDEB might prevent it from being routinely tolerated in at least some. Performance of DEXA scans seems to be an easily performed and well tolerated diagnostic study in EB patients, regardless of their degree of clinical severity, since they may be performed without the need to remove dressings on the skin. In those patients found to have osteoporosis, it would be prudent to obtain laboratory tests to exclude those endocrinopathies (hyperthyroidism; hyperparathyroidism) which have been associated with diminished bone density.

Anemia

It is well known that anemia occurs in patients with severe EB, particularly RDEB-HS and JEB-H, but also to a lesser extent in some other subtypes. In most patients this appears to be multifactorial in origin, leading to anemia of chronic disease. Factors contributing to anemia in these patients include but are not restricted to chronic blood, iron, and protein loss from open wounds on the skin and through erosions present within the intestinal tract, and poor intake and absorption of iron and other nutrients. In one study, erythrocyte survival time was reduced in two patients with RDEB; this has yet to be confirmed by others. Variable degrees of anemia may occur, with some of the most severely affected EB patients having hematocrits in the 18–20% range. Such profound anemia undoubtedly contributes to chronic fatigue, a lack of feeling of well being, and impaired wound healing.

Iron supplementation by mouth appears to be of only limited benefit, even in those patients about to tolerate the gastrointestinal side effects of such replacement therapy, necessitating in some patients the need for chronic transfusions. Iron overload, however, appears to be a very infrequent complication. Parenteral administration of hematopoietin appears to have only a very modest impact on these patients, although it is used commonly by some physicians.

Gynecological considerations

Delayed puberty is the norm in adolescents with RDEB-HS and JEB-H, and may have a negative impact psychologically, as these young women are striving to meet the physiological milestones of their peers. Despite the severity of their disease, many women with generalized RDEB, including those with Hallopeau-Siemens disease, are sexually active. Somewhat surprisingly, with the exception of women with inverse RDEB, in whom severe perineal erosions are exceedingly common, most other patients are able to have intercourse without developing clinically significant erosions or blisters within the vaginal vault.

Having EB appears to have no negative impact on the ability of women to bear children. Indeed, vaginal delivery is well tolerated by most. As such, there appears to be no need for pregnant EB women to routinely undergo delivery via Caesarian section. Similarly, breast feeding may be tolerated by at least most of these women, if they choose to do so. There are no consistent data that suggest that pregnancy by itself is associated with either improvement or worsening of EB, although anecdotes have been reported for both outcomes.

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2.2.7 Premature death in epidermolysis bullosa

Jo-David Fine

It is well known that inherited EB may result in death, especially during early childhood. Unfortunately there are currently only a paucity of published evidence-based data available about the natural history of EB which will assist physicians and parents in determining not only the major risks for death but also the most frequent timing in which these risks present themselves. As a correlate, many of these medical complications are heavily influenced by EB subtype. A better knowledge of EB-associated risks of premature death will allow for more optimal surveillance and earlier intervention, thereby hopefully improving the prognosis of those affected by this disease.

In this chapter, each of the major causes of death will be identified, using data generated by the American National EB Registry (NEBR), the world's largest population-based cohort of well characterized EB patients, who were systematically followed for this purpose from 1986–2002.

Overall causes of death and years-of-life-lost (YLL) assessment

The major causes of death which are attributable to EB include sepsis, pneumonia, respiratory failure other than pneumonia, failure to thrive, renal failure, and metastatic squamous cell carcinoma [6]. Frequencies are highly dependent on EB type and subtype, as will be discussed subsequently.

Years-of-life lost calculations are commonly employed by insurance companies, using actuarial data, to demonstrate the impact of given events on overall

mortality. These are conventionally performed with a cutoff point of age 65. In the setting of EB, however, such a high cutoff may permit inclusion of other causes of death, such as atherosclerotic heart disease, stroke, EB-unrelated cancers (i.e. breast, ovarian, colon, and prostate), and car accidents, which may otherwise confound any assessment of the impact of EB itself on the risk of premature death. For this reason, a cutoff of age 50 was used for most YLL calculations on the NEBR database, with the data derived then compared to that of the overall American population [7].

Among all EBS types, the overall estimate of YLL was negligible (0.3 years). When separated into localized and generalized EBS subtypes, the highest impact was only 0.8 years, and seen among those patients with generalized EBS, primarily EBS-DM. This can be contrasted with the overall YLL observed in JEB by age 50, which was 19.4 years.

When separated in JEB-H, JEB-nH, and indeterminate JEB subtypes, YLL by age 50 were 19.8, 7.6, and 22.6 years, respectively, and rose to 30.6, 13.0, and 25.6 years when a cutoff of age 65 was employed. The highest YLL among these three JEB subtypes, which was seen in indeterminate JEB, is not surprisingly, since this subtype is routinely used as a diagnosis for those infants with JEB who die before they are old enough to have developed clinical features sufficiently characteristic to allow further subclassification. As such, they represent what many years ago was simply named EB letalis.

No significant YLL was observed among DDEB patients, regardless of whether age 50 or 65 was used for calculations. Among all RDEB patients, YLL were 10.3 and 29.9 years by ages 50 and 65. When separated into RDEB-HS and all other RDEB subtypes, YLL observed were 17.1 and 37.8 years, and 4.2 and 26.3 years, respectively, for ages 50 and 65. Consideration of the YLL at the higher cutoff is clinically relevant for RDEB since, as will be discussed, the major risk of death among these particular patients during adulthood is metastatic SCC, and this risk continues to rise well beyond age 50.

Risk of premature death, all causes

Lifetable analyses have been recently performed on each EB subtype in order to determine the cumulative risk of death (inclusive of all causes), for each of the major EB subtypes during childhood [4]. These curves are depicted in Fig. 2.2.7-1. No deaths were seen during the first 15 years of life among EBS-WC and DDEB children, consistent with the relative mildness of skin and extracutaneous disease activity in each of these two patient populations. Even by age 50 the overall cumulative risk of death was only 0.6% and 0.4%, respectively (Fine JD, unpublished data, 2008). Among those with EBS-DM and EBS-K, the cumulative

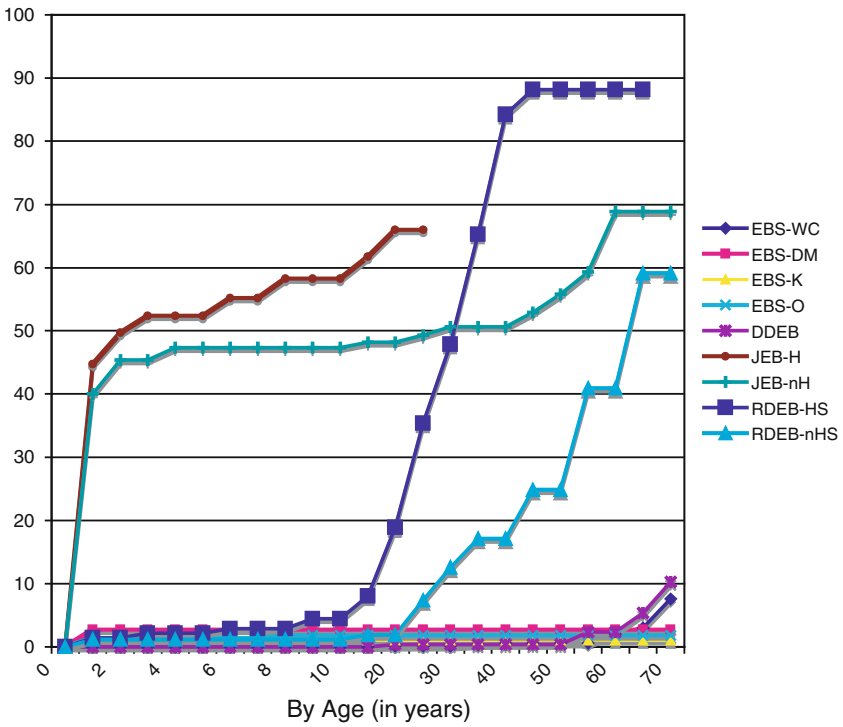

Fig. 2.2.7-1. Cumulative risk of death (all causes) in EB, as stratified by major EB subtype

risks for both subtypes at ages 1 and 50 were 2.8% and 1.1%. These data are consistent with published case reports by others suggesting that EBS-DM may be rarely lethal during infancy.

In striking contrast, the cumulative risks of death among JEB children by age 1 were 44.7% in JEB-H and 40.0% in JEB-nH, with cumulative risks rising to 61.8% and 48.2% in JEB-H and JEB-nH, respectively, by age 15 [4]. The highest conditional risks of death for patients with JEB-H and JEB-nH were within the first year of life (44.7% and 40.0%, respectively), with the second highest conditional risks between ages 1 and 2 (JEB-H, 9.1%; JEB-nH, 9.0%).

These very high cumulative risks of death in JEB infants may be compared to RDEB-HS patients, where the cumulative risks were 1.4%, 2.1%, 4.5%, 8.0%, by ages 1, 5, 10, and 15, respectively, but then rapidly rose to 18.8%, 35.4%, 47.9%, 65.3%, 84.2%, and 88.2% by ages 20, 25, 30, 35, 40, and 45 and above (Fine JD, unpublished data, 2008). Lower cumulative risks were seen in RDEB-nH patients, although by ages 55 and 65 they had risen to 41.0% and 59.1%.

Risk of death from failure to thrive

Failure to thrive is seen almost exclusively in JEB and may result in death during early infancy. Among NEBR participants, both cumulative and conditional risks of death attributed solely to failure to thrive were 16.7% and 4.0% in JEB-H and JEB-nH, respectively, by age 1 [4]. By ages 2 and above, the cumulative risks of death from failure to thrive rose to 20.5% in those with JEB-H, and remained at 4.0% for those with JEB-nH.

Risk of death from sepsis

Even as late as the 1980s, sepsis was considered to be a major clinical issue in EB infants and children, and was touted by many authorities as the leading cause of death. Although this may still be true in some countries, the widespread availability and use of broad spectrum anti-infective drugs, coupled with the routine use of sterile semisynthetic dressings, have reduced considerably the frequency with which sepsis arises and is untreatable in patients with EB. The most common pathogens encountered are *Staphylococcus aureus* and *Streptococcus*, both of which colonize the skin and may enter the bloodsteam via non-healing erosions and ulcerations. *Candida* may also be a cause of septicemia in some EB infants, usually a result of contamination of longstanding indwelling catheters and intravenous lines. Methicillin-resistant Staph. aureus (MRSA) is becoming an increasingly frequent pathogen, especially in those EB infants who acquire these as nosocomial infections via prolonged hospitalization, or as a result of chronic use of extremely potent topical antibiotics (most notably mupirocin) over widespread areas of the skin.

Within the NEBR population death from sepsis was observed in varying frequencies within most EB subtypes, with cumulative risks by age 1 ranging from 0.4% in RDEB-HS to 19.5% in JEB-nH [4] (Fig. 2.2.7-2). The highest risks of death from sepsis during childhood were observed in JEB. Among those with JEB-H, the cumulative risk was 11.4% by age 1 and 17.5% by age 8 and above, whereas the cumulative risk in JEB-nH reached levels of 22.9%, 26.1%, and 35.4% by ages 4, 30, and 60, respectively (Fine JD, unpublished data, 2008). Of note, the majority of deaths occurring in EBS-DM could be attributed to sepsis, with 1.9% of all such children having died of this cause by age 1.

In contrast, the risk of death from sepsis was much lower in RDEB-HS, even though the overall extent and severity of skin involvement in those patients was nearly identical to that of JEB-H. Among those with RDEB-HS, the cumulative risk of death from sepsis was only 0.7% through age 19, rising to 3.2% by age 20 and 8.5% in those aged 35 and above. An even lower risk was seen in RDEB-nHS (0.4% through age 24, and 1.8% thereafter).

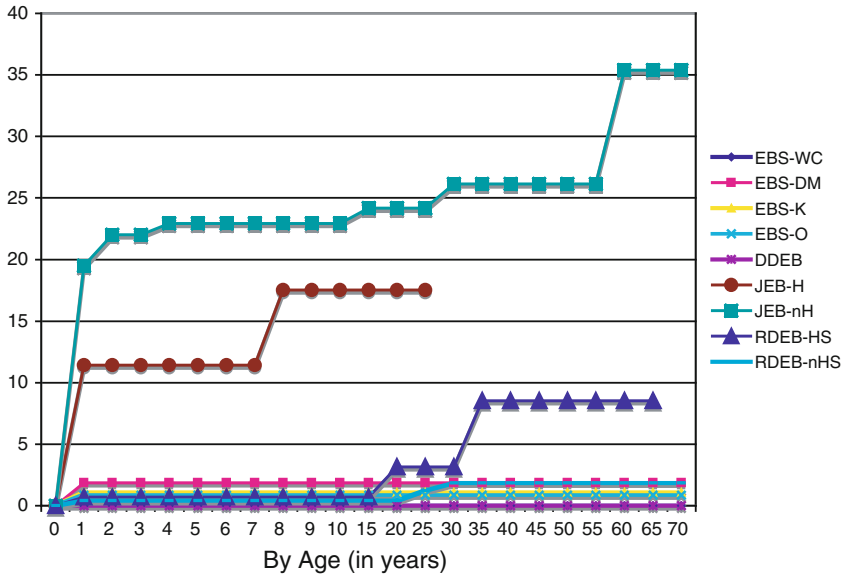

Fig. 2.2.7-2. Cumulative risk of death from sepsis, as stratified by major EB subtype

Death from respiratory causes

Two main causes of pulmonary death were recorded among the NEBR population, pneumonia and respiratory failure. No such deaths from pneumonia were reported among patients having any EBS subtype or DDEB. Through age 14, this was primarily a concern among JEB children, with cumulative risks of 3.0% for JEB-H children aged 1 and above, and 1.7% in JEB-nH patients aged 2 and above [4]. In older EB children and adults, death was attributed to pneumonia in higher frequencies almost exclusively among those with RDEB. By age 40, for example, the cumulative risk of death from pneumonia was 20.2% and 2.5% in RDEB-HS and RDEB-nHS (Fine JD, unpublished data, 2008). An unexplained increase to 10.0% and 9.6% was observed among those with RDEB-nHS and JEB-nH on or after age 55. It is possible that many or all of the deaths attributed to pneumonia in patients with RDEB may have been the result of sepsis secondary to endstage metastatic squamous cell carcinoma rather than primary bacterial infection of the lungs.

Respiratory failure was listed as the cause of death mainly among those with JEB and RDEB. Precise causes of respiratory failure could not be determined from the NEBR database, since those data were based primarily on death certificate entries. Among those with JEB, cumulative risks of 8.7% and 9.0% by age 1, and 14.1% and 13.8% on or after age 6, were reported in JEB-H and JEB-nH [4]. Since these curves are very similar to those for the cumulative risk of tracheolaryngeal stenosis or obstruction in these two JEB subtypes [2], it is very probable that the data on death

from respiratory failure reflect the risk of death from EB-associated upper airway obstruction, rather than from any other respiratory etiology. Of note, by age 1 the risk of death from respiratory failure was 0.9% for EBS-DM, which is intriguing, since it is known that tracheolaryngeal stenosis can rarely develop among EBS-DM infants, as well as more typically among those with JEB. In contrast, virtually no deaths were attributed to respiratory failure in RDEB patients prior to age 15, thereby mimicking the curves for risk of squamous cell carcinoma among those with RDEB. The highest cumulative risks for death from respiratory failure among RDEB patients were observed in those with RDEB-HS (8.4%, age 35) and RDEB-nHS (10.0%, age 55) (Fine J.-D., unpublished data, 2008).

Risk of death from renal failure

As discussed in Chapter 2.2.6, renal failure is a known complication of RDEB and, to a lesser extent, JEB [1]. Among JEB-nH patients, the cumulative risk of death from renal failure was 0.7% on or after age 1. In contrast, no deaths were attributed to renal failure among RDEB patients until age 20. By age 35, however, the cumulative risk of death from renal failure was 12.3% and 1.2% among those with RDEB-HS and RDEB-nHS, respectively.

Death from squamous cell carcinoma

As discussed in greater detail in Chapter 2.1.3, squamous cell carcinomas (SCCs) arise primarily in the skin of patients with RDEB [5]. When the entire NEBR population was considered, rather than confined only to those having already been diagnosed with SCC, the earliest risk of death from metastatic SCC (0.03% by age 15) was seen among those with RDEB-nHS [3]. By ages 25 and 55, the cumulative risks among those with RDEB-HS, RDEB-nHS, and RDEB-I were 5.1%, 1.4%, and 0.0%, and 78.7%, 21.5%, and 11.7%, respectively. When similar analyses were performed on only those patients previously diagnosed with SCC, the cumulative risks of death from SCC by age 55 among those with RDEB-HS, RDEB-nHS, and RDEB-I were 87.3%, 60.0%, and 50.0%. These latter data emphasize how lethal cutaneous SCCs are when they arise among those with RDEB.

It is also known that SCCs may infrequently arise among patients with JEB, particularly those with JEB-H. Within the NEBR population, however, no deaths among its JEB patients have yet been attributed to metastatic SCC.

Summary

Some EB subtypes are definitely at higher risk of premature death than others. The causes of death among these EB patients may also be disease subtype-

specific. By better understanding the natural history of each EB subtype, particularly as it relates to the risk of death, it is hoped that earlier and more effective treatment can be implemented, leading to overall reduced mortality among these patients. It is no longer acceptable for the family of a newborn with EB to be globally advised that death during infancy or childhood is likely, since this risk does not apply to every EB subtype, and the psychological burden of receiving such an unqualified assessment may be devastating, especially during that difficult window of time when parents are only first beginning to accept their child's diagnosis and are struggling with the lifelong implications of having a child with EB. Similarly, decisions on whether to perform major medical or surgical interventions should never be based solely on the diagnosis of EB, since the natural history of this disease varies considerably even among individual subtypes, and early aggressive intervention for one complication may prevent another potentially fatal one from occurring.

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2.2.8 Psychological and sociological aspects

Jo-David Fine

It is well known among specialists who treat large numbers of patients with EB that rather profound psychological and socioeconomic issues may arise which impact on not only the patients themselves but also on their parents, their unaffected siblings, and their extended families. Psychological and psychiatric complications may accompany all EB types and subtypes, not just those with Hallopeau-Siemens recessive dystrophic EB (RDEB-HS) and Herlitz junctional EB (JEB-H).

Psychological impact on parents of having a child with EB

Interpersonal relationships

In a recent study of 425 EB patients, about 60% of all parents of children with JEB, dominant dystrophic EB (DDEB), and RDEB admitted to having interpersonal relationships with their spouses negatively affected by their childrens' disease [2]. Specifically, they reported a significant change in their private lives, to include lack of time or interest in talking to one another or engaging in any activities not directly related to the daily care of their children. Lack of interest and physical fatigue were cited as contributing factors. These parents believed that they were less emotionally close to their spouses following the birth of a child with EB, and up to half of all parents of non-EBS (EB simplex) children reported that their sex lives were significantly hampered as a result of the burden of caring for their affected child or children.

Decision not to have additional children

Approximately one quarter of EBS and JEB parents, and at least half of those with children with dystrophic EB, reported that they chose not to have additional children because of having already born one affected child [2]. This decision was not necessarily based just on the risk of having another affected child but also on their concerns about being able to provide a nurturing and financially supportive environment for additional children within their household.

Risk of divorce

The overall divorce rate among the major EB types ranged from 17–31% [2]. Whereas none of the parents of EBS children cited their child's disease as a factor in their decision to divorce, 50–87% of the other EB parents listed this as a major reason for the divorce. A variety of issues negatively impacted on these couples' relationships. In addition to the loss of emotional closeness and altered sexuality, many blamed their spouses for their child's having EB even though most of those patients had autosomal recessive disease which would have been arisen as the result of silent mutations passed on through both parents' genes. Others felt that their spouses didn't adequate understand their child's disease or couldn't accept and share in the burden of the child's day to day care. A much smaller minority (12–30%) attributed worsening of their personal finances to their decision to seek a divorce. Of note, even though it was common for parents of severely affected children to choose not to have additional children, this was not reported as a factor in the decision of some to divorce.

Impact on the family unit

Psychological and social impact

It is not uncommon for parents of severely affected children to focus much of their daily efforts toward their care. As a result, about 25–50% of all EB parents expressed a feeling that they sometimes neglected their other children because of the pressing needs of their affected child [2]. When parents unintentionally provide unequal attention to some of their children, this can negatively impact in many ways on those children who are unaffected by EB. Some may indeed feel neglected. As a result, some, at least during early childhood, may resent their affected sibling for competing for their parents' affection. Others may feel guilt in not having EB. With increasing age, some unaffected children may also have to assist in caregiving for their affected siblings, especially following divorce of a parent, further limiting or restricting their own day to day activities.

Social ostracization is not uncommon among families having severely affected children; in the National (USA) EB Registry cohort (NEBR) 30–50% reported this having happened to them (Fine JD et al., 2007, unpublished data). Many EB children are shunned from contact with unaffected children due to fear by others of the disease (or of accompanying secondary infection) being communicated to others. Disturbingly, many parents have been falsely accused of child abuse by other adults who were unaware of the nature of these childrens' skin disease, given the frequent similarity of EB wounds to those induced by severe trauma. Some parents also reported decreased social interactions with previously close friends, although no obvious changes were reported among relationships with relatives who were outside of the immediate family unit. While some forms of social isolation or alienation may be subtle, in other situations it may be far more blatant. In one family followed by the American EB Registry, for example, in which both children had JEB-H, the family was shunned from participating in their rural church, for fear by the congregation that the childrens' non-healing wounds represented the mark of Satan. Although this is certainly an extreme example, variations on this theme are frequently recounted by parents.

Financial impact

Having even one child with EB may financially cripple a family. The costs associated with wound care and nutritional support are oftentimes extremely high, and at least within the United States are only partially covered by governmental and most private health insurance programs. Many EB families cannot afford to hire even a parttime nurse or other skilled professional to assist them in the care of their affected children. This may eventually force one or both parents to leave fulltime employment in order to properly care for their child. Unfortunately, lack of employment of both parents adds even further to the financial pressures and woes of these families.

Even in those rare families that are able to financially survive in such a setting, the costs of caring for a single severely affected child can lead to many other unexpected economic sequelae within the family unit. These may include but not be limited to their inability to afford to often venture outside of the home for meals, take family vacations, participate in common recreational family activities such as attendance of movies, or be able to afford to own homes or purchase items, such as automobiles or new appliances, when the needs arise. These tenuous finances may also hinder unaffected children within the family from attending private elementary or secondary schools, or eventually attending college without the assistance of financial scholarships. Similarly, lack of funds may prevent one or both parents from pursuing additional education or training in order to better their own careers and the well-being of their families.

Impact on religious beliefs

It is common for patients of severely affected EB children at some point in time to question why their children have their disease. When we interviewed parents, many admitted to blaming God for their child's affliction. On the other hand, many of those who expressed this view surprisingly claimed that they became more religious as a result of having had an affected child.

Unfortunately, given the amount of time involved in the care of a severely affected EB child, over a quarter of all families that we have followed reported that they eventually had to give up routinely attending church, thereby adding to their social isolation and the lack of support from their communities.

Impact on patients with EB

Loss of self esteem and worth

Patients with more severe EB subtypes may develop loss of self esteem and develop symptoms of body dysmorphism as a result of their lifelong debilitating disease. This may be heightened in some during adolescence, when development of secondary sexual changes are delayed in comparison to others within their peer group. This may also arise if or when they encounter difficulties in the pursuit of common activities which we take for granted, to include achieving or maintaining employment, or attending college without physical or financial assistance.

Inability to cope

Given the rather incredible physical burden placed on EB patients and their families, it is remarkable how well some learn to cope with their affliction. At times, though, this becomes no longer possible, and is made worse if there is inadequate support available from others in the extended family unit or from the patients' caregivers.

Depression

Patients with EB, especially those with more severe, generalized subtypes, frequently admit to being depressed. This may be manifested in many ways. Some choose to withdraw from social contacts with others, including attending public schools. For others, depression may in part be characterized by increased laxity in their adherence to daily treatment routines or even in their activities of daily living [1], such as personal grooming. Some 40–45% of all JEB and RDEB

patients who were studied by the NEBR admitted to having at least once wished that they were dead, and in smaller minority (10–20%) of non-EBS patients, suicidal ideations, gestures, and even attempts were reported.

Summary

The psychological and socioeconomic impact of inherited EB may be devastating for patients and their families. Challenges begin at birth, first with the recognition of the disease and subsequently with the emotionally painful acceptance by parents of having born a potentially severely disabled infant. These challenges sadly do not end in infancy, however. Instead, they evolve over time, as new issues arise, both medically and socially. All health care professionals need to be aware of these many needs, since they are not always as immediately obvious as the medical and surgical issues that continuously surround EB children and adults. EB patients and their families need not only medical support but also the assistance and guidance of psychologists, psychiatrists, and social services specialists, in order for them to live as normally as possible, despite their oftentimes profound physical debilities.

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3. THERAPEUTICAL APPROACHES

3.1.1 Wound healing

Christoph M. Lanschuetzer

All forms of epidermolysis bullosa (EB) are characterized by skin fragility and cutaneous injury. Hence, wound healing is the dominant issue of disease management in all types of EB. Due to ongoing blistering, persistent inflammatory activity, polymicrobial colonization with frequent infections, poor nutritional status, and oxygen supply, EB lesions often become chronic, non-healing wounds. This causes considerable pain, daily extended wound dressings with costs to the patient, family, and healthcare providers [36].

Potentially life-threatening sepsis may occur in infants and children with severe generalized EB, especially in those with JEB. The organisms usually are introduced through chronic nonhealing skin wounds. The risk of septicemia is presumably enhanced by the presence of impaired cellular immunity in more severely affected EB children [52]. Common organisms include *Staphylococcus aureus* and beta-hemolytic *Streptococcus*. The presence of longstanding indwelling catheters and intravenous lines may also predispose to septicemia from *Candidal* species.

Prevention of blistering

When managing EB, protection of fragile skin is of utmost importance; i.e. minimal and gentle handling is absolutely necessary. A cool environment, avoidance of overheating, and skin lubrication to reduce friction can all help lessen blister formation. A water or air mattress padded with foam may help reduce friction, as will a soft fleece covering or percale sheet placed over the mattress. Sheepskin is excellent for padding car seats, infant seats, or other hard surfaces. The young child with EB should never be picked up under the arms, but should be lifted from the bottom and the back of the neck and carried on soft,

non-irritating material. Clothing should be made of soft, non-irritating fabric, easy to put on, and simple in design. Socks and mittens can be used to prevent the infant from rubbing his hands and feet and from scratching his face. It may not be advisable to use a diaper on a baby with severe EB; instead a pad can be placed under the buttocks and the diaper area left uncovered. When a diaper is used, the area must be kept dry and clean.

Biology of cutaneous wound repair

Cutaneous wound healing proceeds in four subsequent phases (inflammation, reepithelialization, tissue formation, and tissue remodelling) [50]: In EB wounds all four phases of cutaneous wound healing can be impacted, leading to chronic non-healing wounds.

Inflammation is caused by mediators such as platelet and endothelium derived growth factors, vasoactive and chemotactic factors generated by the coagulation and activated-complement pathways and by injured or activated parenchymal cells. Substances such as fragments of extracellular-matrix protein, transforming growth factor β, and monocyte chemoattractant protein 1, recruit inflammatory leukocytes to the site of injury, activate fibroblasts, and initiate the formation of granulation tissue. Persistent inflammatory activity due to repeated ongoing friction or colonization and infection often arrests healing of EB wounds.

Gene expression profiles showed a fivefold increase in expression of arginase-1 (ARG1) in chronic wound samples as compared to non-wounded skin from two individuals with HS-RDEB [56]. Further, expression of seven other genes relevant to L-arginine metabolism also showed differences greater than twofold. L-Arginine is known to have a critical role in the synthesis of nitric oxide as part of normal tissue repair. ARG1 up-regulation is believed to impair wound healing by competing for arginine, the substrate for inducible nitric oxide synthetase, the latter having an important role in several aspects of wound healing including re-epithelialization.

Within hours after injury, epidermal cells from skin appendages undergo marked phenotypic alteration that includes retraction of tonofilaments, dissolution of most desmosomes, and formation of peripheral cytoplasmic actin filaments, which allow cell movement [23, 26]. Furthermore, epidermal and dermal cells no longer adhere to one another, because of the dissolution of hemidesmosomal links. The expression of integrin receptors on epidermal cells allows them to interact with extracellular matrix proteins (e.g. fibronectin and vitronectin) [11, 13, 41]. Within a few hours keratinocytes start to secret large amounts of laminin 332 (laminin-5). The activated leading keratinocytes migrate in a GTPase RhoA-dependent manner into the wound using the collagen-binding integrin α2β1 and

release a track of laminin 332 for the following keratinocytes. The laminin 332 deposited in the fresh wound was found to be unprocessed, whereas in the flanking healthy skin only processed laminin-332 could be detected [40]. Laminin 332 containing the unprocessed α3a subunit is expressed at the leading edge of actively migrating epithelial cells and has been shown to promote epithelial cell migration and proliferation through interaction with α3β integrin and stimulation of the mitogen activated protein (MAP) kinase signalling cascade [27]. Laminin 332 containing the processed form of α3a stimulates keratinocyte–matrix attachment and hemidesmosome formation [25]. This processing event involves proteolytic cleavage within the LG repeat region (between LG3 and LG4) and leads to a switch from interaction with integrin α3β on migrating cells to integrin α6β4 within hemidesmosomes of stationary cells. Recent *in vivo* studies [29] highlight the pivotal role of laminin 332 in the regulation of keratinocyte migration and basement membrane restoration for efficient epidermal wound repair. Laminin 332-deficient keratinocytes derived from patients with junctional EB-Herlitz demonstrated striking changes of morphological cell polarity and severely reduced average migration velocity by adoption of a specific saltatory mode of migration. These observations might provide an explanation for the severely impaired wound healing and chronic erosions with frequent development of exuberant granulation tissue often seen in JEB-H [47].

The migrating epidermal cells dissect the wound, separating desiccated eschar from viable tissue. The path of dissection appears to be determined by the array of integrins that the migrating epidermal cells express on their cell membranes. The degradation of the extracellular matrix, which is required if the epidermal cells are to migrate between the collagenous dermis and the fibrin eschar, depends on the production of collagenase [44], as well as the activation of plasmin by plasminogen activator, both produced by the epidermal cells [8]. Plasminogen activator also activates collagenase (matrix metalloproteinase 1) and therefore facilitates the degradation of collagen and extracellular matrix proteins. One to two days after injury, epidermal cells at the wound margin begin to proliferate behind the actively migrating cells. Growth factors such as epidermal growth factor, transforming growth factor α and keratinocyte growth factor may also stimulate these processes [55]. Cytokines and growth factors, such as interleukin 8, basic fibroblast growth factor, hepatocyte growth factor, granulocyte monocyte colony stimulating factor, leukotriene B4, and prostaglandin E2 were detected in blister fluid of EB patients [32]. Moreover, basic fibroblast growth factor was found to be elevated in the urine of patients with recessive dystrophic EB and it was hypothesized that this may contribute to the development of squamous cell carcinomas in these patients [4].

As re-epithelialization ensues, basement membrane proteins reappear in a very ordered sequence from the margin of the wound inward, in a zipperlike fashion [10]. Indeed, if the basement membrane is injured, laminin 332 produc-

tion increases rapidly. It then serves as a scaffold for cell migration, initiates the formation of hemidesmosomes, and accelerates basement membrane restoration at the dermal-epidermal junction. Finally, epidermal cells revert to their normal phenotype, once again firmly attaching to the re-established basement membrane and underlying dermis.

New stroma, often called granulation tissue, begins to invade the wound space approximately four days after injury. Numerous new capillaries endow the new stroma with its granular appearance. Macrophages, fibroblasts, and blood vessels move into the wound space at the same time. The macrophages provide a continuing source of growth factors necessary to stimulate fibroplasia and angiogenesis; the fibroblasts produce the new extracellular matrix necessary to support cell ingrowth; and blood vessels carry oxygen and nutrients necessary to sustain cell metabolism. Growth factors, especially platelet-derived growth factor, transforming growth factor β1, and basic fibroblast growth factor, in concert with extracellular matrix molecules, presumably stimulate fibroblasts of the tissue around the wound to proliferate, express appropriate integrin receptors, and migrate into the wound space. In fact, the appropriate integrin receptors that bind fibronectin, fibrin, or both on fibroblasts appear to be the rate-limiting step in the formation of granulation tissue [35, 44].

Fibroblasts are responsible for the synthesis, deposition, and remodeling of the extracellular matrix. Conversely, the extracellular matrix can have a positive or negative effect on the ability of fibroblasts to synthesize, deposit, remodel, and generally interact with the extracellular matrix. The provisional extracellular matrix is gradually replaced with a collagenous matrix, perhaps as a result of the action of transforming growth factor β1 [12, 54, 60]. Once an abundant collagen matrix has been deposited in the wound, the fibroblasts stop producing collagen, and the fibroblast-rich granulation tissue is replaced by a relatively acellular scar. Cells in the wound undergo apoptosis [16].

Management of EB wounds

Most EB wounds are covered by multiple layers of bandages or sterile non-adherent materials. On a daily basis, the dry outer layers of gauze bandages are carefully removed with scissors, and any adherent dressings underneath are gently soaked off, either by bathing (Fig. 3.1.1-1 to -4) or by application of wet (water) compresses. Older patients usually perform their own dressing changes, but parents, and when necessary, nurses or other healthcare givers, assist children in this procedure. The surface of the wound is then often covered by an ointment or cream, which may contain one or more antimicrobial substances (Fig. 3.1.1-6). New sterile dressings are replaced in reverse order, so as to cover and protect open wounds (Figs. 3.1.1-7 to -10). To prevent a tense, intact blister

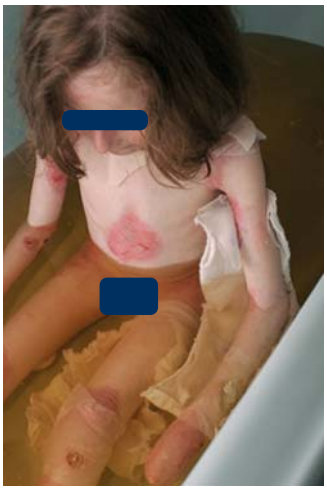

Fig. 3.1.1-1. A girl with RDEB-HS taking a full bath (with an antiseptic in the water) after cutting off her outer dressings

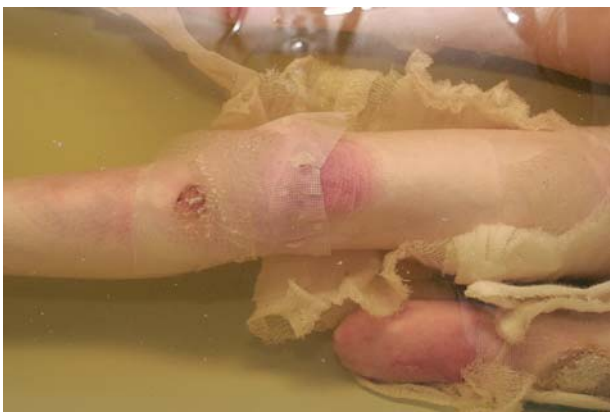

Fig. 3.1.1-2. Slight movement helps to remove any bandages that stick to the skin

from growing bigger and therefore from creating a larger wound, drainage of the blister under sterile conditions is performed (Fig. 3.1.1-5).

Once wounding has occurred, it is necessary to address a number of factors that influence the ability of a patient with EB to heal. An adequate blood supply is required for healing: in the more severe forms of EB, anemia is common and should be corrected as much as possible. Similarly, nutrition is frequently impaired in EB and should therefore be optimized (Fig. 3.4-1). Any necrotic tissue or foreign material, e.g. fibers, should be removed from ulcerated areas to facilitate healing.

Fig. 3.1.1-3. Removal of the gauze dressing by the patient herself

Fig. 3.1.1-4. Any remaining gauze is removed by a skilled nurse

An ideal dressing for EB should aim to maintain appropriate moisture levels, be nonadherent and atraumatic, promote a healthy wound bed, reduce pain, be available in appropriate sizes for the areas to be covered, increase the speed of re-epithelialization, and be inexpensive. Dressing choice will also depend on the type of EB, the relative need for mechanical protection, the level of exudate, and the presence of critical colonization or infection. Family and individual preferences, as well as cost, are also factors determining the choice of dressings. Soft silicone dressings (e.g. Mepitel, Mepilex, Mepilex Transfer, Mepilex Border (Mölnlycke Health Care, Dunstable, UK)) fulfill many of these requirements, and are therefore widely used on EB wounds [48]. Mepitel can be employed as a primary dressing in order that other therapeutic dressings, which otherwise might adhere, can be used safely.

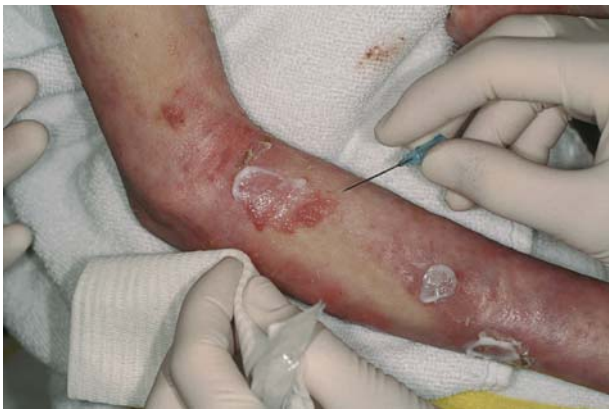

Fig. 3.1.1-5. Tense blisters are opened with a sterile needle and the contents drained

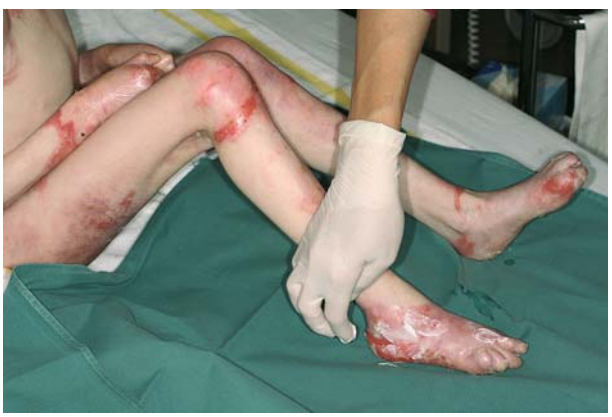

Fig. 3.1.1-6. The eroded skin areas are covered by an antiseptic cream

Fig. 3.1.1-7. The wound is then covered with a sterile nonadherent dressing (Mepitel)

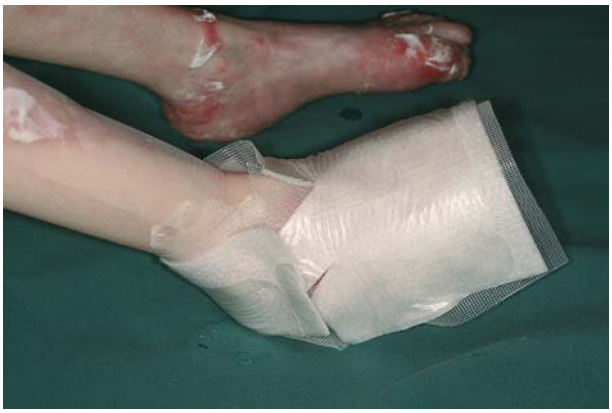

Fig. 3.1.1-8. Additional dressings are applied to better cushion the affected areas

Fig. 3.1.1-9. Sterile tubular gauze is wrapped over the nonadherent dressings until the entire areas are covered and well padded, so as to prevent further injury

Appropriate moisture balance is important to facilitate the healing of EB wounds, and can be influenced by the choice of dressings used. Hydrogel dressings (e.g. Flexigel (Smith and Nephew, London, UK), Vigilon (Bard, Covington, GA, USA), Curagel (Tyco, Gosport, UK)) are useful on drier wounds, helping to maintain a moist healing environment, as well as reducing pain and itching. Second-generation hydrogels (e.g. ActiFormCool (Activa, Burton-upon-Trent, UK)) are able to donate or absorb moisture from the wound and may provide some pain relief. For moderately moist wounds, padded dressings (e.g. Mepilex (Mölnlycke Health Care), Allevyn (Smith and Nephew)) can be useful, as they wick excess exudate away from the wound bed. Heavily exudative wounds may require especially absorptive dressings (e.g. Eclypse (Avancis)) to cope with

Fig. 3.1.1-10. The arms, wrists, and hands are now fully dressed

excess fluid and prevent the maceration and breakdown of surrounding skin. Barrier preparations (e.g. Cavilon (3 M)) may be helpful to protect intact skin in this context. EB simplex may present particular problems, as blistering around the edges of, or underneath, conventional dressings may occur. Cornstarch sprinkled on to pierced blisters may help them dry out and will reduce friction and prevent sticking, thus avoiding the need for dressings.

Bacterial colonization and infection will delay or prevent healing and should be confirmed with swabs if clinically suspected [37]. Generally, topical antibiotics should be used for only short periods of time because of problems with resistance and the potential for sensitization with agents such as neomycin and mupirocin. Acetic acid in a 0.25% solution can be used to bathe wounds (Fig. 3.1.1-1 to -4) or as a compress for 15–20 min daily, and may be helpful to reduce bacterial carriage, especially if pseudomonal overgrowth is suspected. If stinging occurs, lower concentrations can be used. Bleach, which also has bactericidal and viricidal properties, can be employed in a similar fashion, using 5–10 mL in 5 L of water and rinsing thoroughly afterwards. Silver has been used extensively for infected wounds, in both specialized silver dressings and as silver sulfadiazine cream. There are theoretical concerns relating to the systemic absorption of silver from these products, although data are scarce in patients with EB. Where available, 1% lipid-stabilized hydrogen peroxide cream may be useful in reducing bacterial colonization and infection. Ointments and dressings containing medical grade honey may also have a useful antimicrobial and anti-inflammatory effect [30]. Honey has also been shown to deodorize wounds and promote debridement in chronic EB wounds. Honey may reduce the pain of chronic wounds, although some patients may find increased pain because of the acidity of honey and its strong osmotic “pull”.

When healing does not occur despite the above measures, and malignancy has been excluded if appropriate, it may be necessary to adjust therapy. Occasionally, cellular wound dressings may be helpful to heal stubborn wounds (e.g. Apligraf (Organogenesis Inc., Canton, MA, USA), OrCel (Ortec)) [18, 49]. Cadaveric donor skin and xenografts (e.g. Oasis (Healthpoint, Fort Worth, TX, USA)) have also been used in EB as temporary dressings to try and promote healing [48].

It is important to remember that patients with EB, particularly recessive dystrophic EB, are at greatly increased risk of developing cutaneous squamous cell carcinoma. Tumors may resemble normal nonhealing EB skin, and therefore biopsies for histologic evaluation must be taken from any clinically suspicious areas of broken skin or areas which have failed to heal after appropriate wound care measures have been taken (Chapter 2.1.3).

Phenytoin

Phenytoin (diphenylhydantoin; Dilantin) is a anticonvulsant agent used in the treatment of grand mal and psychomotor epilepsy. In dermatology, phenytoin has been used to treat ulcers, epidermolysis bullosa, and inflammatory conditions. Its mechanism appears to involve its ability to inhibit collagenase. Its topical use for the promotion of wound healing seems promising but requires further trials. Common side effects include gingival hyperplasia, coarsening of the facies, and hirsutism. Rarer cutaneous side effects include drug-induced lupus, purple-hand syndrome, pigmentary alterations, and IgA bullous dermatosis. Phenytoin can cause generalized cutaneous eruptions that include a maculopapular exanthem, Stevens-Johnson syndrome, toxic epidermal necrolysis, vasculitis, and fixed-drug eruptions. This drug has also been linked to a hypersensitivity syndrome manifested by fever, rash, and lymphadenopathy. Patients receiving phenytoin may develop pseudolymphoma or, rarely, malignant lymphoma and mycosis-fungoides-like lesions. Prenatal exposure may result in a spectrum of structural, developmental, and behavioral changes known as the fetal hydantoin syndrome [46].

In some patients with EB, levels of collagenase are increased [17, 31]. Phenytoin inhibits collagenase *in vitro* [6]. By inhibiting collagenase activity, phenytoin has been theorized to stabilize collagen fibrils and thus decrease blister formation [6]. Phenytoin reduces the contraction of recessive dystrophic EB fibroblast-populated collagen gels [51] and modulates connective tissue metabolism and cell proliferation in human skin fibroblast cultures [38]. When fibroblasts are embedded within freely contracting, relaxed, type I collagen matrices, they are insensitive to phenytoin. However, if fibroblasts are grown in collagen matrices that are nonretracting and under tension, phenytoin stimulates cell proliferation and inhibits collagenase activity [24]. Patients with EB treated with phenytion had lower levels of inflammatory mediators such as

arachidonic acid in plasma and erythrocyte phospholipids than did untreated epidermolysis bullosa patients [15].

Some studies have shown that phenytoin is useful in treating recessive dystrophic EB and in reducing its blister count [1–3, 14]. The aforementioned studies not withstanding, however, a large study conducted by the Epidermolysis Bullosa Study Group did not find phenytoin effective in treating dystrophic epidermolysis bullosa [9] although this may have reflected problems in patient selection and recruitment.

Phenytoin may be effective in the treatment of other EB variants. It has also been used to treat junctional EB [5, 21, 28, 39, 42, 61]. One study found it effective in treating two patients with generalized atrophic benign EB (GABEB) and ineffective in treating two patients with Herlitz disease [21]. Others have found it ineffective for treating junctional EB. It has been used to treat the Dowling-Meara variant of EB simplex [33]. Topical phenytoin (2%–5% cream twice daily) has been found helpful in treating the ulcers of EB simplex [34].

Thymosin β4

Topical application of thymosin β4 is thought to enhance wound healing in patients with inherited EB. Suspected mechanisms include the promotion of migration and adherence of keratinocytes on wounds, or the upregulation of one or more extracellular matrix proteins known to play pivotal roles in the maintenance of the integrity of the dermal-epidermal junction.

Since late 2005, a clinical trial has been under way to ascertain whether the topical application of thymosin β4 can promote wound re-epithelialization in EB [20]. The study design is a randomized, double-blind one, involving three concentrations of the agent and a placebo control. Fifteen clinical centers US-wide are participating. Approximately 35–40 patients with RDEB or JEB, aged 2 or above, are being sought. The trial involves the treatment of a solitary wound 14–60 days old with a surface area of 5–50 sq. cm. The wound will be followed clinically and photographically on a weekly basis for up to 56 days (unless healing has already occurred), and serial computerized surface area measurements will be performed, to quantitatively document the rate of improvement.

Anti-inflammatory antibiotics in EB

After an initial report on two siblings with a generalized form of EBS who were treated with oral tetracycline for acne and who experienced a markedly decreased skin blistering activity [22, 45], a placebo controlled, double-blind study in patients (of whom only 6 completed the trial) with EB simplex

Weber–Cockayne found a positive response with reduced blistering tendency with tetracycline (1.5 g daily for 4 months) [53]. Four of these 6 experienced a reduction in their total number of EB lesions (mean reduction 59.5%, range 40.6–71.1%). In contrast, two experienced an increased number of lesions after 4 months of active therapy. Although a definite trend suggesting efficacy of active treatment was seen, confirmed by subjective reporting in the same four subjects experiencing objective reductions in blister counts, statistical significance was not achieved.

Anecdotically, treatment of dystrophic EB with sulfamethoxazole-trimethoprim was reported to be beneficial [7]. A double-blind, placebo controlled study is currently performed to rigorously test the efficacy of trimethoprim 4 mg/kg/day given twice daily in RDEB patients. Preliminary results from 7 patients showed a tendency for reduced blister formation, without however statistical significance (communication Dr. Elena Pope, Dermatology World Congress, Buenos Aires 2007).

In one case report systemic thalidomide treatment was documented to have had an excellent response to EB pruriginosa, a distinctive clinical subtype of dystrophic EB. Thalidomide was started at a dose of 50 mg once daily at night, with a reduction to 50 mg on alternate nights. The patient reported substantial reduction in pruritus (75% as subjectively assessed) and her leg pains resolved. Over a total of five years there has been a progressive improvement in the appearance of all affected areas [43].

Experimental wound care strategies

A simple protein-based approach for treating RDEB was recently tested in a mouse model. Intradermally injected human recombinant type VII collagen localized to the basement membrane zone of mouse skin and a RDEB human skin equivalent transplanted onto mice. Interestingly, such type VII collagen was organized into human anchoring fibril structures and reversed the features of DEB disease in the DEB skin equivalent [57, 58].

An alternative strategy to treat widespread wounds, i.e. to intravenously inject molecularly engineered RDEB fibroblasts (overexpressing human collagen VII) into the patient's circulation that home to the skin wounds and deposit the transgene product, was tested in the above mentioned mouse model. Injected fibroblasts homed to murine skin wounds and continuously delivered collagen VII at the wound site, where it incorporated into the skin's basement membrane zone and formed anchoring fibril structures. Wounds made on murine or grafted human skin demonstrated accelerated healing when the animals were i.v. injected with gene-corrected DEB fibroblasts [59].

Experimental wound care strategies have been recently summarized [19]. Such approaches include a tissue-engineered "chimeric" skin equivalent, containing keratinocytes from dystrophic EB patients and healthy donor fibroblasts, that is thought to supply sufficient collagen VII, to make the skin-equivalent graft therapeutically useful.

Further, several products containing autologous or allogeneic fibroblasts are currently tested in clinical trials to treat chronic wounds. Recent progress has been made in developing skin substitutes and "smart" systems, such as nanoparticles and microspheres, to deliver growth factors, drugs, and/or nucleic acids. Finally, the discovery that transforming growth factor-β3 (TGFβ3),which is high in fetal keratinocytes and fibroblasts but low in adults, dramatically improves the healing of surgical scars, might be valuable to future EB wound care.

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3.1.2 Pain management in epidermolysis bullosa

Elke Nischler

Pain and discomfort are universal features in all forms of EB, and their management is central to the well-being and quality of life of the patient [21]. Chronic pain can be a burden for children and families and can impair social functioning and school attendance. During the last decades researchers have discovered much about the causes, mechanisms, and treatment of pain. Additionally, much more is now known about the safe and effective management of pain in infants and children. However, bridging the gap between this knowledge and everyday clinical practice in patients with EB remains a major difficulty.

The sources of pain in patients with EB are multiple and often difficult to treat. Pain may be acute (e.g. from cutaneous or oral cavity blisters and wounds, gastrointestinal reflux, esophageal stenosis or spasm, tooth disease, corneal erosions, or anal fissures), chronic (e.g. from persistent inflammation of the skin, neuropathic pain, bone pain, constipation, or contractures) or procedural (e.g. related to dressing changes or bathing) [17]. Even the milder variants of EB may cause considerable suffering and limitation of normal childhood activities. EBS is often considered to be the mildest subtype of EB but Horn et al. showed that, in her Scottish cohort, EBS had a more marked negative effect on the quality of life than the non-Hallopeau-Siemens variants of DEB [11].

Pain measurement

Commonly used definitions of pain emphasize its personal sensory, emotional, and contextual nature, placing much reliance on an individual's ability to express what he or she feels when in pain [1]. Therefore individual quantification of pain

is central to the investigation of pain and analgesia. Self-reporting, usually by use of a linear visual analog scale, is regarded as the most reliable estimate of pain, but only children who have attained a certain degree of cognitive ability are able to accurately provide information this way [6]. Children eight or more years of age can generally use the same visual analog scales used by adults, which involve rating the intensity of pain along a horizontal ruler. For children from three to eight years old, self-reported measures use either "faces" scales (composed of a series of photographs or drawings of faces showing increasing degrees of distress) or color analog scales (e.g. rulers with increasing intensity of red color signifying increasing intensity of pain) [15, 22]. Infants and pre-verbal children may be among the most vulnerable to unrecognized pain. Here behavioral observations (e.g. facial expression, limb and trunk motor responses), changes in physiologic characteristics (e.g. heart rate), or combination of these indirect measures are used [12]. However, behavioral observations may underrepresent the intensity of persistent pain, as compared with self-reports [3]. Thus, pain assessment in neonates, infants, and children less than four years of age remains a challenge.

Frequency and severity of pain in EB

Fine JD et al. studied EB-related pain in 425 patients with inherited EB who had been followed longitudinally every 2 years as part of the NEBR project from 1992 to 2002. An estimated average daily level of EB-related pain was assessed using a linear scale of 0 (no pain) to 10 (unbearable pain) [9]. Cutaneous pain during childhood was most often a feature of RDEB. Among this particular patient cohort, only 5% of all RDEB children and 9% of RDEB adults reportedly were pain-free, compared to 12–14% of those with EBS, JEB, and DDEB. This observation is consistent with the extent of chronic surface denudation in most patients with RDEB, as well as the relative depth of lesions in this particular type of EB, which might be expected to be associated with exposure of dermal cutaneous nerve endings within such wounds [9].

Whereas 14–19% of all children with EBS, JEB, and DDEB were graded with pain levels of more than 5 (on a scale of 0–10), 32% of all RDEB children reportedly suffered this much pain [9]. A similar distribution in pain severity was seen in adult EB patients, although there was a tendency for affected adults to report greater pain. This finding may be consistent with the extent of skin surface areas usually involved in such patients, as well as the relative depth of those wounds that are chronically non-healing.

Pain prevention strategies

Besides pharmacological and psychological treatment strategies, pain prevention plays a very important role. Good dressing techniques are probably the

most important factors in the long-term pain management of patients with EB [10]. Dressings should be changed regularly in order to control blistering, and to prevent infection and potentially debilitating contractures from arising. Atraumatic nonadherent dressings (Chapter 3.1.1) should be used to provide comfort to the wounds, as well as to reduce procedural pain at dressing changes. Often dressings are removed in the bath (Fig. 3.1.1-1 to -4) or with wet compresses so as to minimize discomfort, but despite optimal nursing care these procedures can still be painful and poorly tolerated [7]. When analgesics are necessary, a combination of paracetamol and a suitable non-steroidal analgesic (NSAID) may be sufficient for dressing changes associated with small wounds. For pain associated with large dressing changes, especially postoperative dressings, potent analgesia and sedation, such as a combination of oral morphine solution and oral midazolam given 30–45 min prior to the procedure, may be required [10].

Physiotherapy and hydrotherapy are other approaches which may be beneficial in pain management and to maintain mobility and decrease contracture formation [10, 17]. A soft diet may be useful to overcome oral pain and dysphagia. Constipation, anal fissures and perianal ulceration (Chapter 2.2.4) can be managed with laxatives and increased dietary fiber [10, 17]. Another source of long-term chronic pain is dental and periodontal disease (Chapter 2.2.3). Therefore all patients should be encouraged to pursue good dental hygiene, even it itself may be very painful [10, 17].

Local analgesia

In the acute phase of blister formation, the expanding and oftentimes tense bulla can cause considerable pain and discomfort. Lancing of blisters will often provide some relief, and ethyl chloride spray used immediately beforehand may also help to reduce the intensity of pain [17]. Opioids may also be used topically for their effect on peripheral opioid receptors; 10 mg of morphine sulphate mixed with 15 g IntraSite gel (Smith and Nephew) can be applied directly to open wounds daily at dressing changes [17, 20].

Oral pain secondary to erosions and ulcerations may be reduced by the use of teething gels and local anaesthetic sprays or mouthwashes (e.g. benzydamine hydrochloride 0.15%). Gastroesophageal reflux, a complication that may occur in all types of EB but particularly in those with severe JEB and RDEB, should be treated with an H-2 blocker such as ranitidine or cimetidine and, if necessary, with the addition of domperidone and a proton pump inhibitor. Pain from anal fissures can be helped by softening the stool, lubrication, and the use of topical anaesthetics. If these fissures are severe and persistent, surgical intervention by a colorectal surgeon may be required. Corneal erosions can be particularly

painful and require ophthalmologic review (Chapter 2.2.1). Lubricating eye drops and ointments (Fig. 3.3-8c) may be helpful in milder cases.

General analgesia

For general analgesia, a stepwise approach is favored [10, 17]. For mild pain, simple analgesics, such as acetaminophen (paracetamol), can be used alone or in conjunction with a non-steroidal anti-inflammatory drug (NSAID), e.g. ibuprophen or diclofenac. For more severe pain, opioids, such as codeine phosphate or morphine sulphate, may be required. As with adults, the risk of addiction appears low among children receiving opioids for pain. Common side effects are mild respiratory depression, nausea, ileus, itching, urinary retention and, when used for a longer period of time, opioids often cause chronic constipation associated with painful defecation and abdominal pain [7, 14].

For brief painful procedures, fentanyl may be helpful, as it has a swift onset of action. Nitrous oxide is occasionally used for procedures, but should be avoided for more long-term analgesia.

Analgesic medications reduce the sense of pain, but feelings of anxiety and helplessness may remain. Therefore, when severe anxiety is present, a combination of analgesia, cognitive behavioral interventions, and an anxiolytic agent such as midazolam are often useful. Satisfactory anxiolysis by midazolam may be observed as soon as 10 min after oral administration and the duration of the effect is about 30 to 45 min [7]. However, administration of sedatives such as midazolam or chloral hydrate is not recommended outside a hospital setting. The major risk is excessive sedation or respiratory depression due to inadvertent overdosing or concurrent use of other medications.

There is also growing evidence that tricyclic antidepressants, most notably amitriptyline, and several anticonvulsants (to include gabapentin) may be beneficial for long-term pain control (e.g. neuropathic pain) [2]. Great care must be taken in their use, however, since there is now one report of death in an EB child attributed to the use of amitriptyline [19]. The use of other antidepressants, psychotropics and other drugs commonly used for chronic pain in other situations (such as in postherpetic neuralgia) has not been well researched in the management of EB, but it is our impression that they might be effective in this disease, although they can have side effects [10, 17].

Some patients have reported that intravenous or oral bisphosphonates help reduce bone pain caused by osteoporosis, although the effects on bone density have not yet been evaluated in EB [17] and these drugs may rarely cause avascular osteonecrosis of the mandible.

Psychological methods

In addition to pharmacological pain management, psychological methods of pain control may be of great benefit. Cognitive-behavioral strategies such as distraction, guided imagery and hypnosis by trained practitioners are useful in some situations and are generally liked by children [13]. They help the patient achieve a sense of personal control over the pain and any accompanying anxiety. For infants and younger children, comfort measures such as cuddling, swaddling, and suckling may reduce behavioral and physiologic responses to acute pain [4, 8, 16]. They are not regarded as a substitute for analgesia but are generally easy to implement and can be particularly effective at reducing the unpleasantness and distress of painful events [12].

Parents are increasingly recognized as important participants in pain management [18]. However, parents may sometimes underestimate and undertreat pain. To prevent that from happening, parental pain assessment tools have been designed so as to help parents better recognize pain and guide in the pain management of their children [5].

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3.1.3 Pruritus in epidermolysis bullosa

Elke Nischler and Anja Diem

Introduction

Pruritus or itching is defined as an unpleasant cutaneous sensation leading to the desire to scratch. It serves as a physiological selfprotective mechanism, as do other cutaneous sensations like pain, touch, vibration, cold, and heat, to help defend the skin against harmful external agents (e.g. parasites and plants) [38]. Pruritus can be directly evoked in the skin by chemical mediators and by physical and thermal stimuli. In addition, it can arise in the diseased peripheral nerve or even in the central nervous system [30, 35, 41].

Previously, it was believed that itch was received in skin by free nerve endings of cutaneous sensory C-nerve fibers in and around the dermo-epidermal junction as well as intra-epidermally [20, 31]. However, recent work suggests that the epidermis itself, especially the keratinocytes which form the bulk of the epidermis, constitute the itch receptor [18]. Keratinocytes express a range of neuropeptide mediators and receptors, which appear to be involved in pruritus, including opioids, nerve growth factors (NGF), substance P and receptors including vanilloid receptors and proteinase activated receptor type 2 (PAR 2) and voltage-gated ATP channels [16]. Thus, the epidermis and its associated ramifications of fine intraepidermal C-neuron filaments can be looked upon as the "itch receptor". The close interaction of sensory nerve fibers with keratinocytes and other skin cells is involved in cutaneous elicitation of itch and thereby represent potential targets for antipruritic therapy [31, 34].

When pruritus becomes chronic it has been shown that additional psychological factors are of increased importance [39]. Examples of factors that have

been associated with increased pruritus are stressful life events, emotional stress, psychiatric symptoms such as depression and anxiety, and personality features, such as interpersonal sensitivity, neuroticism and hostility [2, 15, 33]. Therefore supportive psychological or psychopharmacological interventions may be of potential benefit in the treatment of chronic pruritus.

Pruritus in EB

Pruritus is a major symptom of many skin and systemic diseases, but it is also a common and often very disturbing symptom in EB [26]. It is particularly troublesome in patients with dystrophic EB (DEB) and the Dowling-Meara form of EB simplex (EBS), but itching has also been noted in all other forms of EB [8, 14, 17, 22, 23, 32]. Supposed reasons of pruritus in these cases are the constantly necessary wound healing process, dryness of skin in the wound area and mild inflammation with activation of mast cells. Moreover, chronic rubbing and scratching leads to secondary skin lesions such as erosions, excoriations, crusts, hyperpigmentation or hypopigmentation, lichenification, prurigo, and scars and causes the release of inflammatory mediators that potentially induce or aggravate pruritic sensations resulting in an itch-scratch cycle [34].

Epidermolysis bullosa pruriginosa

In 1994 McGrath et al. described a distinct clinical subtype of DEB termed EB pruriginosa (EBP), which is characterized by intense pruritus, nodular prurigo-like lichenified lesions, which are mostly localized on the lower extremities and extensor forearms, nail dystrophy, and the variable presence of albopapuloid lesions [24]. Although the clinical signs had a clear overlap with the pretibial form of DEB [11], the lesions are more widespread and always associated with intense pruritus. The diagnosis of EBP may be difficult, particularly because intact blisters are rare and the onset of clinical features may only be evident some years after birth. In some cases the onset is much more delayed until the second or third decade of life [24, 25]. Thus EBP can be mistaken for acquired disorders, such as nodular prurigo (prurigo nodularis), lichen simplex chronicus, lichen planus, hypertrophic scarring, or dermatitis artefacta. As yet the cause of intense pruritus in EBP patients is not known. Some studies had implicated elevated IgE levels and immune predisposition to atopy in the pathogenesis of the pruriginosa phenotype, but others did not [4, 9, 25, 40]. In EBP to date, more than 15 different mutations with autosomal dominant, autosomal recessive and sporadic inheritance patterns have been reported in the literature [24]. In almost all patients glycine substitution mutations could be found within the region of consecutive Gly-X-Y repeats in the collagenous triple helical domain of COL7A1 [36]. However, identical COL7A1 mutations can cause remarkably heterogeneous

clinical phenotypes. Muarata et al. [27] described two patients where glycine substitution mutations by different amino acids in the same codon of COL7A1 lead to EBP in the one and to classical type of DEB in the other patient. Due to this high intrafamilial and interfamilial variability of expression, it is suggested that additional factors, such as genetic, environmental, metabolic, immunologic, hormonal, or other cutaneous or systemic factors, may be relevant to the resulting phenotype.

Therapy

General measures

Medical management of pruritus in EB, and especially in EBP, is often very difficult and mostly symptomatic. The first and most important steps in pruritus treatment are general measures that reduce itching (summarized in Table 3.1.3-1) and the specific treatment of a possibly underlying cause of pruritus, e.g. iron deficiency, atopy or others (as summarized in Table 3.1.3-2). Identifying and treating the underlying cause is the most effective therapy for pruritus.

In general a patient with a generalised itch should be advised to keep the body cool since the intensity of itching is usually enhanced if the skin is warm [13]. Cool compresses and cool baths may help relieve the itch. In addition, a cool environment at home and workplace helps. Light and non-irritating clothing, light bedclothes and a cool shower before bedtime keep the patient more comfortable at night. Topical menthol in calamine cream is also appreciated by most patients,

Table 3.1.3-1. General measures to prevent and treat pruritus

Avoid dry skin	Adequate intake of fluids Avoid overheating of rooms Avoid dehydrating agents (alcoholic solutions, powders, soaps) Avoid long and hot baths or showers
Cooling of skin	Use cool compresses (saline solution, vinegar or black tea) Cool baths Cool and non-irritating clothing and bed linens (cotton, silk)
Interrupt itch-scratch cycle	No scratching, but pressing, tapping, or softly massaging the skin Scratching an object instead of the skin Cutting fingernails short Use of cotton gloves at night Use of relaxing methods (autogenic training; progressive muscle relaxation; other)

Table 3.1.3-2. Specific causes of pruritus

Skin disorders	Psoriasis, atopic dermatitis, eczema, polymorphous light eruption, urticaria, mastocytosis, lichen planus, herpes simplex, herpes zoster, other
Systemic diseases	Cholestasis (e.g. primary biliary cirrhosis), chronic renal failure, Hodgkin's or other lymphomas, malignant tumors, polycythemia vera, iron deficiency, diabetes, thyroid disease
Drugs	ACE-blockers, AT-II-antagonists, antibiotics, anticonvulsives, analgesics (aspirin, celecoxib, diclofenac, ibuprofen, indometacin, ketoprofen, naproxen, piroxicam), immunosuppressive agents (tacrolimus, cyclophosphamide, cyclosporin, methotrexate, mycophenolate mofetil, thalidomide), antihypertensives, antiarrhythmics, beta blockers, calcium-antagonists, antidiabetic drugs, diuretics (hydrochlorothiazide), statins, hormones, hydroxyl ethyl starck (HES)
Pregnancy	
Neuropathy	e.g. brachioradial pruritus, vulvodynia, multiple sclerosis
Psychiatric disorders	Depression, schizophrenia, anorexia nervosa

since it causes a cooling sensation via thermosensitive TRP afferent nociceptor receptor channels [5, 37].

Dry skin, especially in EB patients, is invariably itchy and should be corrected by moisturisation. Patients should also avoid frequent and hot baths and excessive use of soap and other irritating agents that further dry or de-fat the skin.

Although difficult to implement, the importance of breaking the itch-scratch cycle should be clearly explained, as scratching leads to more itching. Tapping, pinching or gently massaging the affected area may help temporarily. Additionally, shortly trimmed fingernails and wearing of cotton gloves during nighttime may help to prevent unintended scratching during sleep. The learning of relaxation methods or psychotherapy may help the patient in coping with chronic pruritus.

Topical treatments

Topical corticosteroids may provide some relief from itching, e.g. a 1/10th dilution of 0.025% beclomethasone dipropionate ointment, with or without wet wrap bandaging [26]. In EBP patients, potent topical corticosteroids and intralesional triamcinolone have been reported to reduce the pruritus in some cases, but

did not produce sustained improvement [24]. Moreover, long-term use of topical corticosteroids compounds the skin fragility by inducing significant cutaneous atrophy and may cause systemic problems such as acne, hyperglycemia, growth retardation, and adrenal insufficiency when applied to large areas of skin [21].

Topical tacrolimus has been reported to be successful in the treatment of a patient with EBP [1] and may also be tried in EB patients with therapy resistant pruritus or concomitant atopic dermatitis. Tacrolimus acts on skin mast cells challenged with anti-IgE antibodies to inhibit histamine release in a concentration-dependent manner and impair *de novo* synthesis of prostaglandin D2 [7]. Additionally, it has been shown that tacrolimus diminishes the enhancing effect of interleukin-3 on anti-IgE antibody-induced histamine release from basophils [10].

For extremely pruritic papules and plaques some authors recommend a cryotherapy series with liquid nitrogen on localized prurigo lesions and under local anesthesia, as is oftentimes done in patients with nodular prurigo [6]. This would, however, result in blistering of the skin, which might not be welcomed by patients with underlying EB.

Systemic agents

Systemic therapy with antihistamines, corticosteroids, or etidronate have been tried with variable success in EB, but had no sustained effect [24, 26]. Antihistamines are usually poorly effective unless the pruritus is principally mediated by histamine, although the sedative action of the first-generation H1 antihistamines may be useful in some cases.

Low-dose oral cyclosporin has also been reported to be effective in EB associated with pruritus [3, 40]. However, since we know that neoplasia formation is one of the risks of long-term use of cyclosporin and DEB patients have an increased risk of developing squamous cell carcinoma, this treatment should be used with care and only for short periods.

In another patient with EBS Dowling-Meara and generalized pruritic blistering, dapsone (up to 150 mg daily) was reported to be beneficial [23]. Since, however, anemia is a common side effect of this drug, it should be used only with the greatest care in those subtypes of EB prone to multifactorial anemia.

For very resistant cases, especially EBP patients, where a vicious cycle has developed, in which excoriation leads to progressive deterioration in the skin, and where there is a substantial decrease in quality of life, oral therapy with

thalidomide may be considered [29]. The exact mechanisms of action that underlie the immunomodulatory, anti-inflammatory, antiangiogenic and anti-pruric properties of thalidomide are unclear [12]. There is some evidence that thalidomide may increase keratinocyte proliferation and migration, possibly via IL-8 [28]. Thalidomide also inhibits the production of interferon-gamma. Interferon-gamma knockout mice display accelerated collagen biosynthesis with increased TGF-beta production when wounded, which may be an explanation for the effect of thalidomide in wound healing [19]. However, one should be aware of peripheral neuropathy, an important and potentially irreversible side effect of thalidomide treatment.

In addition to the above mentioned therapies there are quite a lot of other specific and unspecific substances, especially ointments, lotions and bath additives, used by EB patients. It is certainly not easy to find a satisfying solution for this challenging problem, but in many cases at least some relieve of pruritus can be achieved by a combination of general and specific measures.

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3.2 debra-austria and the eb-haus Austria

Gabriela Pohla-Gubo, Rainer Riedl, and Helmut Hintner

History

Confronted with more and more patients with epidermolysis bullosa (EB) at the Department of Dermatology in Salzburg the father of one of our youngest patients told us about a patient support group in Great Britain called DEBRA-UK. At that time many of the Austrian EB patients went to London to find help and medical support. We were taught that this group has raised significant funds for an interdisciplinary clinical unit at the Great Ormond Street Hospital for children in which patients with EB were treated. This was the point when we decided to follow their example. In 1995 Franz Feichtlbauer and Helmut Hintner founded "debra-austria" as an Austrian patient support group. The most important goal at that time was to bring together families who suffer from EB and to facilitate the exchange of experiences between individuals who are confronted with an incurable disease. But very soon two major missions were defined by the new group: to provide the best medical support for patients and their families and to fund research in order to find a cure for this severe rare disease.

Thanks to the help of the experienced English DEBRA group we learned how to raise money, and came in contact with DEBRA Europe, the international network of EB support groups. Very soon we got to know all the relevant people in EB care and EB research. In the following years we established a multi-disciplinary team of doctors and medical specialists in Salzburg, which allowed us to take care of the Austrian patients in a professional way. We started to educate the new team and, perhaps even more

Fig. 3.2-1. A group of "butterfly children"

important, patients and their relatives in how to deal with this often devastating condition.

In 2001 the current president of debra-austria, Dr. Rainer Riedl, came up with the idea to establish an EB centre for medical care and research in Salzburg. Before the so-called "eb-haus Austria" could open its doors four years later – 1.8 Million € had to be raised. That was when debra-austria began a nationwide direct mail campaign and started a number of fund raising events in order to collect this enormous amount of money. It was at that time that the term "Schmetterlingskinder" ("butterfly children", Fig. 3.2-1) was introduced in Austria. The broad public was not familiar with expressions like epidermolysis bullosa or DEBRA but understood quickly what it means to live as a "butterfly child", having skin as fragile as the wings of a butterfly.

Two of the best-known advertising agencies in the country, Ogilvy & Mather and Lowe GGK, created what should become one of the most successful advertising campaigns ever in Austria (Fig. 3.2-2). This led to a situation where not only debra-austria was working very dedicated but kindergardens, schools, companies, social clubs and private individuals donated money to build the eb-haus. Shortly before the opening of the eb-haus financial support came also from the Austrian government by the help of the Austrian President, Dr. Heinz Fischer, and the Governor of Salzburg, Mag. Gabriele Burgstaller, who spoke for debra-austria to the Ministry of Health (Fig. 3.2-3).

Currently there is approximately 450 sq. meters of dedicated space for the eb-haus, situated on the campus of the Salzburger Landeskliniken (SALK) and affiliated with the Department of Dermatology of the Paracelsus Private Medical

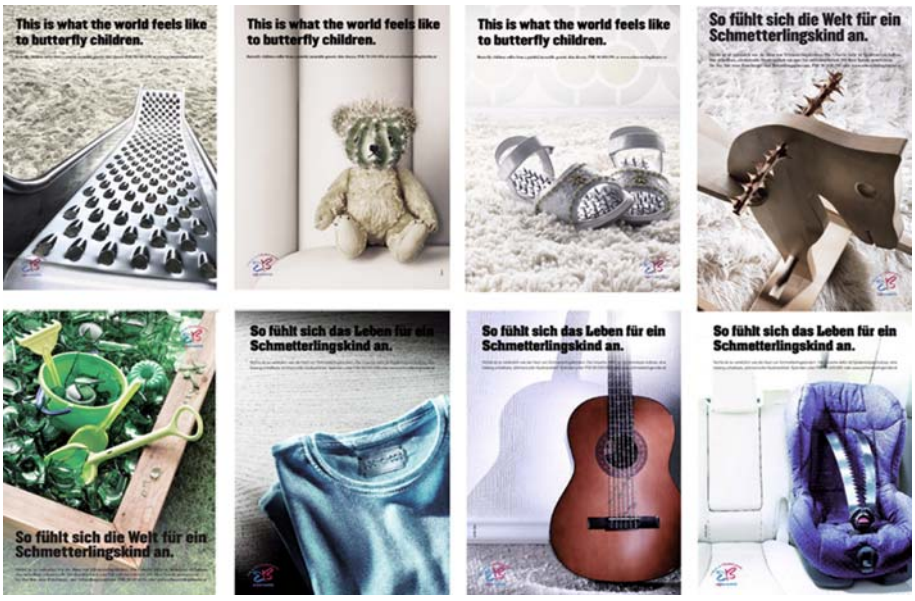

Fig. 3.2-2. Advertising campaign for debra-austria (created by Ogilvy & Mather and Lowe GGK)

Fig. 3.2-3. (left to right). Governor of Salzburg, Mag. Gabriele Burgstaller, Austrian President, Dr. Heinz Fischer, patients, parents and medical team at the eb-haus Austria

University (PMU), Salzburg. Within the eb haus (Fig. 3.2-4a, b), in addition to diagnostic and research laboratory space (Fig. 3.2-4d), are two examination rooms (Fig. 3.2-4c), one treatment room equipped for medical and surgical interventions not requiring general anesthesia (to include dental prophylaxis),

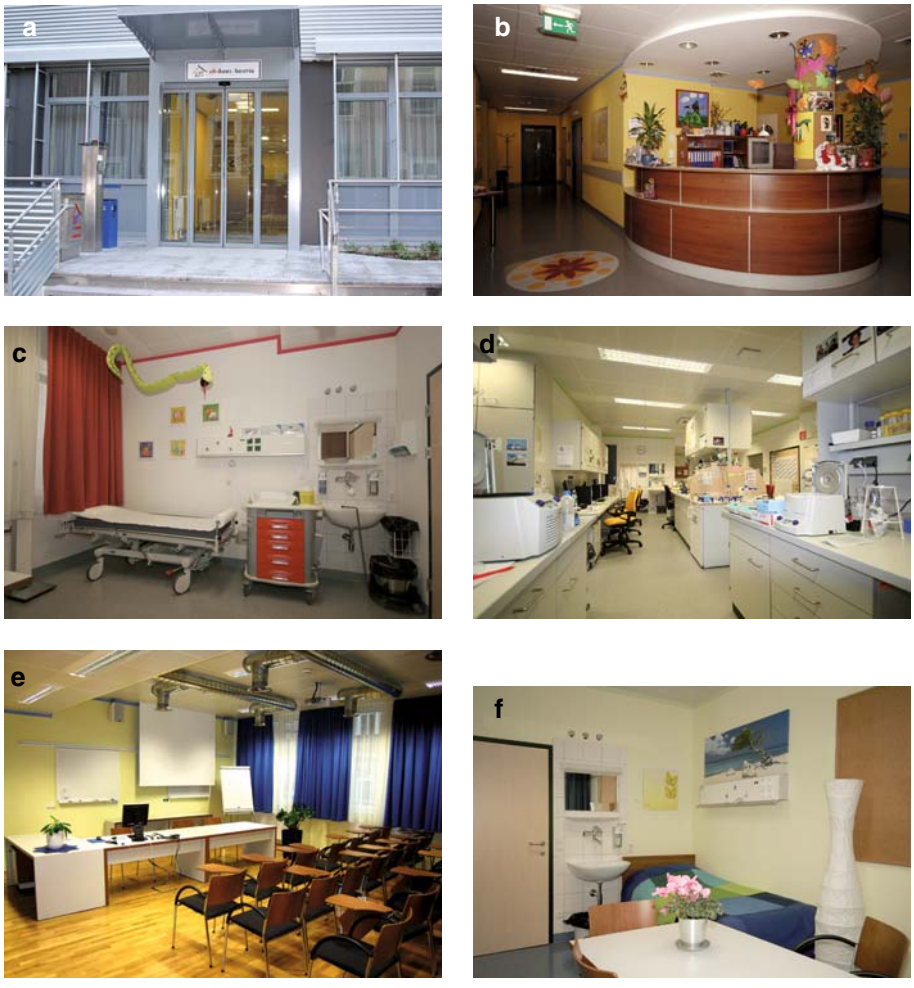

Fig. 3.2-4. **a**) Entrance to the eb haus Austria, **b**) office, **c**) outpatient unit, **d**) laboratory, **e**) seminar room (academy), **f**) multipurpose room

a steam room to facilitate dressing changes, a seminar room (Fig. 3.2-4e), and another multipurpose room (Fig. 3.2-4f), for both consultations and use by patients and their family (including kitchen and sleeping quarters).

Medical care and research at the eb-haus Austria

Medical care for people with EB demands a multi-disciplinary approach. Therefore the main goal of debra-austria and the Department of Dermatology of the PMU Salzburg is to provide top medical advice in all fields of medicine. The latter

works with a network of dedicated doctors and other medical specialists of the SALK. Debra-austria has to raise about 0.5 million Euro every year to cover the current personnel and material costs: this covers the costs for two EB specialists and two nurses, as well as a secretary and a team of scientists. The specialists of the eb-haus Austria are not only consulted by patients from Austria but also by EB sufferers from throughout the entire European Union and other countries. During the last three years the eb-haus Austria has developed into an international centre of excellence for EB care and research.

The activities in the eb-haus Austria focus on three major tasks:

- State of the art medical advice, treatment, and support are provided in the *EB Outpatient Unit*, which is managed by Dr. Anja Diem, EB physician. At present a multi-disciplinary team of about 40 medical doctors and other medical specialists offer what we call "all in one day" clinics (i.e. the patient can consult different specialists in quite a short time) or "all in one surgery" (for example, esophageal dilatation, hand surgery and dental treatment). In addition, clinical studies are performed. In the last two years many projects and studies including problems like pain management, itching, diagnosis and treatment of aggressive skin cancers have been started.
- With the ultimate goal to find a cure by developing a successful molecular therapy, a very dedicated team under management of Johann Bauer, Professor of Molecular Biology, is working at the *EB Laboratory*. Together with the Department of Dermatology and the Institute of Pathology of the Paracelsus Private Medical University Salzburg, this laboratory also provides routine diagnostics like histology, antigen mapping, electron microscopy, and mutation analysis.
- The main task of the *EB Academy*, which is managed by Dr. Gabriela Pohla-Gubo, is to provide continuous multi-disciplinary education and training for laypersons and experts. Establishing and maintaining national and international contacts with medical experts, scientists and patient support groups ensure that we improve daily our knowledge and experience in EB. This is obtained by inviting specialists, organising workshops and conferences, supporting public relations and increasing information on our website (www.eb-haus.eu). Of great importance is the establishment of an Austrian national EB-Registry, modeled after the US National EB Registry (NEBR), which has been a valuable source of epidemiologic and scientific information for over twenty years.

3.3 Surgical interventions

Barbara Ludwikowski

Patients with epidermolysis bullosa (EB) and particularly those with the recessive dystrophic form (RDEB) often require surgery within the oral cavity and gastrointestinal tract and on the hands. These patients are best approached by a multidisciplinary team. Preoperative preparation must be individualized, with special attention given to the potential anesthetic problems that are pertinent to EB patients. Among the challenges for the anesthesiologist are microstomia, ankyloglossia, intraoral blistering and sloughing (Fig. 3.3-1), and the possible need for tracheostomy. If endotracheal anesthesia is needed, the anesthesiologist should be prepared to perform fiberoptic intubation to overcome the problem of microstomia. Preoperative discussion with patients and parents of all potential problems and complications is very important.

For the preoperative preparation of children with microstomia, we recommend daily exercises with wooden spatulas to maximize the oral opening. When procedures under anesthesia are planned, it is best to coordinate as many surgical procedures as possible to avoid repeated anesthesias. For example, a dentist may be needed to treat dental caries, and surgeons to dilate the esophagus, place a gastrostomy tube to overcome dysphagia and malnutrition, or to release pseudosyndactylies to enhance the functionality of the hands. This team should be assembled and coordinated in advance; perfect cooperation of different specialists is required.

We usually admit the EB patient to the hospital the day before the operation in order to perform radiographs and blood tests, and to confirm the planned interventions. The staff that is involved in the treatment of these patients should be aware of the skin fragility and of the need to avoid the use of adhesive tape of

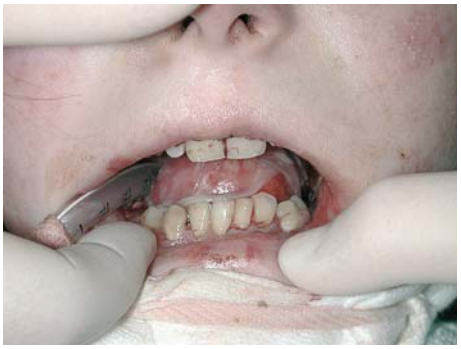

Fig. 3.3-1. Difficult intubation because of microstomia and ankyloglossia. Note sloughing of tongue and lip epithelia

any kind (Fig. 3.3-2) [2, 7]. This is important for fixing intravenous lines, routine anesthesia monitoring, draping of the patient in the operating room, and using electrocautery during surgery. Some patients have also an ophthalmologic

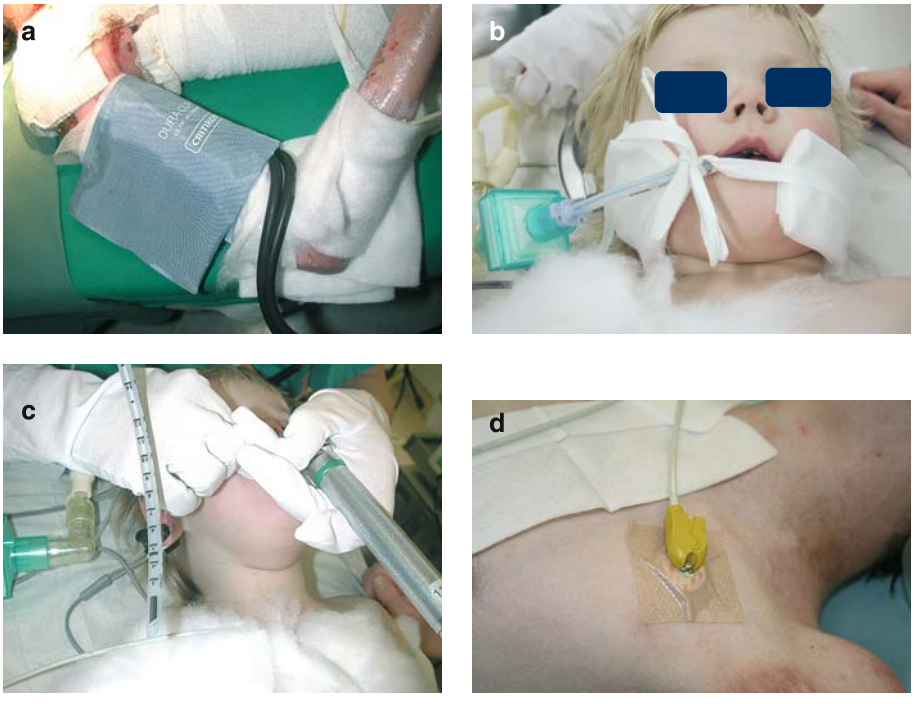

Fig. 3.3-2. **a** Cotton wool is placed underneath the blood pressure cuff to prevent further skin trauma. **b** Intubation tube is fixed with cotton ties. **c** Cotton gloves are used during intubation to protect the skin. **d** Electrocardiogram electrodes are attached with Mepifoam

involvement with dry eye syndrome, corneal erosions or blisters [6]. During the procedure the eyes should be lubricated (Fig. 3.3-8c) and postoperatively an ophthalmologic evaluation is recommended. The postoperative care of the patients should also be multidisciplinary.

Hand surgery for webbing and contractures

The most severe deformities of the hands are seen in children with RDEB (Chapter 2.2.5). Within this group there is a broad range of severity, depending of the subtype, ranging from nearly normal hand activity to severe pseudosyndactylies with total "skin cocooning" of the hand and adjacent wrist contractions [7, 8]. Typically children of preschool age are brought in by their parents for the first time to the surgeon to give them the ability to learn writing in school. Before school age most children can handle their lives and there is no wish for a release of the digits. Children with RDEB should be evaluated within the first 1 or 2 years of life, so that digital dressings (Fig. 3.5-3 to 7; Chapter 3.5) and splinting can be begun, so as to delay web formation [2]. Hand deformities may be cutaneous or articular or both. Photographic documentation and radiographic investigation are recommended before surgery in order to make a precise diagnosis. Cutaneous involvement may include retractile scars, absence of nails, atrophy of soft tissue of the finger tips, dermal cocooning and pseudosyndactyly. Articular manifestations may include flexion contractures of the fingers in the interphalangeal and metacarpophalangeal joints. Longstanding contractures result in soft tissue loss, because of inappropriate use of hand muscles, sometimes in shortening of flexor tendons or subluxation in the joints [19]. The neurovascular bundles of the fingers can also be contracted and result in ischemia when fingers are retracted. Hand surgery should be performed before deformities become irreversible, but this is very difficult to estimate. In most patients, contracture of the fingers has little effect on deep structures, permitting good function of the fingers after release of the skin and subcutaneous tissue.

Operative treatment

In the literature there is no consensus as to how to treat these hands. The aim of operative treatment is to improve hand function, particular to grasp and to pinch. For that, abduction and extension of thumb and fingers are necessary. Dorsal flexion of the wrist and extension of the palm of the hand is desirable, to gain a maximum of mobility and use of the hands in daily life. Surgical approaches depend on the severity of involvement, and always include the release of skin and soft tissue, thereby preserving the neurovascular bundles. Some surgeons prefer to perform hand operations using a well padded arm tournique in order to make the surgical field bloodless [13, 18]. We do not use this approach and prefer instead to achieve hemostasis with bipolar tweezers. Traction sutures hold the

fingertips and can avoid further blistering of the skin by minimizing mechanical trauma. The adductor contracture of the thumb is released by deep incision of the skin of the palmar and dorsal first web space. Pseudosyndactylies are often easily released by blunt dissection, and flexion contractions of the fingers by sharp X-shaped incision of the palmar side of the fingers between the proximal and distal interphangeal joint. Tendons are stretched during surgery. Surgical tendon elongation or corrective osteotomies of the axis are reserved for selected cases. It should be kept in mind that the blood supply is affected by stretching the vessels, and wound healing in these patients is prolonged, if compared to the normal population, with a higher risk of postoperative infections. The use of skin grafts is controversial. For lateral defects of the fingers after release of pseudo-syndactylies no grafts are recommended [18]. Split skin graftings for palmar secondary defects of the fingers and hand are used by most surgeons [13, 18, 19]. The use of a dermatome or freehand technique with a knife depends on the experience of the surgeon. This leads to further skin defects that may lead to atrophic scarring (Fig. 3.3-3); rarely, these surgical wounds heal only after rather prolonged periods of time. To avoid this problem different techniques, with only lifting the epidermis, are descibed [9, 13]. Early reports about skin graft therapy in EB had encouraging results, with a healing time of 10–14 days, and with stimulation of autologous closure of the wounds [9, 10]. Long-time results were not reported. Acellular allograft dermal matrix has been used in RDEB and may restore some features of normal dermal architecture, but the reported follow-up was only one year. We have seen a patient who underwent previous hand surgery in Russia without grafting; we later performed split skin grafting on this patient, with the same functional result. Since then, we have successfully used skin harvested from the fingertips, which are blistered during the procedure, to cover the tendons, as was described by Greider and Flatt [10, 11]. We have also chosen to leave the defects without skin grafts, as described by Vozdvizhensky and Albanova [20], Marin-Bertolin et al. [14], and Siepe et al. [17]. In these instances we covered the defect with silver sulfadiazine cream (Flammazine©; Silvadene©) to avoid postoperative infection. A non-adhering dressing (such as

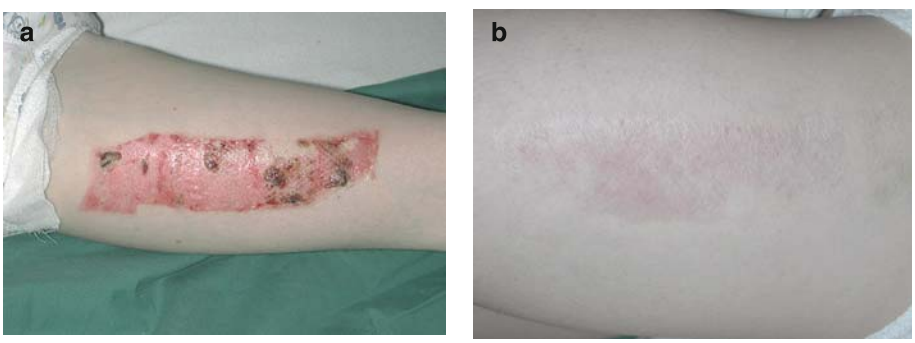

Fig. 3.3-3. **a** One week after split skin grafting of the right upper leg. **b** Two months after split skin grafting

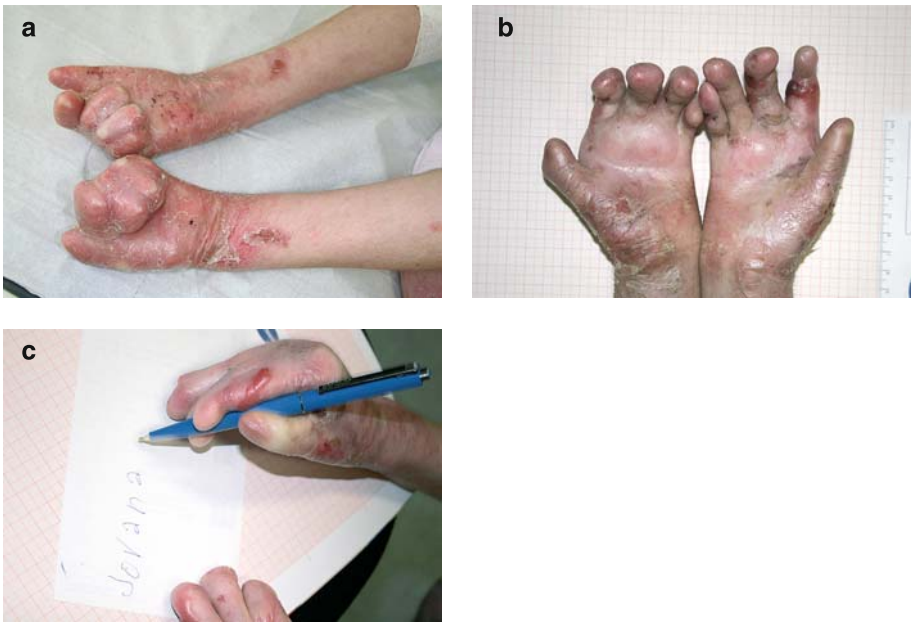

Fig. 3.3-4. A 16 year old girl with RDEB-HS with pseudosyndactyly and contractures before (**a**) and after (**b**) bilateral hand operation. A very satisfying effect of the hand operation was achieved (**c**)

Adaptic®) and cotton gauze were then applied. Using the retracting sutures, the thumb is extended and abducted, the fingers are extended, and the wrist is placed in dorsal or straight flexion. The position of the hand is then fixed with a cast. The time schedule for changing dressings is not uniform within the literature. Postoperative dressing changes vary from daily [8], weekly [13] to biweekly [18]. Because our first dressing change is performed under general anesthesia and dressing changes in EB children are oftentimes traumatic, we prefer to extend the time for the first dressing change to 2 weeks postoperatively and then 10 days later (Fig. 3.3-4 to -6). After that time anesthesia is seldom required for dressing changes. After 4 to 6 weeks the hands are usually fully healed, the fragility of the skin is improved, and the thermoplastic splints (Fig. 3.3-7) can be adjusted. At that time normal function of the hand should be exercised with the help of physical therapists (Chapter 3.5); during both day and night the splints should be used. Successful postoperative care includes management with static and dynamic splints. Education and involvement of both patients and their families are essential. Optimally, patients should be cared for by an multidisciplinary team including surgeons, nurses, physical therapists, and psychologists. The results are influenced by the input of the patient. The postoperative period, and the consistent use and regular adjusting of splints, may be very difficult for the patient, his or her parents, and the therapists. Unfortunately, hand surgery has only a tempory result; contractures tend to recur over time. Within no more than

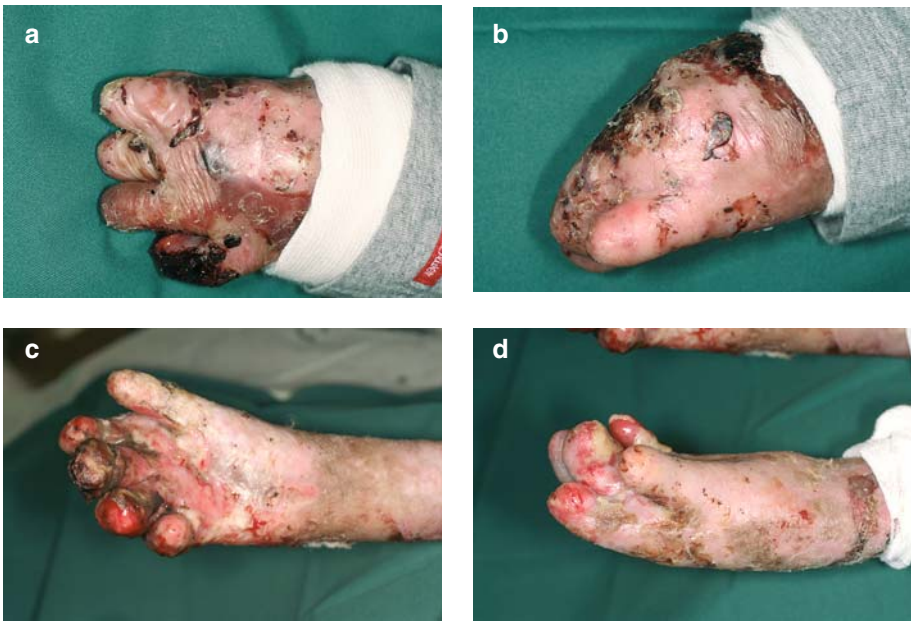

Fig. 3.3-5. A 23 year old man with RDEB-HS with severe hand deformities before (**a & b**) and after (**c & d**) hand surgery

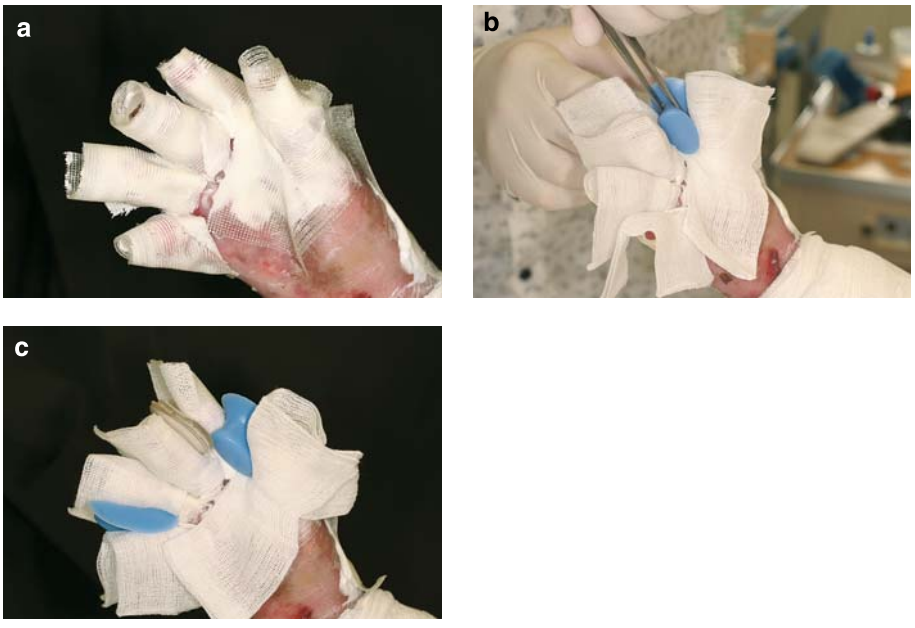

Fig. 3.3-6. **a–c** Wound care after hand operation

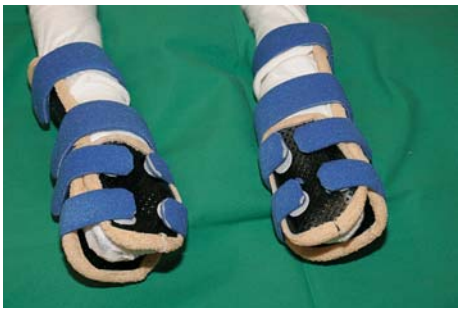

Fig. 3.3-7. An example of hand and wrist splints

five years most of these patients require further surgery to maintain optimal function [3, 20]. Recurrences may be more frequent in the dominant than in the non-dominant hand [11], because repeated blistering of the hands results in development of pseudosyndactylies [2]. On the other hand, we have seen in some patients that the non-dominant hand developed earlier contructures and pseudosyndactylies than the dominant one, because presumably in these patients the greater use of the dominant hand might have had a protective effect. Many of our patients prefer to use dressings on their hands more or less permanently, to avoid further blistering.

Treatment of esophageal strictures

Esophageal stenosis is treated by various methods including steroids, esophageal balloon dilatation or replacement procedures [15]. Steroids, tetracycline and phenytoin have been used in the past for systemic therapy, but none has proven beneficial [5]. Esophageal dilatation is indicated when strictures lead to dysphagia, varying degrees of obstruction, and/or an odor from accumulated food or saliva above the level of the stricture [2]. Tangential shearing forces induced by bougienage and endoscopy should be avoided whenever possible. The use of inflatable dilatator balloons, which produce vertical pressure, seems to be less mechanically traumatic [5]. Before dilatation we highly recommend a radiographic investigation of the entire esophagus with water soluble contrast media to evaluate the level, number and severity of strictures and webs, and to answer the question of whether gastroesophageal reflux exists. In addition, dissection of the esophageal wall, which can also lead to dysphagia, should be excluded. In the latter instances dilatation is useless and the patient is treated instead with H-2 blockers. Because most strictures (50%) arise within the proximal part of the esophagus and aspiration is likely, we prefer to use water soluble contrast medium instead of barium. As described before, balloon dilatation is the current treatment of choice. We used to perform bougienage with Savary-Gilliard dilators without complications, but more recently have changed to balloon

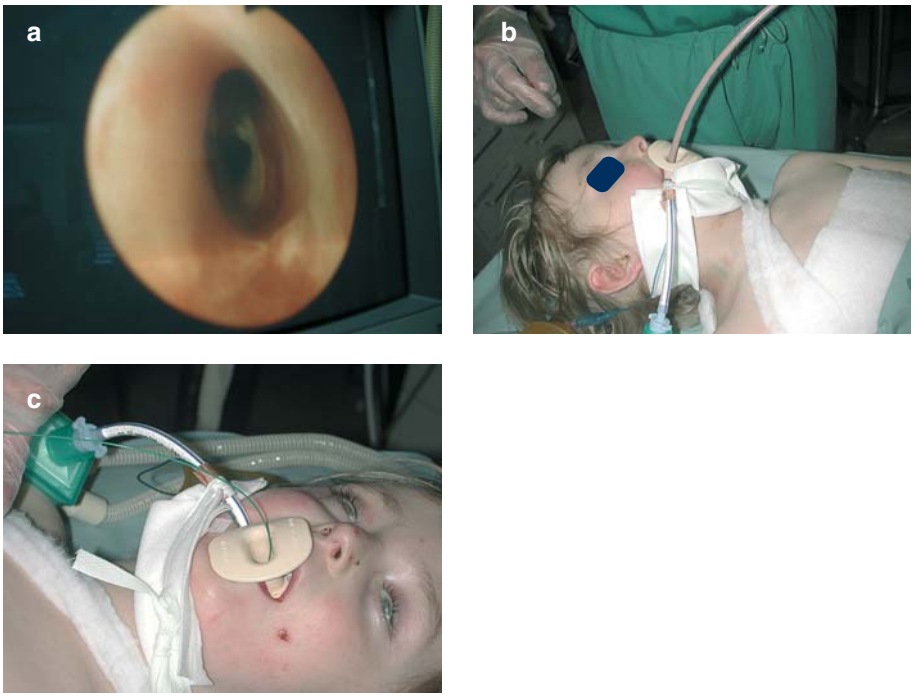

Fig. 3.3-8. **a** Intraoperative endoscopic demonstration of esophageal erosions and stenosis. **b** and **c** Dilatation of esophageal stricture. Note (**c**) the protection of the external surface of the eyes with sterile ophthalmic ointment

dilatation so as to avoid longitudinal shearing forces, especially in short strictures (Fig. 3.3-8). Perforation of the esophagus has been described with both methods. Balloon dilatation is difficult to perform in long strictures; for these cases the Savary-Gilliard dilators are still used [16]. It is important to recognize the presence of perforations early, so as to stent the esophagus with a tube in order to allow it to heal. Early antibiotic prophylaxis prevents mediastinitis. For that reason we perform a combination of esophago-gastroscopy with hydrostatic balloon dilatation. The balloon is filled with water soluble contrast medium; under radioscopy the stricture is then dilated. After dilatation, esophagoscopy with a baby gastroscope is performed to exclude a perforation.

From Great Ormond Street Hospital in London, Denyer and colleagues have described their experiences with fluoroscopically guided balloon dilatation under general anesthesia; their average interval between dilatations was about 10 months (15 days to 3.4 years). Azizkhan has reported on the use of fluoroscopically guided balloon dilatation [1] under general anesthesia; since 2001 his patients have received steroids (at a dose of 1–2 mg/kg/day of prednisone) at the time of dilatation. This is followed by a five day tapered daily dose of liquid

prednisolone, with the initial dose being 1–2 mg/kg/day. To reduce gastric acidity, his patients also received a proton pump inhibitor or H-2 blocker. The mean interval between dilatations was 13 months (range, 2 months to 6 years), with one patient remaining symptom-free for 6 years. An advantage of this method is the elimination of oropharyngeal trauma caused by endoscopy. In Salzburg we prefer very careful endoscopic control, to exclude esophageal perforation after dilatation.

Gastrostomy tube

A gastrostomy tube should be placed when height or weight gain falls below the expected rate, parents have intolerable stress by feeding and re-feeding, the child refuses prescribed oral medications, or when there is intractable painful defecation due to constipation [12]. The advantage of early gastrostomy placement is a greater acceptance of the device, with less awareness of altered body image by the child. Early gastrostomy also leads to less aversion to eating, and undernutrition and growth failure are more likely improved. As a result, wound healing may improve because of better nutritional support. Disadavantages of a gastrostomy tube are the dislike of the button by the patients, the risk of infection around the button site, difficulties with the equipment, reduced oral intake, bed-wetting when feeding time is overnight, and worsening of gastro-esophageal reflux. Gastrostomy makes nasogastric tube placement unnecessary, avoiding discomfort, and makes esophageal replacement (i.e. colonic interposition) unnecessary. The latter is desirable since the proximal anastomosis often heals poorly. Demirogullari et al. [4] reported on 10 RDEB patients who underwent esophageal replacement with colonic interposition, in whom 44% then developed postoperative anastomotic problems.

Techniques for gastrostomy tube placement

Different approaches for gastrostomy tube placement have been successfully used in patients with EB. Open, laparoscopic or percutaneous gastrostomy (PEG) can be performed, with the latter carried out using either an endoscopic or non-endoscopic technique. Preoperative radiographic investigation is necessary to exclude gastroesophageal reflux, because this can worsen after gastrostomy. Twenty-four hour pH monitoring is also performed, with particular care given when the probe is inserted [4]. We prefer an open gastrostomy to avoid endoscopy, but if a dilatation of the esophagus is required, then a PEG will be the choice. Laparoscopy has no advantage in children with EB. A standard Stamm gastrostomy is placed and we use balloon catheters. After the gastrostomy tract has healed and is well established, the original tube can be replaced with low profile gastrostomy buttons (Fig. 3.3-9). Button devices are also used primarily by some [4]. Feeding with water starts 24 hours after the procedure and then with

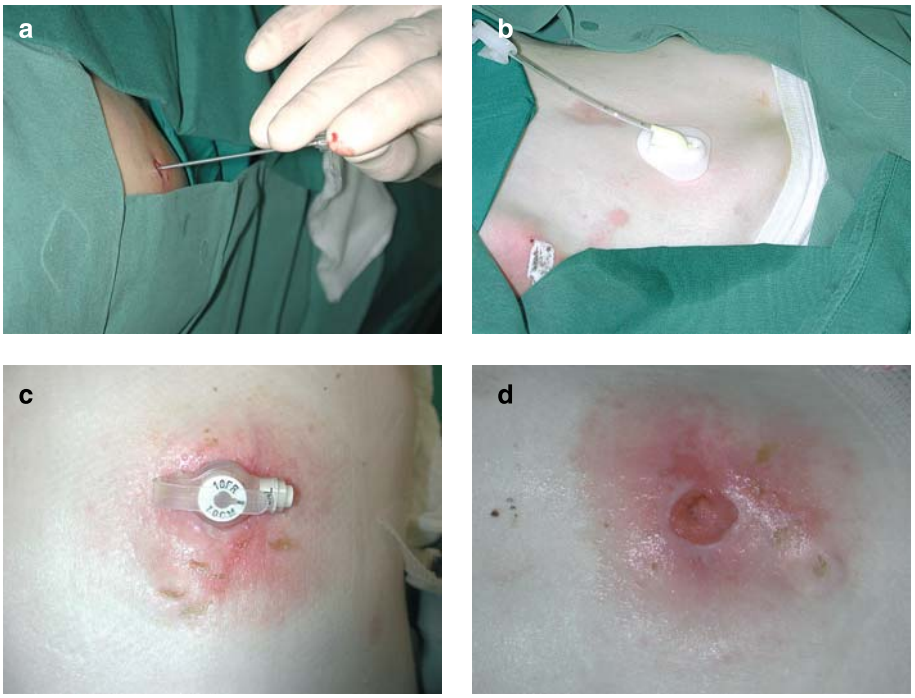

Fig. 3.3-9. **a** Percutaneous insertion of a gastrostomy tube. **b** The polyurethane PEG tube after connection to a silicone retention plate. **c** Skin erosion around a gastrostomy because of leakage of the balloon after four months. **d** Prolapse of gastric mucosa at the gastrostomy site

nutrient-dense feed. The child should have regular mealtimes, with the rest of the calories given via gastrostomy. We avoid feeding overnight if gastroesophageal reflux is known, because of the risk of aspiration. Complications of the procedure are iatrogenic oropharyngeal and esophageal lesions by insertion of a PEG; for that reason open gastrostomy is preferred.

Stoma management for gastrostomy buttons

The following are the recommendations given to patients with a gastrostomy:

- Mepilex is applied around the stoma, changing the dressing twice daily.
- No other dressings are applied around the stoma
- Prontosan Gel (containing hydroxyethylcellulose, glycerin, Polihexanid, water), a barrier cream, is placed on adjacent intact skin to prevent erosion from leakage.
- The skin around the stoma is cleaned only with water.
- It is not recommended that the button be turned daily in EB patients, to avoid blistering. Our experience has shown that it is better left alone.

- Ensure that the balloon is filled properly to avoid leaking and movement of the tube, which may otherwise cause granulation tissue to develop.
- Consider mucosal prolapse (Fig. 3.3-9d) in the case of possible excessive granulation tissue, as they both look very similar.
- If mucosal prolapse occurs, it is best to do a surgical revision or to place the tube in a different part of the upper abdominal area.
- It is best to remove granulation tissue surgically.
- Maxitrol eye ointment (dexamethasone 0.1%, neomycin 0.35% (as sulphate), polymyxin B sulphate 6000 units/g) can be used to control granulation tissue.
- It has been suggested that there are more healing problems due to the tubes being made out of silastic (for sensitization and allergy reasons) instead of latex, which causes a better biological scar.
- A common mistake if the site is leaking is to insert a larger tube. This can aggravate the problem as it makes the tract larger and may make leakage worse. It is best to take the tube out for several hours so that the hole shrinks by 20–30% and then add a one size smaller tube.
- Cutting a keyhole dressing from Acticoat, which is pre-moistened with water, can help the stoma to shrink down.
- Leakage may be caused by a problem with delayed gastric emptying; if this is suspected, the assistance from a gastroenterologist is needed.

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3.4 Nutritional support for children with epidermolysis bullosa

Lesley Haynes

Introduction

The most serious types of epidermolysis bullosa (EB) have been described as "severe, recalcitrant nutritional deprivation unparalleled in all of clinical medicine" [43]. Although potentially, this remains true today, a greater awareness of the role of nutritional support in the management of these children now exists. The dietitian is an important member of the multi-disciplinary team (MDT) whose collective expertise is needed for optimal management [23].

Nutritional support that is applied proactively has the best chance of optimising aspects such as resistance to infection, growth and sexual maturation, wound healing and overall quality of life. However, attempts to improve nutritional status are often sabotaged by factors beyond the dietitian's control, such as inadequate skin care, dental caries and periodontal disease, gastro-esophageal reflux (GER), fecal loading and overflow incontinence, and psychological and psychosocial issues. In addition, parents of very severely affected children inevitably prioritize aspects of care, and compliance with dietetic advice may be given low precedence. Cohesive MDT working can minimise these difficulties, but realistically, even the most well coordinated nutritional intervention may exert limited impact.

Nutritional compromise

Nutritional compromise is directly proportional to EB severity and occurs mainly in generalized forms of recessive dystrophic EB (RDEB) and junctional EB

(JEB) [4]. In these patients, multiple organ system involvement directly and indirectly affects the child's physical and emotional state. Infants and younger children with the Dowling-Meara sub-type of EB simplex (EBS) also often experience difficulties in maintaining satisfactory nutritional status. Conversely, excess weight may be gained in these EB types when lesions are mainly confined to feet, enforcing a predominantly sedentary lifestyle [23]. Nutritional compromise is the result of:

- the hypercatabolic, inflammatory state in which open skin lesions, with consequent losses of blood and serous fluid, increased protein turnover, heat loss and infection, all contribute to increased requirements. As in the patient with thermal burns, nutrient needs reflect the severity of lesions [18].
- the degree to which oral, oropharyngeal, esophageal and other gastrointestinal (GI) complications limit intake or affect absorption. Fecal loading, chronic constipation and painful defecation are extremely common and frequently cause apathy and secondary anorexia [3, 10].

Figure 3.4-1 illustrates the interactions between causes and effects of inadequate nutritional intake in severe EB.

In general, nutritional support aims to:

- minimize nutritional deficiencies
- alleviate the stresses of feeding
- promote normal body composition
- optimize growth
- promote pubertal development
- optimize bowel function
- optimize immune status
- optimize wound healing

These aims should be modified for Herlitz JEB (JEB-H) in view of its prognosis. Table 3.4-1 summarizes the nutrition-related complications of different types of EB, with the required dietetic interventions.

Denudation of the intestinal epithelium has been said to lead to impaired absorption of amino acids and other nutrients [15]. Some authors believe only the columnar epithelium of the GI tract to be affected [33] whereas others believe the abnormality is confined to stratified squamous epithelium, i.e. skin, mucous membranes, esophagus, bronchus and anus [32]. Inflammatory bowel disease has been reported in DEB [39] and GI mucosal biopsy findings have shown inflammatory changes that may be due to defective cell adhesion, perhaps resulting in abnormal mucosal permeability [35].

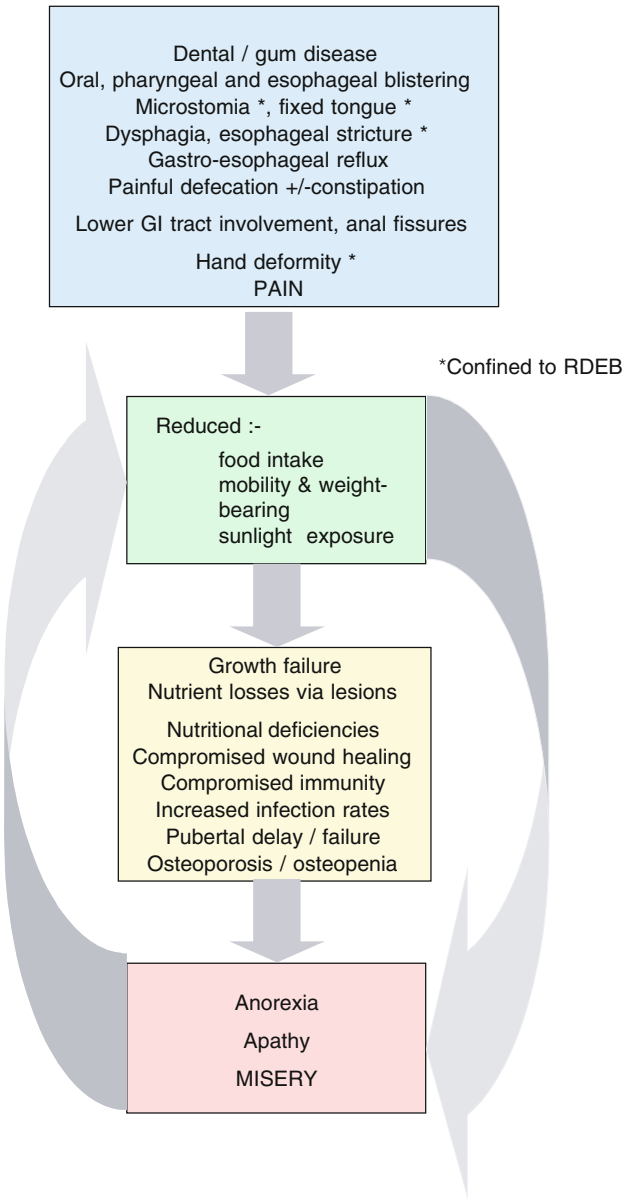

Fig. 3.4-1. Interactions between causes and effects of inadequate nutritional intake in severe EB (*Reproduced with permission of Blackwell Science*)

Some strictures arising in the upper third of the esophagus may result in part from the trauma of ingested food, explaining why many EB children prefer to eat only soft or puréed foods. Strictures in the distal esophagus may reflect damage from reflux of gastric acid [11]. As such, some physicians recommend pro-active

Table 3.4-1. Main complications and nutritional interventions in different types of EB (*Reproduced with permission of Blackwell Science*)

EB type	Complications affecting nutritional status	Nutritional interventions
Weber-Cockayne EB simplex (EBS-WC)	Lesions, usually confined to feet and hands, may be severe, especially in hot weather, often severely limiting mobility. Frequently painful defecation ± constipation.	Due to reduced mobility and activity, advice on weight maintenance/reduction may be required. Age-appropriate fiber (and fluid) intakes.
Dowling-Meara EB simplex (EBS-DM)	Generalized blistering tending later to become confined to hands and feet. Feeding problems often severe in infancy (especially gastro-esophageal reflux (GER) but generally resolve before teenage. Often painful defecation ± constipation.	As for RDEB (see below) in early years. Due to reduced mobility and activity, advice on weight maintenance/reduction may be required. Age-appropriate fiber (and fluid) intakes.
Herlitz junctional EB (JEB-H)	Recurrent moderate to severe lesions. Dental pain due to abnormal tooth composition. Good initial weight gain usually followed by profound failure to thrive; possible protein-losing enteropathy. Opioid analgesia often exacerbates constipation. Massive sepsis and respiratory complications. Survivors often profoundly anemic with osteoporosis/osteopenia consequent to immobility and possibly to malabsorption.	As for RDEB (see below), but as part of palliative care to improve comfort and quality of life rather than to intention improve nutritional status. Gastrostomy placement not routinely advised as likely to result in very poor healing around entry site, skin breakdown and leakage of gastric contents. Specialized formula feeds and exclusion diets may have a role if protein-losing enteropathy is suspected.

Non-Herlitz junctional EB (JEB-nH)	Recurrent mild to severe lesions. Dental pain due to abnormal tooth composition. Possible protein-losing enteropathy (PLE).	Global supplementation often required except in mild cases. Specialized formula feeds and exclusion diets have been used experimentally in suspected PLE.
Junctional EB with pyloric atresia (PA)	Mild to severe lesions. PA. Usually fatal in infancy, but there are exceptions.	As for JEB-H
Dominant dystrophic EB (DDEB)	Usually mild lesions. May have oral and esophageal involvement. Anal erosions/fissures can cause painful and reluctant defecation ± constipation.	Intervention generally not indicated other than age-appropriate fiber (and fluid) intakes.
Recessive dystrophic EB (RDEB)	When severe, recurrent moderate to severe lesions heal poorly with generalized scarring and contractures. Internal contractures cause microstomia, dysphagia and esophageal strictures. Anal erosions/fissures often cause painful and reluctant defecation ± constipation. Some develop inflammatory bowel disease/colitis. Refractory anemia. Osteoporosis/osteopenia in less mobile patients.	Global supplementation usually required except in mild cases. Esophageal dilatation ± gastrostomy feeding often indicated. Specialized formula feeds and exclusion diets have been used experimentally with patients with inflammatory bowel disease/colitis.

anti-reflux therapy. Esophageal dilatation is recommended whenever esophageal signs and symptoms indicate the presence of clinically significant strictures.

Abnormal hematological and biochemical findings have been reported not only in patients receiving no nutritional supplementation [3, 8, 14, 27] but also in those apparently receiving oral supplements and gastrostomy feeds [25]. Lack of compliance with recommendations may be the reason for a poor response in supplemented patients, and if suspected, should be investigated. Regimens should be adjusted to minimize undesirable side-effects e.g. zinc-induced nausea and iron-induced constipation.

Nutritional requirements

Nutritional requirements are difficult to assess due to [24]:

- the multi-system, inflammatory, infection-prone nature of the disease
- the variability over time of individual patient's requirements as a reflection of age, extent of skin lesions, presence of infection, need for catch-up growth, etc.
- the difficulties associated with estimating desirable weight gain when height is compromised due to chronic inflammation, pain, joint contractures and osteoporosis
- the difficulties associated with conducting clinical trials in such small patient numbers

Extrapolations from work with pressure ulcers and thermal burns form the basis of current practice. However, a rigorous evidence base is lacking even in these patients with normal skin. As in burns, nutrient requirements in EB are assumed to reflect the severity of lesions [18]. However, the megadoses of some nutrients recommended in burns are likely to be inadvisable in the long term. Despite improvements in nutrition, few EB children report associated improvements in wound healing rates. This is disappointing, but unsurprising, considering the intrinsic flaws in EB skin.

In spite of this, it would be wrong to assume that nutrition has no impact on the complex events surrounding attempted tissue repair. Efforts should always be made to optimize nutritional intake if deficiency or imbalance is suspected. More work is required to identify both the clinical conditions and the doses of individual micronutrients that actively promote or accelerate healing [37, 44]. "Immune enhanced" formulas containing nutrients such as arginine, glutamine and essential fatty acids are marketed for adults as promoting healing, optimizing immune status and exerting a beneficial effect on inflammatory conditions [40]. Although these would be highly advantageous in EB, their efficacy has not yet been tested.

When considering provision of adequate nutrition to optimise both growth and healing, best practice currently involves 3 main facets:

- Evaluation of factors compromising nutritional intake using a scoring system such as *THINC* (*T*ool to *H*elp *I*dentify *N*utritional *C*ompromise). *THINC* highlights the EB child at actual or potential risk of nutritional compromise. *THINC* should be used by the dietitian or health professional supervising the child's care, in cooperation with other appropriate professionals. *THINC* should aid, not replace, clinical judgment and be used with reference to Clinical Practice Guidelines for Nutrition Support in Infants and Children with EB [24]. *THINC*'s scoring chart rates 3 main aspects of the child's state; weight and length or height, gastroenterology, and dermatology. The maximum possible total score is 100; the higher the score, the greater is the likelihood of nutritional compromise. According to the total score, algorithms illustrate suggested care plans.

- Comparison of nutritional intake with that of age/height age and gender-matched unaffected children, with the addition of a factor to allow for increased requirements of certain nutrients [8, 23]. A formula estimates energy requirements using a calculation based on weight-for-height age and additional factors: blistering, sepsis and requirement for catch-up growth [8]. This provides a working figure, but the scoring of skin involvement is subjective and the formula is somewhat complex:

  Weight (kg) × (kcal/kg for height age) × [1 + (sum of 3 additional factors)]
  Additional factors =

  1. Ratio of blisters to body surface area (BSA):
     20% BSA = 0.19, 40% BSA = 0.5, 100% BSA = 0.95
  2. Sepsis: mild = 0.2, moderate = 0.4, severe = 0.8
  3. Catch-up growth: 0.1–0.2

  Example: 6 year old boy; weight 13 kg; height age (25th centile) = 4.7 years;
  20% BSA blistered; mild sepsis; stunted.

  Apply formula: $13 \times 90 \times [1 + 0.19 + 0.2 + 0.2] = 1860$ kcals = 143 kcals/kg.

  Using a simpler method, based on chronological age and UK Dietary Reference Values [2], increases in weight can be achieved by providing 100–150% estimated average requirement (EAR) for energy. Using the above example to estimate energy requirement:

  100–150% of energy requirement for chronological age = 1810–2715 kcals/day
  = 139–209 kcals/kg/day (average 174 kcals/kg).

Even the lower end of this range is likely to provide a significantly higher intake than that to which the child has been accustomed, so it is advisable to start here and monitor weight gain, increasing kcals/kg until consistent weight gain is achieved.

Protein requirements should be based on 115–200% the UK reference nutrient intake (RNI) for protein, using chronological age.

Using the above example:

22.7–39.4 g protein/day = 1.7–3 g/kg/day. Children with extensive/infected lesions will require an intake at the higher end of the range.

- Evaluation of biochemical and haematological parameters [23, 25], although interpretation of results is problematic due to the effect of the acute phase inflammatory response. Nevertheless, certain parameters should be monitored. Table 3.4-2 lists the investigations that should be routinely carried out, with suggested sampling intervals. Frequency of sampling depends on disease severity and the need to evaluate intervention such as individual nutrient supplementation or gastrostomy placement.

Assessing adequacy of growth

Measurements of weight and height velocity are the most practical means of assessing growth, and these should be taken every 3–6 months, more frequently

Table 3.4-2. Biochemical and hematological investigations in EB children; suggested investigations and sampling frequencies (*Reproduced with permission of Blackwell Science*)

6–12 monthly	Yearly	1–2 yearly
Urea and electrolytes	Vitamin B1	Vitamin E
Creatinine	Carnitine	
Calcium, phosphate (±Vitamin D3)	Vitamin B12	Vitamin A
Total protein, albumin	Folate	
Alkaline phosphatase		
Zinc, selenium		
Serum iron, ferritin, full blood count		
Hypochromic red blood cell		
Transferrin receptors		
Mean corpuscular volume (MCV)		
Reticulocytes, red cell folate		
Erythrocyte sedimentation rate (ESR)		
Free erythrocyte protoporphyrin (FEP)		

N.B. The above is a guide. Sampling frequency depends on individual disease severity and the need to evaluate intervention.

in babies and younger children. Head circumference should be measured in children under the age of 2 years and values plotted on nationally-approved growth charts [17]. Although the aim is for the child to grow according to his genetic and ethnic potential, longitudinal growth in particular is often poor in severely affected EB children. Chronic inflammation and pro-inflammatory cytokines are thought to play a role, but the mechanism is uncertain. It has been postulated that RDEB children are significantly lighter at birth than unaffected children, and that the compromise in growth begins *in utero* [16]. So although plotting the growth is useful, this should always be considered in the context of disease severity, and goals adjusted accordingly.

Painful fixed flexion contractures around joints and osteoporosis in severely affected children make height difficult or impossible to measure. A supine stadiometer or segmental measuring may be more practical [41]. Body mass index (BMI), weight (in kg) divided by height (in meters) squared, gives an indication of relative weight for height, but does not differentiate between lean and fat tissue and is not routinely used (see Optimizing growth and mobility, Calcium and Vitamin D, Osteoporosis and Osteopenia).

Nutritional support in EB infants

Severe lesions, particularly with sepsis, are likely to increase requirements [27]. Energy requirements range from 130 to 180 kcal/kg/24 h (115–150% Estimated Average Requirement or EAR, and may be up to 225 kcal/kg. Protein requirements are 2.5–4 g/kg, (115–200% RNI]) and fluid 150–200 ml/kg. Extensive blistering causes fluid loss and may require correspondingly larger feed volumes.

Breast feeding should be encouraged for the many benefits it confers [1]. However, rooting may cause or exacerbate facial lesions and suckling may lead to blistering of the mouth, tongue and gums. Babies should be allowed to suckle on demand; applying white soft paraffin or Vaseline to the lips and to the nipple reduces friction. When satisfactory feed intake is compromised by pain (oral and general), GER and constipation, this should be addressed promptly with regular recalculation of drug dosages in line with weight.

Except in mild cases, breast milk alone often fails to satisfy increased requirements. A more nutrient-dense feed should be provided (ideally in association with an ongoing intake of breast milk, either direct from the breast or expressed) and this can be achieved in several ways [23]:

- fortify expressed breast milk (EBM) with a proprietary whey-based powdered infant formula at a concentration of 5 g/100 ml.

- reconstitute proprietary whey-based powdered infant formula to a higher concentration than normal (e.g. 15% instead of the usual 13%).
- add glucose polymer such as Maxijul (Scientific Hospital Supplies International) at 2–5 g per 100 ml to increase further the energy content, giving a total carbohydrate content of 10–12 g/100 ml feed.
- add fat emulsion such as Calogen or Liquigen (SHS International) in 1% increments to give a total fat concentration of 5-6% (i.e. 5-6 g fat per 100 ml feed) if the gut function is normal. A long chain fat emulsion (Calogen) is generally favored over medium chain fat (Liquigen) for its lower osmotic effect and source of essential fatty acids.

Care should be taken when modifying feeds, as diarrhea can result with an osmotic load of over than 500 mOsm/kg water. Ready-to-feed, nutrient-dense formulations such as Infatrini (Nutricia) or SMA High Energy Formula (Wyeth) provide alternatives to modifying EBM or term formula. Products such as Nutrini or Nutrini Energy (Nutricia) are suitable for infants over 1 year or 8 kg. Regular review of weight and feed adequacy is essential.

To avoid the feeding nipple adhering to the infant's lips and causing damage on its removal, it should be moistened with cooled, boiled water, feed, white soft paraffin or Vaseline before feeding. An age-appropriate oral analgesic gel can be applied (on the nipple or in the mouth) if painful oral lesions cause reluctance to feed. A specialized feeder designed for a cleft lip or palate such as the Haberman Feeder (Athrodax Healthcare International) is extremely useful. This type of feeder's shape minimizes trauma to the gum margin, and its internal valve and long shaft allow the carer to control the flow of feed, so that even a weak suck will deliver a satisfactory milk flow. Carers should be warned against squeezing the feeder's shaft so strongly that feed is delivered to the back of the throat, stimulating the gag reflex and/or causing feed aspiration. If such a feeder is not available, the hole in a conventional feeder can be enlarged using a sterile needle. Babies who cannot suck from a nipple may need to be fed from a spoon or dropper. Nasogastric tubes are not routinely placed (see Nasogastric (NG) and gastrostomy feeding).

Vitamin requirements for infants thriving on breast milk or regular infant formulas are unlikely to be greater than normal. However, if in doubt, an age-appropriate multivitamin supplement can be given, provided that total intake (particularly of vitamin A) does not exceed recommended safe upper limits [2]. Although precise requirements for EB babies have not been determined, "best guess" assumes that more severely affected babies need increased amounts of all vitamins [14, 23], especially vitamin C, whose role in enhancing iron absorption [34] and in collagen synthesis [28] is recognised. The provision of 150–200% of the RNI ensures that intakes are still within recommended safe limits.

Increased concentration feeds provide increased amounts of all vitamins, possibly nearing 150% RNI if large volumes are consumed. If a satisfactory intake is in doubt, and if skin lesions are significant, then a comprehensive preparation such as Ketovite liquid and tablets (Paines & Byrne) should be prescribed. Ketovite tablets can be crushed, mixed with a small amount of feed or water and given from a syringe or spoon. If this is not possible, a liquid vitamin preparation such as Abidec drops (Chefaro) can be used, although this provides an incomplete range of vitamins A, B1, B2, B6, Niacin, C, and D. When considering vitamin D intake, it should be remembered that extensive dressings may prevent significant sunlight exposure. Where there are significant blood losses, iron and zinc status should be assessed and supplemented if required (see Iron and anemia and Zinc).

Solid foods can be offered to an EB baby at the same time and in the same form as for an unaffected baby, excluding hard and abrasive foods. Acceptance of new foods is often very slow and parents need reassurance to allow the baby to progress at his own pace. A shallow plastic (not brittle) spoon with rounded edges should be used and babies who have an extremely fragile mouth may feed more confidently from the parent's fingertip or from a piece of soft food, e.g. a cooked carrot or potato.

Reluctance to try new foods is often a legacy of previously (or on-going) poorly controlled GER [30] and/or a very fragile, painful mouth. Scarring and tongue fixation can cause an uncoordinated swallow, with the risk of aspiration [13]. Weaning foods containing soft lumps in a liquid matrix are difficult to control in the mouth, and can lead to panic and subsequent gagging and choking, thereby compounding negative associations with feeding. Parents should be advised how to increase dietary protein and energy content without increasing bulk (see *Nutrition for babies with epidermolysis bullosa*, DebRA UK publication). The expertise of a Pediatric Speech and Language Therapist is invaluable in promoting confidence with different food textures.

Based upon our clinical service's experience at Great Ormond Street Hospital for Children, nutritional support unfortunately appears to have little or no impact in JEB-H. As such, interventions such as placement of a feeding gastrostomy are futile, likely only to increase the parental burden. Any nutritional intervention in an infant or baby with JEB-H should be as part of palliative care to enhance the quality, rather than the quantity, of life [50]. Protracted diarrhea is often a hallmark of JEB-H and it has been suggested that a deficiency of intestinal integrin is responsible [26]. Replacement of dietary long chain triglyceride (LCT) by medium chain triglyceride (MCT) may alleviate this, by virtue of its metabolic pathway via the portal vein rather than via the lymphatic system, but such a maneuver remains experimental.

Dietary assessment

This should be comprehensive and sensitively probing in order to elicit an authentic picture. The proforma in Clinical Practice Guidelines [24] suggests specific points to address when carrying out a dietary assessment. Older children with significant oral and esophageal complications seldom maintain satisfactory nutritional status using normal foods. Liquidized foods tend to be low in all nutrients unless large volumes are consumed. Advice, initially, should aim to improve the nutritional value of the child's normal food intake (see *Diet for Epidermolysis Bullosa – for Children over 1 Year*, DebRA UK publication). Dietary modifications must be practical and tailored to the family's individual dynamics. The emphasis should be on increased protein and energy intakes, with improvements in vitamin and mineral intakes as indicated by dietary assessment and laboratory results. Milk often figures prominently in EB children's diets, so protein and calcium intakes are generally satisfactory (but see Calcium and Vitamin D). Conversely, the intakes of those who dislike milk, etc., and who have difficulties chewing and swallowing meat invariably require supplementation.

Realistically, severely affected children rely heavily on multinutrient supplements such as Build-up (Nestle UK), Fresubin (Fresenius) and Fortisip (Nutricia). Glucose polymers, e.g. Polycal (Cow & Gate), and fat emulsions, e.g. Calogen (Scientific Hospital Supplies International), are useful if an increase in energy only is required. Children who regularly consume multinutrient supplements receive extra vitamins from these and this should be considered before further supplementation is advised. Those with anything more than the mildest form of EB should receive extra vitamins [3, 23], aiming for 150–200% of the RNI for age (see Vitamins).

Children who maintain adequate oral nutrition frequently do so only by the extraordinary efforts of their parents; this becomes increasingly harder to maintain. It is important not to set unrealistic targets, and the effect of recommended intervention should be monitored and given an agreed time limit (e.g. 2–3 months). If the desired improvement is not achieved, intervention such as esophageal dilatation and/or gastrostomy placement should be considered. Percutaneous endoscopic gastrostomies (PEGs) are generally unsuitable because of the shearing damage to the esophagus caused at their placement. Button devices inserted as a primary procedure are generally well tolerated, but should be placed via a laparotomy rather than endoscopically [21].

Optimizing growth and mobility

Determination of optimal growth rates for children with severe EB is difficult and should always be considered in the context of the individual child's

disease severity. Nutritional support by gastrostomy feeding has definite advantages in EB, but may be associated with central fat deposition with poor linear growth. The reasons for this are multifactorial and interrelated and less likely to be due to gastrostomy feeding *per se* than to disturbances in growth hormone production mediated by cytokines and increased cortisol production inherent in chronic inflammatory illness [45]. The child whose weight centile deviates upwardly by more than 2 centiles from his height centile may be less mobile and more likely to depend on a wheelchair. It is important to maintain a balance between mobility, growth and nutritional status, as these three aspects are interrelated and interdependent. Lack of weight bearing and wheelchair dependency compounds the low bone mass frequently seen in children with RDEB and JEB [12]. An increased propensity to bone pain and fractures leads to further immobility. Conversely, undernourished children may fail to attain puberty and to benefit from its associated protective hormonal effect on bone health.

Iron and anemia

The degree of anemia corresponds to the severity of EB. RDEB and JEB sufferers experience continual blood losses from skin lesions, from the upper gastrointestinal tract and possibly also from the lower bowel (especially where there is anal fissuring). The resulting anemia is usually microcytic and hypochromic, believed to be related to the so-called 'anemia of chronic disease' [4, 19]. Coexistent inflammation makes interpretation of the laboratory iron store assays difficult in severe EB. However, iron is generally supplemented enterally when there is reduced hemoglobin, low mean corpuscular volume (MCV), low ferritin and high total iron binding capacity (TIBC). A liquid preparation is preferable (e.g. Sytron, Link, Horsham, UK), and a dose of 1 ml/kg body weight (5–6 mg iron/kg) is a useful guide (up to approximately 30 kg). Iron supplements are often associated with gastric irritation and constipation. Consequently, compliance is poor unless appropriate action is taken (alternative preparations, stool softeners etc.). Iron related parameters that are commonly checked are listed in Table 3.4-2.

Debate continues over the merits of daily [20] versus weekly iron administration [7]. The latter view relies on the hypothesis that a mucosal 'block' occurs in intestinal cells which then cannot absorb therapeutic daily doses of iron until they are renewed by cell turnover at roughly three day intervals. In practice, medications given on a less than daily basis are prone to be forgotten, so it is safer to prescribe on a daily basis as a part of the regular routine. Undesirable side effects may be eased by dividing a daily dose into two, increasing fiber intake, and prescribing appropriate laxatives and/or stool softeners [23] To improve iron absorption, a rich source of vitamin C (blackcurrant or orange juice) can be taken concurrently, provided that this does not irritate the oral mucosa.

There is no clear consensus regarding separate or joint administration of iron and zinc supplements [48]. These two micronutrients have the potential to interact, leading to reduced absorption of both. A review article concluded that, although some trials have shown that joint iron and zinc supplementation has less of an effect on biochemical or functional outcomes than does supplementation with either mineral alone, there is no strong evidence to discourage joint supplementation [46]. Whichever regimen is adopted, it is important to establish that there is good compliance. If the scheme proves impractical or the physiological response is poor, the regimen should be modified accordingly.

Zinc

Zinc is an essential co-factor for over 200 enzymes, playing vital roles in growth, wound healing, immune function and membrane stability, where its antioxidant properties are crucial [29, 36]. However, the degree of desirable supplementation is unclear [44] and intakes over 200% RNI are probably unnecessary, as improvement in wound healing may only occur in deficiency states. More importantly, excessive intakes have been reported to impair immune responses in adults [9] and may interfere with copper and iron absorption [47]. Nausea and vomiting are common side effects of zinc supplementation and are frequent reasons for non-compliance. Flavored zinc lozenges may be better tolerated (e.g. Holland & Barratt, 23 mg zinc per lozenge). These dissolve slowly in the mouth, but their chalky "mouth feel" is unacceptable to many children and their slightly rough texture may irritate a fragile oral mucosa. Plasma zinc estimation should be undertaken to provide a baseline, although the interpretation of results is complicated by factors such as low plasma albumin which causes an associated spuriously low zinc result. In such a situation, it is inappropriate to supplement zinc alone; energy and protein intake should also be increased. A liquid supplement is preferable, such as 5–10 ml of zinc sulfate solution (30 mg zinc in 5 ml) or a proprietary preparation such as 1/2–1 effervescent tablet of Solvazinc (Cortecs) (45 mg zinc per tablet). To optimize absorption and minimize side effects of nausea, the daily dose should be split into two. It may be prudent to advise giving zinc supplements, if not on different days from iron supplements, then at least at different times of the day, although no trial has yet demonstrated clearly the merits of either regimen (see Iron and anemia).

Selenium and carnitine

A small number of severely affected RDEB children have developed fatal dilated cardiomyopathy (DCM), thought to be associated with deficiencies of selenium and carnitine [31]. However, these children were chronically malnourished, so the cause of their cardiomyopathy may have instead been multifactorial. More

recent work [38] supports the hypothesis that selenium and carnitine are implicated in the development of DCM. An association has been made between concurrent administration of amitryptiline (for relief of chronic pain) and cisapride (for GER and gastric stasis) and the development of DCM in EB, as both drugs are potentially cardiotoxic [42]. The authors claimed that nutritional deficiency was an unlikely cause of DCM because previously reported cases were under close nutritional supervision. However, an assumption that nutritional supervision automatically ensures compliance with supplements and medications or their efficacy, is flawed. Indeed, the case described was extremely malnourished and all aspects of parental management were extremely poor. Global malnutrition may well be a contributory factor in the development of DCM [5]. In the absence of further data, monitoring of selenium and carnitine status is advisable in all EB children whose nutrition is compromised and/or who rely on nutritional support (e.g. gastrostomy feeding). Where there is biochemical evidence of deficiency, supplementation should be provided and monitored regularly. Selenium supplements which also contain vitamin A should be given only if intake of vitamin A from other sources is not significant. Pure selenium is currently available in the UK only in 50 mcg tablets. Carnitine is given as Carnitor Paediatric solution (Shire) 50–100 mg/kg daily.

Calcium and Vitamin D, Osteoporosis and Osteopenia

Children with the more severe forms of EB have low bone mass and are at risk of abnormal bone mineralization and fractures. This is believed to be due to factors such as poor nutrition, delayed puberty, reduced levels of mobility and weight-bearing exercise, and reduced exposure to sunlight. Although EB children receiving enteral feeds via gastrostomy obtain their theoretical requirements for calcium, elevated cytokine concentrations secondary to chronic infection or inflammation may adversely affect bone turnover, and gastrointestinal complications may interfere with absorption. Current management involves determination of bone mass by dual X-ray absorptiometry (DEXA scan), biochemical estimation of calcium and Vitamin D status and administration of bisphosphonates with a combined calcium and Vitamin D preparation [12]. Cacit D3 (Procter & Gamble), containing 500 mg calcium and 11 mcg colecalciferol) is a granular, effervescent preparation that is preferable to chewable tablets.

Nasogastric (NG) and gastrostomy feeding

NG tubes should not be routinely placed. Despite great care, they can cause trauma and are difficult to secure (only non-adhesive dressings such as Tubifast (Seton Scholl, Oldham, UK), are suitable, the tube being secured by winding a length of Tubifast around it where it enters the nostril). Alternatively, silicone

tape recommended for fragile skin can be used. Internal friction and scarring by the tube can interfere with oral feeding. Resulting discomfort is thought to be associated with later food aversion and an increased tendency to develop esophageal strictures. However, temporary NG feeding may be unavoidable in babies who do not take satisfactory volumes of oral feeds and in those whose mouths become excessively traumatized by suckling. It may be used as an interim measure in the RDEB or non-Herlitz JEB child who it is believed would benefit from gastrostomy placement, but who needs, or whose carers need, evidence of the effects of improved nutrition before agreeing to surgery. A 6–8 week period of NG feeding should be sufficient to demonstrate benefit. A further indication for short term NG feeding is in RDEB, where extensive dental procedures cause significant oral and pharyngeal blistering. Postoperatively, such trauma may preclude a satisfactory oral intake for several days. Since these procedures are undertaken under general anaesthesia, an NG tube can be passed in the surgical theatre and the child fed by this route until adequate oral intake is resumed. Whatever the age of the patient, the tube used should be as soft and of as narrow a gauge as possible, and it should not be re-sited at every feed, but left *in situ*.

Although serial esophageal dilatations can significantly improve swallowing [6], continuing difficulties with chewing, compliance with medications, and maintenance of a satisfactory fiber intake, merit consideration of gastrostomy placement, ideally before the child's growth deteriorates markedly. Some parents initially view this recommendation as a sign of their failure to nourish their child adequately and request continued information regarding alternative oral supplements. Delaying placement may only add to their stress and frustration, engendering increased feelings of failure. With early intervention, the child is more likely to continue with oral nutrition, albeit in small and varying quantities. This is important not only for social reasons but also in anticipation of a time, after the pubertal growth spurt, when they may be able to take sufficient nutrition orally and the gastrostomy can be removed [32].

Following gastrostomy insertion, children may be reluctant to continue with oral nutrition, preferring to rely heavily or entirely on gastrostomy feeds. This probably reflects long term negativity about eating and relief at having an alternative route for nutrition. However, the large feed volumes required to promote optimal growth may be poorly tolerated, whether they are given overnight, during the day, or a combination of both. To optimize feed tolerance and promote continuing oral intake, it is advisable to begin with small volumes; this may mean as little as 200–250 ml of a 1.5 kcal/ml feed for a child under 5 years, and 300–500 ml for an older child. A nutrient-dense feed containing a mixed fiber source, such as Nutrini Multi Fibre, Nutrini Energy Multi Fibre (Nutricia) or Jevity Plus (Abbott), is generally appropriate; for children over the age of about 3 years, these are usually delivered overnight via a pump. Some children require a combination of pumped overnight feeds and daytime bolus

feeds. It is not advisable to give pureed food via a gastrostomy due to the likelihood of blockage and the danger of microbial growth. Nocturnal feed volumes over 800–1000 ml are often associated with the need to pass urine during the night (involving broken sleep for the child and for the parent whose assistance is required), or with bedwetting and occasionally with defecation. Families' priorities should firstly be established and detailed discussions regarding the pros and cons undertaken before surgery takes place. Although gastrostomy placement is not a panacea, it is an important management tool and its potential benefits should be explored.

Painful defecation with or without constipation

Chronic constipation (often more accurately termed fecal loading) with painful defecation is one of the most frequent, yet underestimated, complications of all types of EB [10, 22]. It should be treated without delay if the vicious cycle of pain, conscious ignoring of the gastrocolonic reflex, and secondary anorexia (Fig. 3.4-1) is to be avoided. In babies, extra fluid should be offered in the form of water, or if this is refused, as one teaspoon of fresh fruit juice diluted in 100 ml water or ready-to-feed baby juice diluted with an equal volume of water. Lactulose should be prescribed, starting with 2.5 ml every other day to 2.5 ml twice daily. A fiber-containing feed such as Pediasure with Fibre (Abbott) or Nutrini Fibre (Nutricia) should be introduced at 6–8 months. Whether the infant is constipated or not, it is prudent to introduce a fiber source at 9–12 months of age, since constipation is such a likely complication of all types of EB, plus a stool softener such as lactulose or, for the over-2's, Movicol Paediatric (Norgine).

In the older child, overflow incontinence is often mistaken for diarrhea. As a result, caregivers often reduce or stop the prescribed laxative therapy, inadvertently exacerbating the situation. Oral lesions, dysphagia, and requirement for a low bulk, nutrient-dense intake may prevent the EB child from consuming a conventional high fiber diet. An increase in fiber intake is important, however, and a fiber-containing feed (preferably one based on a mixed fiber source) should be introduced, orally or by via gastrostomy, but only after the extent of fecal loading has been investigated and addressed. In addition to the feeds mentioned above, such products include Fortisip MultiFibre (Nutricia), Jevity, Jevity Plus, Enrich (Abbott) and Nutrison Multifibre (Nutricia).

A pure fiber source, e.g. Resource Benefiber (Novartis Medical Nutrition), is virtually tasteless and blends well into water, juices and feeds. If the child then progresses onto a diet sufficiently high in fiber to promote comfortable defecation, then the fiber source can be phased out. Resource Benefiber can be highly successful in normalizing stool consistency and frequency of defecation and in promoting confidence in defecation. Using the US formula of age (yrs) + 5–10 g

daily [49], a 3 year old child will require 8–13 g of fiber per day; adequate fluid intake should be advised concurrently. Before introducing Resource Benefiber, the degree of fecal loading should be assessed (e.g. by giving a carmine marker) and addressed using either a bowel preparation solution such as Klean-Prep (Norgine) as a hospital inpatient, or with a strong laxative such as Picolax (Ferring). If this step is not taken, abdominal pain, GER and vomiting invariably ensue and compliance is jeopardized. Thereafter, a stool softener/laxative is generally necessary in the long term.

Dental aspects

Adequate energy intake may be unachievable without the frequent consumption of fermentable carbohydrate, especially sucrose. Unfortunately, this is highly conducive to the development of dental caries. This apparent conflict of interests between dietitian and dentist can lead to contradictory advice to the child and the caregivers. However, compromise is possible, and the advice of an experienced dentist should be followed regarding use of a fluoride supplement, fluoride toothpaste and a plaque inhibiting mouthwash. Sweets should ideally be restricted to the end of mealtimes and continuous sipping of sugary drinks outside mealtimes discouraged. However, this may be impossible in severely affected children with no gastrostomy, as eating and drinking are extremely slow and one mealtime unavoidably merges into the next.

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3.5 Physical medicine and epidermolysis bullosa

Margret Burger-Rafael

Specialists in Physical Medicine and Rehabilitation play an important role in the multidisciplinary evaluation and care of patients with hereditary EB, first by performing testing which assesses deficits in range of movement and in senso-motoric ability, as well as in perception. Based on these findings, specific therapeutic interventions can then be pursued which will enhance functionality and well-being of the patient.

Functional diagnosis

The development of movement skills is a progressive one. A baby's urge to grasp or try something new leads to total body involvement. As perceptions change, so do the range of movements involved, as well as learned dexterity [3].

The task of diagnostics is to explore possibilities for different approaches to therapy for the individual patient. The main problem for patients caused by dystrophy is contractions, resulting in secondary damage to muscles, tendons and joints [2]. A typical body posture develops as a result of the current condition and this contributes to a malpositioned spinal column and body joints [1]. This can be a special problem in the more severe EB subtypes, as hand and finger movements are especially important in elementary school children. It is also important to remember that whereas all of the toes contribute to walking, the great toe is needed for proper balance and support.

Is based on the above observations, the diagnosis of body postures and dysfunctional movements, which are caused by pain, also influenced by psychological factors like anxiety?

The over-protective approach of some parents and caretakers has to be mentioned here. This applies also to doctors and therapists who have to learn to overcome their own anxieties and concerns. A positive experience with mobility supports the self-esteem and security of the patient and allows patients to develop the courage to try new things and to improve social contact, regardless of age. Properly fitted shoes, equipment, toys, and tools (e.g. tricycles with thick handles) are examples of items which, if properly chosen, can enhance functionality. As another example, for children with RDEB, who are especially at risk of developing progressively restrictive hand deformities, support and maintenance of finger mobility may be enhanced if the child is taught how to play a keyboard instrument or to work on a personal computer.

It is also worth remembering that the capabilities of more severely affected EB children are often underestimated, due to the presence of a poor nutritional state and muscle dystrophies, since they usually appear to be smaller and much younger than their true biological age.

Another goal in functional diagnosis identifies the importance of the surroundings of the patient (see also Chapter 4.2); where do they live (in a large city or small town?) and what does the family situation look like? Is there any danger of the child being over-protected by its caretakers and not allowing him or her to participate in normal activities? It is the assessment and documentation of all these social factors from every day life that are indispensable for the integration of this child into society [4].

Documentation: This component of diagnosis is of importance for the evaluation of the effectiveness of physical therapy and rehabilitative interventions, because there is insufficient published data in relation to patients with EB.

Therapy

Physical therapy

Exercise therapy

The importance of being able to move is crucial to overall well-being and functionality of the EB patient. Therefore, an exercise therapy programme in active, passive and assisted range of motion is offered (Fig. 3.5-1). The proper

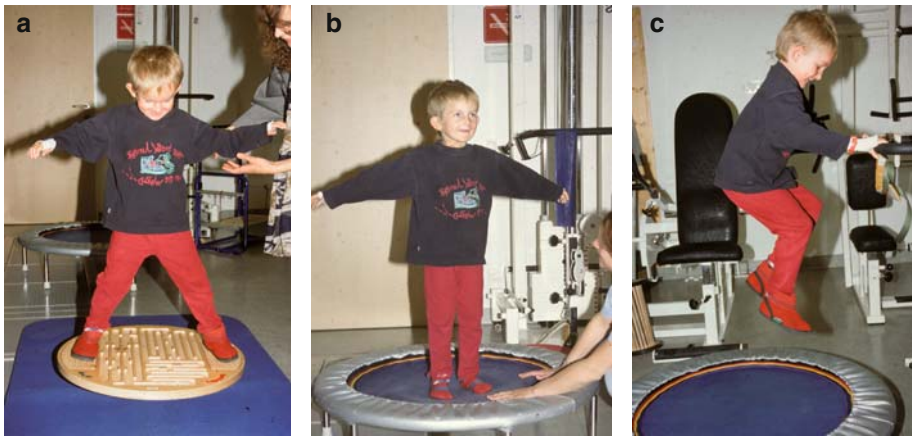

Fig. 3.5-1. **a**) Balancing exercises. **b & c**) Use of a trampoline to exercise the entire body

choice of handling techniques, with large areas of grip that are mechanically not too stressful to the skin, is recommended. Also, suspension equipment like the "Schlingentisch," has proven to be a good option. As mentioned before, soft-padded and wide wrapping material should be used.

Important areas of focus are physical therapy of the joints, regeneration of muscles and strength, improved coordination and balance, and the perception of sensory motor skills. Especially important is the proper "school of walking". The chosen technique must meet the criteria above. Ergonomic parameters should be considered for every therapy, in combination with corresponding instructions.

Hydrotherapy

This form of therapy is especially suited for EB patients, based on the unique physical properties of water. The reduction of body weight and the resulting effortless movements that are possible while immersed in water are of special benefit to these patients. Problematic considerations, though, are the hygienic conditions of the hydrotherapy unit, the temperature of the water used (e.g. blisters may develop if the water is too warm), and the time which is needed for dressing changes to be performed after completion of each therapy session.

Horse back riding

Riding on and with a horse is suitable and beneficial, even for a patient with EB, with the proper equipment, a soft saddle, and a small horse. The goal, along with the objective of learning how to ride, is that the patient is using his or her

sensory motor skills and developing emotional contact with the animal (see also Chapter 4.2).

Low level laser treatment

A new modality which we are now exploring as an adjunct treatment for selected EB patients is low level laser treatment. An adverse thermal side effect to the treated area can be excluded, due to the low power that is employed (e.g. less than 1 Watt). Among the many hypotheses as yet unproven that have been suggested to explain the beneficial effects that are observed in some patients, it is believed that they may be based on effects to the cellular respiratory system, via photosensitivity of the mitochondria, and thus through enrichment of adenosine triphosphate (ATP). This may possibly explain the accelerated healing of blisters and ulcers which we have observed in some of our EB patients who have been treated by this particular modality. Pain relieving effects, if seen, possibly occur through more central mechanisms [6]. The low intensity of this therapy and the higher frequency of treatment that can be safely performed at this dosage are advantages to such a treatment. Acupuncture by laser might possibly be an alternative treatment option in selected patients, based on experiences in others patients who do not have EB.

Magnetic therapy

This controversially discussed therapy is believed to affect cell membranes, leading to regeneration and pain relief. Pulsating magnetic fields with low density of flow are preferred. This therapy is easy to apply to an EB patient, since the patient does not need to undress; therefore complex dressings on the skin will not have to be disrupted. This concept of treatment includes a preparation for the whole body, whereby the patient lies on a mattress and a selective application is performed [5, 8].

Massage

Due to the high mechanical friction to the skin resulting from massage, this particular therapy is only rarely used for EB, since blistering would be expected to occur in most patients who are treated in this manner. Manual lymph drainage to an edematous limb is, however, offered to selected patients, since it can be applied with less pressure and traction to the skin.

Electrotherapy

This traditional physical medicine therapy is less suited for EB patients, as there is a double irritation through skin and electric stimulation caused by the current-carrying electrodes.

Thermotherapy

This therapy can be applied as diathermy, also from the area of electromagnetic rays, because direct contact with the skin is unnecessary. Infra-red light therapy, although not as yet proven to be beneficial for EB, might also a possibility, if not otherwise contraindicated.

Occupational therapy

Maintenance of motion of the fingers is of major importance, particularly for patients with dystrophic EB who commonly develop partial or complete webbing

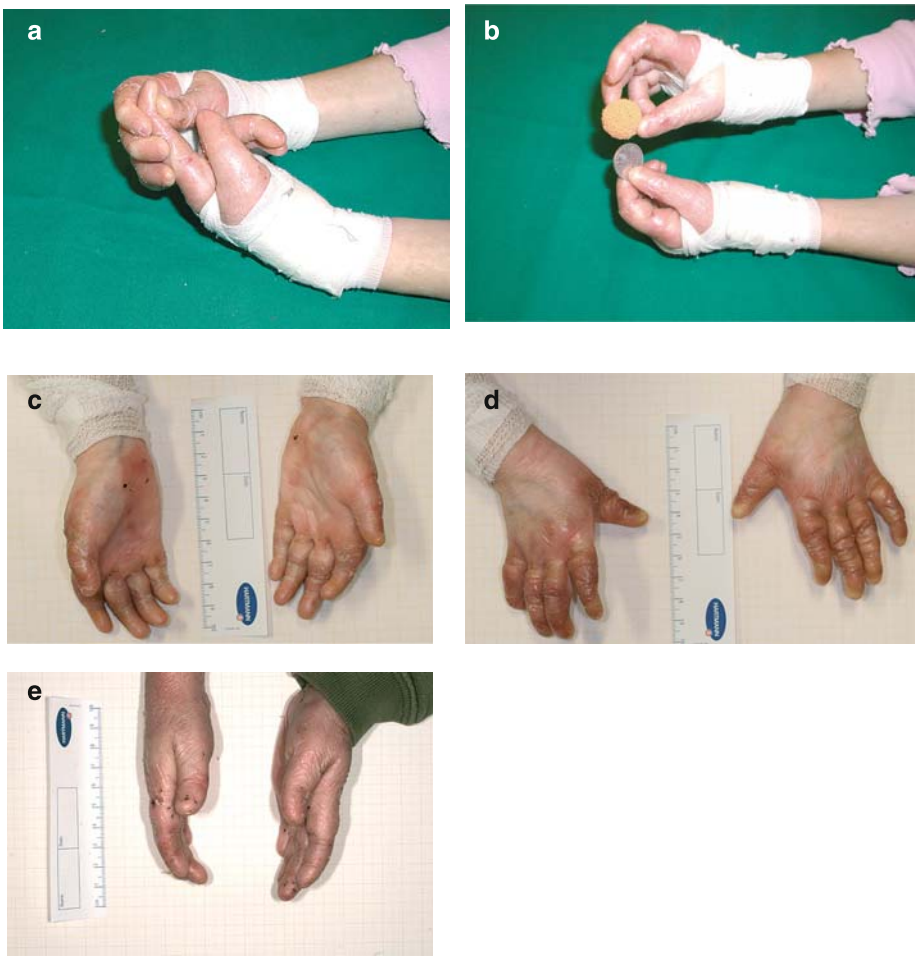

Fig. 3.5-2. **a**) Stretching exercises of the fingers. **b**) Fine grasping movements. **c–e**) Measurements of range of motion

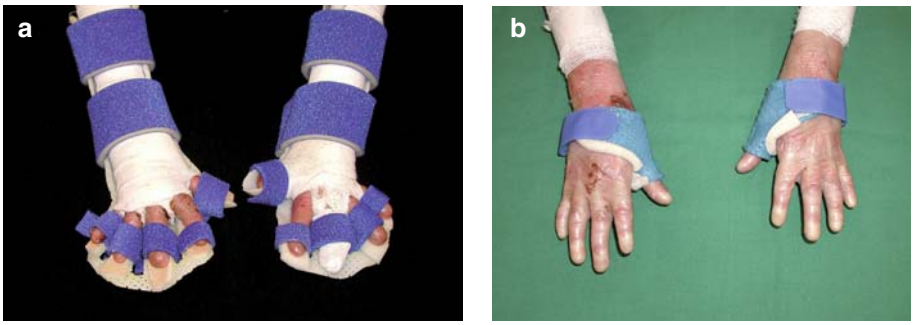

Fig. 3.5-3. (**a & b**) Various hand splints

deformities. There are two important areas of therapy: (1) physical therapies, with active and passive elements; and (2) the application of special splints. Exercise for grip and pull are also important for the functional therapy for the fingers (Fig. 3.5-2). Occupational counselling is another important programme to offer.

Splints act as support for the proper positioning of the hands and fingers, and are used in order to prevent or slow down the growth of pseudosyndactylies (webbing) (Fig. 3.5-3). Functional splints are also helpful in specific circumstances,

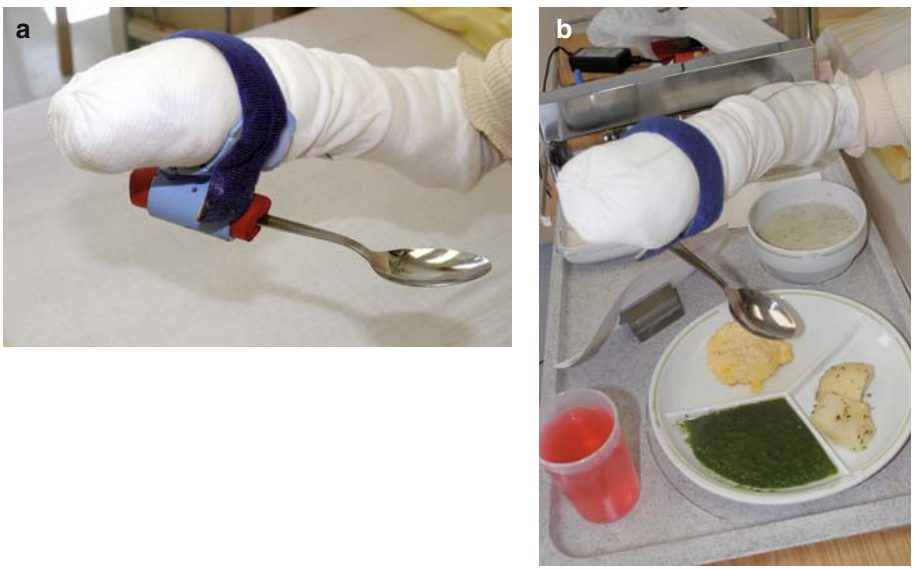

Fig. 3.5-4. (**a & b**) A specially designed device which allows a spoon to be held and used properly by a patient with severe pseudosyndactyly

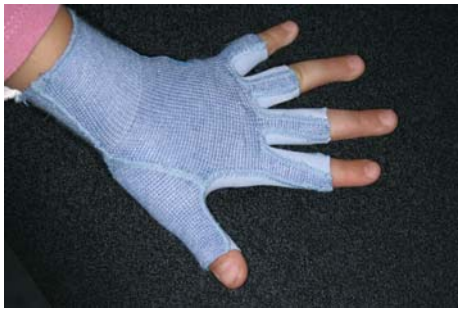

Fig. 3.5-5. Individually tailored compression glove

for instance to support the function of the thumb for writing purposes or of the hand in allowing eating utensils to be held (Fig. 3.5-4). Special care has to be given to the selection of chosen materials. In designing splints, skin hygiene must be considered, to prevent additional skin irritation from developing. This can be achieved by using soft, washable materials.

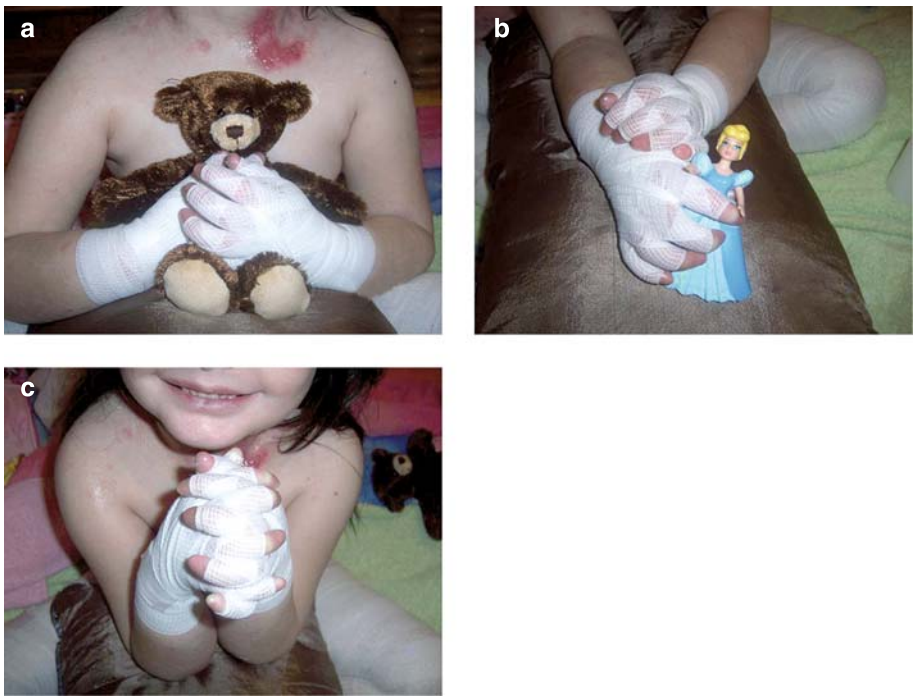

Fig. 3.5-6. (**a–c**) Meticulous wrapping of the fingers and hands can provide an alternative to gloves in some patients, and help to maintain separation of the digits (kindly provided by Mrs. Contreras-Mata)

Fig. 3.5-7. The compression glove is well accepted

The use of splints after surgery on the fingers, also in combination with functional dressing techniques, is important, in order to maximize and prolong the benefits of reconstructive hand surgery (Chapter 3.3) [7].

Another possibility to try to prevent webbing from developing is to use tailored compression gloves (Fig. 3.5-5), which are now an emerging treatment for burn victims. This appears to be a good therapeutic option for those EB patients who are at highest risk of developing pseudosyndactyly. Alternatively, similar results may be achieved with meticulous finger and hand wrappings using thin, narrow, sterile gauze sheeting (Fig. 3.5-6). Compliance with young patients is surprisingly good with either approach (Fig. 3.5-7). The positive perception achieved by wearing these gloves or wrappings is also of importance for the patient [9].

Conclusion

Many of the physical medicine methods employed in patients with other injuries and diseases are playing an increasingly important role in the overall symptomatic therapy for EB, especially those with severe forms of junctional and dystrophic EB. Occupational and physical therapy should become an integral part of the overall management of patients with EB. Their purposes are the prevention of contractions, the training of sensory motor skills, and the maintenance of function. As we obtain more experience with EB patients, we are finding that more therapeutic approaches exist than were originally expected. Given the clinical variability among the many EB types and subtypes, specifically designed treatment programmes should be devised and implemented, based upon the individual needs of each child and adult.

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3.6 Molecular therapy of epidermolysis bullosa

Martin Laimer and Johann W. Bauer

Gene therapy exploits the potential of current biotechnology to manipulate sequences of gene expression for therapeutic purposes. It is thereby considered a novel therapeutic approach to common inherited and acquired human diseases where treatment procedures are not available or not very effective. Considerable technical progress in the field of inherited disorders have been made over the past decade. These advances have produced new insights in genetics and molecular mechanisms of these diseases and furthermore permitted refined molecular classifications with better understanding of genotype-phenotype correlation, improved genetic counseling and prognostication as well as DNA-based preimplantation and prenatal diagnosis (Chapter 1.4.2.3). This ongoing exploration of the genetic background of various genetic diseases represents a prerequisite to develop and apply technical procedures specifically counteracting dysfunctional gene expression. For the latter, rapid advances in vector design, gene delivery, regulation of targeted gene expression, and immune modulation brought attempts of therapeutic molecular manipulation much nearer to clinical use.

The skin represents a particularly attractive target for gene therapy because of both its accessibility as well as instant visualization of results to evaluate the effects of direct topical and injectable gene transfer in a minimally invasive fashion. In addition, a large number of diseases are principally amenable to cutaneous gene transfer (for synopsis on current approaches to cutaneous gene therapy see Fig. 3.6-1). Besides being primarily targeted by focal gene therapy intervention, the cutis may also serve as an "*in vivo* bioreactor" for systemic therapy, by which specifically designed (therapeutic) gene products (like erythropoietin, apolipoprotein E, factor IX or growth factors to promote wound healing) have already been successfully delivered into the circulation [2, 4, 38].

The skin as target for gene therapy reproduced from Laimer et al. [15]

STRATEGY

Ex vivo gene therapy

Propagation of target cells/tissue obtained from affected individuals in culture where genetic manipulation/transduction occurs, re-grafting of transgenic tissue to donor

⊕ Well-established autologous skin grafting techniques and transduction protocols, confirmation of the efficiency of gene transfer prior to delivery, permitting safety studies

⊖ Costs, fragility of engineered epidermal tissue, burden of surgical procedures, risk of infection

In vivo gene therapy

Introduction/transfer of transgene directly into target tissue either by e.g. direct injection or topical application

⊕ Topical application attractive for treating large surface areas, potential utility as a means of vaccination

⊖ Efficiency of prolonged in vivo transduction low, boostering of gene delivery by physical means such as direct injections is restricted to localized treatment (e.g. of severely affected areas) and largely limited to the needle track due to particle size and limited diffusion]

Natural gene therapy

Rescue of disease causing mutation by means of naturally occurring secondary genetic phenomenon (e.g. mitotic gene conversion) mechanistically driven by exposure to exo- and endogenous noxae, disease associated increase of genomic instability, mutation induced accumulation of mutagenic metabolites, presence of mutational hotspots (such as CpG dinucleotides or repeats), selective advantage of reverted cells

Gene replacement

Gene restoration: re-introduction of wild-type gene or functional copy of defective gene in recessive loss-of-function mutations

Gene augmentation: therapeutically favoured gene-overexpression, e.g. in erythropoietin-responsive anaemia

Gene targeting

Gene correction of small genetic lesions by site-specific homologous recombination of corrective DNA/RNA chimeric molecules

Gene inhibition: pre-defined alterations in the genome of pathogenic dominant mutations by ribozymes, antisense techniques and transsplicing

Introduction of therapeutic genes

In target cells to produce therapeutic proteins, modify cellular information and developmental programs, stimulate immune responses and suicidal genes

VEHICLE

Non-viral vectors

Epicutaneous, intra- and subcutaneous delivery of nucleic acid to skin

Improvement of DNA penetration by physical and chemical methods (stripping, dermabrasio, microinjection, microbubble-enhanced ultrasound, electroporation, gene gun DNA particle bombardment, magnetofection; nucleic acid precipitation, lipoplexes, polyplexes and tissue specific peptide ligands favouring penetration and endocytosis)

Examples: DNA vaccines in infectious diseases, allergy and cancer immunotherapy; PhiC31 bacteriophage integrase for unidirectional genomic integration of therapeutic genes; fish transposon/transposase system Sleeping beauty (SB); genomic DNA vectors; artificial chromosome expression systems

⊕ Less immunogenic and cytopathic than viral devices, repetitive application exploiting the accessibility of the skin is possible, unlimited insert (DNA) size, relatively simple manufacturing, storage and quality control

⊖ Efficiencies in transfection and expression generally are not satisfactory, early dissemination and rapid decay of exogenous DNA within several days, limited clinical experience

Viral vectors

⊕ Highly efficient in long-term gene expression even in vivo, modification of e.g. viral capsid proteins or attachment receptors is possible to enable site specific integration

⊖ Immunogenicity/(cyto-)toxicity of vector backbone (and transgene product) with consecutive (auto)immune reactions, biosafety concerns (complementation and risk of systemic infection, dysregulated expression with risk of insertional mutagenesis due to insertion at random sites within cellular genome, especially when multiple vector injections are required), some viruses mediate transgene integration only in dividing cells and not quiescent stem cells (e.g. retrovirus) or remain episomal (e.g. adenovirus) compromising durability of expression, restricted host range and tissue tropism

Hybrid vectors

Constructed to overcome limitations and exploit advantages of individual types of vectors in their combination

Example: virosomes produced by fusion of lipoplexes with UV-inactivated hemagglutinating virus of Japan (HVJ) for both in vitro and in vivo gene transfer

LIMITS

Limits to transgene expression

- Impaired penetration and (local) persistence due to epidermal barrier, compartmentalization, continuous self-renewal, clearing effect on focally injected or released transgenes by dense vascularization
- Vector deficiencies in transduction efficiency, feasability of production and purification, targeting and specificity, regulation of transgene expression (quod vide)
- Identification, targeting, selection and therapeutic manipulation of stem cells in vivo
- Short term transgene expression by suboptimal grafting procedures, integrated transgene silencing (DNA methylation, chromatin condensation), degradation and gene dilution of extrachromosomal non-integrating plasmid/viral vectors in dividing cells, selective growth disadvantage of transduced cells
- Quantitative achievement of level of transgene expression required for correction
- Immunogenicity of neoantigens, especially in case of repetitive administration

Fig. 3.6-1. Introduction to cutaneous gene therapy

Table 3.6-1. Tested techniques in EB gene therapy at present

Method of transduction	Method of correction	Gene[a]	Reference
Retrovirus	cDNA	COL17A1	[73]
		LAMB3	[19, 49, 67]
		ITGB4	[5, 14, 20]
		COL7A1	
PAC Clon	Gene	COL7A1	[53]
Transposase	cDNA	LAMB3	[58]
PhiC31 integrase	cDNA	COL7A1	[56, 59]
Transient	Trans-splicing	COL17A1	[17]
Transient	Ribozyme	K14	[52]

[a]Genes: COL17A1: coding for type XVII collagen; LAMB3: coding for the β-chain of laminin-332; ITGB4: coding for β4 integrin; COL7A1: coding for type VII collagen; K14: coding for keratin 14. PAC: P1-based artificial chromosome

Depending on the level of tissue cleavage, EB has traditionally been divided into three main groups. Detailed discussions of the classification system (Chapter 1.2) employed for EB, the ultrastructural (Chapter 1.4.1.3) and antigenic (Chapter 1.4.1.2) features present within each recognized subtype, and the types of genetic mutations (Chapter 1.4.2.1) which occur in EB [78], may be found elsewhere within this monograph (see also Table 3.6-1).

Easy access to the target organ, the skin, and identification of underlying mutations in EB, facilitated the first cutaneous gene therapy experiments *in vitro* in the mid-1990s. Since then, the leading technology was transduction of the respective cDNA carried by a retroviral vector. Using this approach, the genotypical and phenotypical hallmark features of the recessive forms of junctional EB were corrected first *in vitro* and *in vivo* using xenograft mouse models. Finally, Mavilio et al. [49] published a pioneering clinical Phase I/II trial of retroviral gene therapy in a patient with junctional EB in 2006. These results outlined the current stage of development of retroviral cDNA transfer and its promising therapeutic potential.

Despite the recent advances in human gene therapy, especially in the field of EB (the first successful gene therapy in a non-hematological disease [49]), several problems exist in general that are not convincingly solved, thereby still limiting the prospect of broad clinical application in the instant future. First, histology and physiology of the skin represent a methodical challenge impeding common approaches of targeted transgene delivery and expression. The epidermis, providing a primary stronghold against microbial invasion and desiccation, is poorly permeable especially to water and water-soluble compounds, a fact that highlights the importance of efficiently operating transportation devices for transgenes. The compartmentalization of the epidermis makes it necessary that any introduced therapeutic gene does not interfere with the highly regulated

developmental program of proliferation and differentiation illustrated by the sequential expression of a series of genes from the basal to the upper epidermal layers affecting adhesion, growth and cellular specification [4]. The continuous self-renewal process of the epidermis within 2 to 4 weeks further demands efficient identification, isolation, targeting and subsequent transduction of long-lived stem cell populations, since stable transmission to and expression in descendant cells is essential to achieve prolonged expression of the transgene and long-lasting phenotypic correction. To solve the latter problem, methods have recently been developed and cellular markers been found that identify stem cell-like fractions (holoclones) in mouse and human keratinocyte populations [21, 23, 44, 55, 77]. In addition, it has been shown that through *ex vivo* selection using clonal analysis, transduced keratinocytes can be obtained that have the capacity to replicate for more than 30 generations and are able to reconstitute a fully differentiated epidermis on nude mice for more than 40 weeks [40].

Other difficulties, however, remain to be solved. The *ex vivo* approaches to cutaneous gene therapy necessitate the transfer of the transduced epidermal sheets back to the patient's "skin bed" using surgical procedures with potentially scarring sequelae, a risk that is of special significance in EB patients. Even for moderately invasive surgical procedures such as Timed-(**T**echnique for the **I**mplementation of **M**easured **E**lectrosurgical **D**ata)-Surgery [28] that allows the selective removal of the epidermis from the underlying dermis without generating scars by maintaining the integrity of the dermal papillae, we will need more experience to prove the feasibility to be used in EB patients routinely. Moreover, there is always the potential problem of infection at any state of the transplantation procedure. Therefore, in the long run, it is evident that *in vivo* approaches of gene therapy would be favored in EB patients, especially since so far only "transplantable locations" are apt to gene therapy. Finally, transduction of keratinocytes with vectors containing normal full-length cDNA copies of the defective gene is not feasible for autosomal dominant forms of EB. To neutralize dominant negative mutations, genetic therapies must either suppress the production of the "toxic" proteins (knock down expression of mutant allele) or correct the genetic defect at the chromosomal level (replacement by wild-type gene). In these EB variants, technologies such as ribozyme therapy, antisense oligonucleotides or RNA interference might be used to reduce the amounts of the defective protein and thus to ameliorate the disease phenotype. These technologies, however, have not yet been tested thoroughly for skin gene therapy approaches, especially not in EB (reviewed in [42]).

In other genetic diseases, immune rejection of a therapeutically administered protein has been demonstrated. Elimination of Factor VIII has been seen in haemophilic patients and haemophilic mouse models induced by neutralising antibodies to Factor VIII (or Factor IX), preventing stable phenotypic correction following gene therapy (reviewed in VandenDriesche [81]). In the haemophilia

model, the risk of antibody formation varies depending on the type of vector, vector serotype, vector dose, expression levels and promoter used, route of administration, transduced cell type and the underlying mutation. In EB research, no animal models are available at present to investigate these immune reactions. Therefore, induction of autoimmune bullous skin diseases by the transgenic protein is a serious concern in EB gene therapy. In addition, immune responses to viral vector determinants might reduce the transgene longevity (for a comprehensive review see [47]). One possible strategy to overcome this immune response is CD40 blockade [82].

In vitro and *in vivo* attempts of gene therapy in EB according to the protein/gene to be corrected

Keratins 5 and 14

Dominant-negative mutations in the genes for keratin 5 or 14 are underlying most cases of EBS by causing loss of the functionality of the keratin heterodimers in basal keratinocytes [34]. Correction of these interindividually quite variable molecular aberrations would require unique reprogramming of the genetic profile for each patient, e.g. by RNA interference, oligonucleotide-mediated gene correction or antisense technology. In terms of clinical benefit, however, the efficiencies of the latter techniques hitherto have been reported to be rather low. Recently, however, Hickerson et al. [31] showed that single-nucleotide-specific siRNA targeting of a missense mutation within the keratin 6 gene can potently and selectively block mutant allele expression in a dominant-negative murine model of pachyonychia congenita. Moreover, the authors were able to demonstrate that wild type-specific siRNAs also knocked down the expression of pre-existing endogenous keratin 6 in human keratinocytes.

Alternative approaches have also been reported. Using a keratinocyte cell line established from an EBS patient, it was investigated whether desmin, the muscle-specific intermediate filament protein, would be sufficient to functionally compensate a mutant keratin 14 in cultured keratinocytes. Stably transfected EBS cells formed an extended keratin-independent cytoskeleton. When desmin-transfected cells were subjected to heat shock, the mutant keratin filaments showed a transient collapse while desmin filaments were maintained unchanged. Thus, defective keratin filaments and the wild-type desmin filaments appear to coexist in cells without interference [16, 48]. *In vivo* complementation of a keratin 5 knockout mouse by desmin did not rescue the phenotype. The authors reasoned that in this model insufficient desmin was expressed to compensate for the loss of keratin 5 [39]. Several other reports also describe principles of handling EBS patient cells for procedures in gene therapy experiments. To facilitate *in vitro* experiments by extension of the cell lifetime, EBS patient cells have been

transduced with human telomerase (hTert). The hTert(+) cells had a normal karyotype and the cells have undergone more than 80 population doublings following hTert retroviral transduction, whereas control cells exhibited senescence-associated proliferation arrest after 8 population doublings. In organotypic culture, the hTert(+) cells were capable of forming a stratified epithelium, illustrating the preservation of their ability to differentiate [35]. Cao et al. reported the generation of a mouse model that allows focal activation of a mutant keratin 14 allele in epidermal stem cells upon topical administration of the synthetic progesterone RU-486, resulting in EBS phenotypes in treated areas. Using laser capture microdissection, they show that induced blisters healed by migration of surrounding non-phenotypic stem cells into the wound bed, demonstrating a growth advantage of normal cells [9].

Type XVII collagen/BPAG2

Most JEB patients belong to either of two main subgroups: Herlitz JEB (JEB-H) and non-Herlitz JEB (JEB-nH). Patients diagnosed with the former disease usually die within the first two years of life, whereas the latter diagnosis is associated with a better prognosis and a tendency towards further improvement during adult life. Initial observations describing reduced expression of BPAG2 in some patients suffering from JEB-nH [64] were followed by the identification of mutations in the gene COL17A1 coding for type XVII collagen in those patients [50]. The clinical phenotype of JEB-nH can also be generated by mutations in the genes coding for laminin-332 [15]. For the COL17A1 gene, phenotypic reversion of the ultrastructural characteristics of JEB-nH keratinocytes have been demonstrated by retroviral transduction [73]. Restoration of full-length BPAG2 protein expression was associated with adhesion parameter normalization of primary BPAG2-negative human keratinocytes which were then used to regenerate human skin on immune-deficient mice.

Laminin-332

Laminin-332 (formerly named laminin-5) is composed of three distinct polypeptides, the alpha3, beta3 and gamma2 chains, which are encoded by three different genes, LAMA3, LAMB3 and LAMC2, respectively. Loss of one of these genes can lead to complete loss of function of the laminin heterotrimer, which critically depends on correct peptide assembly. In pioneering work by Dell'Ambra et al. [19], keratinocytes were isolated from a patient suffering with JEB-H, characterised by a homozygous mutation of the LAMB3 gene resulting in a complete absence of the beta3 polypeptide. These beta3-null keratinocytes were unable to synthesise laminin-332 and to assemble hemidesmosomes, maintained the impairment of their adhesive properties in culture, and displayed a decrease in their colony-forming ability. A retroviral construct expressing human

beta3 cDNA was used to transduce such primary beta3-null keratinocytes. Beta3-transduced cells were shown to synthesize and secrete mature heterotrimeric laminin-332. In addition, those keratinocytes regained the capacity to adhere and to assemble hemidesmosomes. Clonal analysis demonstrated that holoclones expressed the transgene permanently, suggesting stable correction of epidermal stem cells [19]. Meneguzzi's group analyzed the retroviral expression of the beta3 chain of laminin-332 in human JEB-H keratinocytes in an organotypic culture and showed that reconstituted epidermis closely adhered to the mesenchyme and built mature hemidesmosomes bridging the cytoplasmic intermediate filaments of the basal cells to the anchoring filaments of the basement membrane [79]. To evaluate gene correction *in vivo*, primary keratinocytes of JEB patients were transduced with a retroviral vector encoding laminin beta3 and used to regenerate human skin on severe combined immunodeficient (SCID) mice. Tissue regenerated from beta3-transduced JEB keratinocytes produced phenotypically normal skin characterised by permanent beta3 expression and the formation of hemidesmosomes. In addition, beta3 gene transfer corrected the distribution of a number of important basement membrane zone proteins, including BPAG2, integrins β4/β1 and laminins alpha3/gamma2 [67].

Those experiments made the first clinical application of gene therapy possible with an *ex vivo* gene therapy approach in a patient with junctional EB. The technology recently reported by Mavilio et al. [49] proved to be feasible and potent for structural and functional correction of the disease. Epidermal stem cells from the palm of an adult patient affected by laminin-332-beta3-chain deficient junctional EB were transduced with a retroviral vector expressing LAMB3 cDNA, and used to prepare genetically corrected epidermal grafts grown in culture. Importantly, areas of epidermis could be obtained from small biopsies. Engraftment was accompanied by proper synthesis, assembly and normal levels of functional laminin-332, together with the development of a firmly adherent, self-renewing epidermis. The grafts remained stable for the duration of a one year follow up in the absence of blisters (even after induced mechanical stress), infections, inflammation or immune response.

In this special case, attention should be paid to several facts. First, clonogenic cells obtained from the patient's palms were transduced with virtually 100% efficiency, as indicated by immunofluorescence analysis of cytoplasmic LAM5-beta3. *In situ* hybridization additionally showed that vector-derived LAM5-beta3 transcripts were present in virtually all cells of the regenerated epidermis. According to retroviral integration site analysis, epidermal regeneration seemed to be maintained by a defined repertoire of transduced stem cells which may have benefited from a selective growth advantage over resident defective cells [23]. Selected by nature, viral based gene delivery is thus confirmed in being the most efficient transduction device at this point of time. Moreover, new powerful vectors such as lentiviruses are even capable of infecting non-proliferative cells

and concomitantly reducing their genotoxicity by integrating anywhere within transcriptional units and not preferentially near transcriptional start sites (as retroviral vectors do). The latter fact is associated with an increased risk of proto-oncogenic activation [23]. Incorporation of long terminal repeat sequences, strong promoters for (tissue-) specific transgene expression as well as "insulator" elements that prevent transcription of neighboring DNA sequences and position effects like silencing should further stimulate the development of ideal gene therapy vectors [22].

Secondly, the *ex vivo* approach currently harbors undoubted practical benefits. Transduction of cells grown *in vitro* is known to be more efficient and not limited by the presence of extracellular barriers as *in vivo*. The transduced cell population can furthermore be expanded *in vitro* prior to grafting. Another advantage is the avoidance of signals provided to the immune system by *in vivo* administration of recombinant viral vectors.

Thirdly, the patient's compound mutations (i.e. a null allele associated with a missense mutation) allowed residual synthesis (<5% of normal levels) of the laminin-beta3 chain carrying a single amino acid substitution. This may account for the lack of a humoral or cytotoxic immune response to the fully restored laminin-beta3 chain which otherwise would significantly compromise the therapeutic benefit.

Fourthly, removal of LAM5-beta3-deficient epidermal remnants was performed by moderately invasive Timed-surgery [28] under local anesthesia. Although surgical procedures are rather delicate in mechanobullous diseases, the intervention proved, at least in this one JEB patient, to be quite practical. However, the feasibility of its routine application in EB patients with often large affected areas remains to be determined.

Finally, no hints for uncontrolled insertional mutagenesis were found, although such an event remains to be considered as a relevant, sometimes fatal limitation of vector-based gene correction [11, 23, 29]. Despite the above listed reservations, the therapy successes seen to date give us new promising perspectives to counteract aberrant gene expression.

Molecular therapy studies usually examine only a limited number of corrective parameters. To assess the general impact on cellular gene expression for two major molecular therapeutic approaches, gene versus protein delivery in JEB were compared. Both gene and protein replacement of the laminin-332 beta3 adhesion molecule restored normal growth and adhesion of poorly viable JEB cells. Gene expression profiling was then performed using cDNA microarrays. The expression of more genes was normalised after laminin-332 beta3 gene transfer than after protein transfer. As anticipated for laminin-332 beta3 delivery, many of

the genes whose expression was restored to a normal range were those encoding for adhesion molecules and hemidesmosome components. Although gene transfer normalised the expression of a higher percentage of genes than did protein transfer, neither approach fully normalised expression of all genes examined [68].

Although integrating viral vectors can achieve repair on the molecular and cellular level with outstanding efficiency, that method still suffers from logistical and biosafety concerns (e.g. recombination with endogenous viruses; immune or inflammatory anti-viral responses; or genotoxicity/insertional mutagenesis), as illustrated in 2003 when two of the ten patients in a successful gene-therapy trial for severe combined immunodeficiency developed leukemia as a result of insertional mutagenesis caused by the retroviral vector integrating into the 5'-untranslated region of an oncogene [8, 10, 25].

To develop a non-viral approach to EB gene therapy, PhiC31 bacteriophage integrase was used, which mediates unidirectional genomic integration of plasmids containing a specific attB site. An attB-containing laminin-332 beta3 expression plasmid was integrated into the genomes of primary keratinocytes from four unrelated, genetically characterised JEB patients. Thereby, PhiC31 integrase supported genomic integration into epidermal progenitor cells. Regeneration of human skin on immunodeficient mice using these cells produced human skin tissue with restored laminin-332 expression. Furthermore, corrected JEB tissue restored hemidesmosome formation and abolished histological evidence of subepidermal blistering [59]. In another attempt from the same group, the Sleeping Beauty transposable element was used to integrate the LAMB3 cDNA into genomes of epidermal holoclones from six unrelated JEB patients. These cells also regenerated human JEB skin on SCID mice in terms of normalisation at the level of laminin-332 protein expression, hemidesmosome formation and blistering [58].

Integrin β4

Primary beta4-null keratinocytes, obtained from a newborn suffering from pyloric atresia-junctional EB, were stably transduced with retroviruses carrying a full-length beta4 cDNA and hemidesmosome assembly was evaluated on organotypic skin cultures. Beta4-corrected keratinocytes were indistinguishable from normal cells in terms of integrin a6β4 expression, the localisation of hemidesmosome components, and hemidesmosome structure and density, suggesting full genetic and functional correction of beta4-null keratinocytes [20].

Type VII collagen

Much of the efforts in gene therapy for EB has been dedicated to the correction of the lack of type VII collagen in recessive dystropic EB (RDEB). This condition

has attracted the special interest of research because it is a lifelong severe EB variant associated with considerable morbidity and mortality.

An intriguing approach to correct the lack of expression of a gene is to transfer the full genomic region, including all intronic and regulatory regions, into the cell of interest. In that sense, a cosmid clone containing the entire human COL7A1 gene in one piece was tested in transgenic mice. The data showed that the gene construct is capable of directing expression of collagen VII in the skin of fetal and neonatal transgenic mice. Expression of human COL7A1 in these mice was widespread, in a pattern consistent with that found in human tissues, and was in parallel with that of the endogenous mouse gene. Immunostaining using human-specific antibodies showed that human collagen VII protein was present in the skin basement membrane zone of the transgenic mice. Dermal extracts from 19-month-old transgenic mice contained mature human collagen VII protein, and fibroblasts derived from skin biopsies of these mice actively synthesised human collagen VII [71]. A further 'proof of principle' study for genomic DNA vectors as a means of restoring collagen VII production in RDEB skin was published by Mecklenbeck et al. in 2002. The entire human COL7A1 locus in a P1-derived artificial chromosome was transferred to RDEB keratinocytes by microinjection, after which biosynthesis and secretion of procollagen VII was detected for 1 year *in vitro*. Protein chemistry analysis demonstrated that the chain composition, domain structure, N-glycosylation and protein folding of the newly produced procollagen VII were similar to its authentic counterpart, indicating that the transgenic procollagen VII was structurally normal [53]. At present, this approach suffers from the logistical hurdles caused by the microinjection techniques used in these studies.

It had not been made clear whether the COL7A1 cDNA can be integrated into a vector suitable for keratinocytes gene transduction until Chen et al. used a self-inactivating minimal lentivirus-based vector containing the COL7A1 transgene. Transduction of COL7A1 with this kind of vector into RDEB keratinocytes and fibroblasts (in which type VII collagen was absent) resulted in persistent synthesis and secretion of type VII collagen. Unlike RDEB parent cells, the gene-corrected cells exhibited normal morphology, proliferating potential, matrix attachment and motility. These gene-corrected cells were used to regenerate human skin on immunodeficient mice. This regenerated skin had restored expression of type VII collagen and formed anchoring fibrils at the dermal-epidermal junction *in vivo* [14, 24]. However, type VII collagen cDNA rearrangements within the cell genome after gene delivery have not yet been investigated. The size of the type VII collagen cDNA and the presence of numerous repeated sequences may in fact lead to interruptions during the retro-transcription process and the formation of shorter sequences that will integrate into the host genome (rearrangements) [13].

A PhiC31 bacteriophage integrase approach was used to integrate the COL7A1 cDNA into genomes of primary epidermal progenitor cells from four unrelated RDEB patients. Skin regenerated using these cells displayed stable correction of RDEB disease features, including type VII collagen protein expression, anchoring fibril formation and dermal-epidermal cohesion [56]. Another trail to circumvent limitations associated with the size of type VII collagen cDNA used retroviral vectors carrying a recombinant truncated type VII collagen "minigene" (characterized by a 683-amino acid in-frame deletion) to correct the genetic defect in human RDEB keratinocytes [13, 23]. However, for clinical applications the transduction efficiency of non-viral vectors is still too low to be therapeutically efficient and the use of minigenes might raise safety issues.

A new way to overcome the problem of how to reach the cutaneous region of interest (i.e. the dermis) in RDEB was presented by Ortiz-Urda et al. using the PhiC31 integrase approach. Intradermal injection of RDEB fibroblasts overexpressing type VII collagen into intact RDEB skin stably restored correctly localised type VII collagen expression *in vivo* and normalised hallmark RDEB disease features, including subepidermal blistering and anchoring fibril defects [57]. With regard to molecular engineering, dermal fibroblasts have several advantages over epidermal keratinocytes as they are easier to propagate, robust and less susceptible to growth arrest. The question of whether dermal fibroblasts are able to produce type VII collagen in sufficient amounts was also targeted by Woodley et al. Human skin generated by COL7A1 gene-corrected RDEB fibroblasts or normal human fibroblasts combined with RDEB keratinocytes restored type VII collagen expression at the dermo-epidermal junction *in vivo* in SCID mice. Furthermore, intradermal injection of normal human or gene-corrected RDEB fibroblasts into mouse skin resulted in the stable expression of human type VII collagen at the mouse dermo-epidermal junction [86]. Goto et al. demonstrated that retroviral gene-transferred DEB fibroblasts transplanted into nude mice supplied even more collagen VII to the new dermal-epidermal junction than gene-transferred keratinocytes. These results suggest that fibroblasts may be a better gene therapy target of DEB treatment than keratinocytes [27]. Alternative approaches involved *in vivo* intradermal injections of lentiviral vectors carrying the COL7A1 gene [87] and recombinant human type VII collagen [88].

Considering the limitation that severe dystrophic EB causes widespread wounds and treatment thus would require multiple *intradermal* injections, Woodley et al. recently demonstrated that even intravenously injected, genetically engineered autologous DEB fibroblasts over-expressing corrected type VII collagen homed to murine skin wounds and continuously delivered the transgene product at the wound site. There it incorporated into the skin's basement membrane zone and formed anchoring fibril structures associated with accelerated wound healing. This strategy suggests that "protein therapy" and "cell therapy" approaches could be possible for treating EB patients and that one day

genetically corrected bone marrow stem cells may offer another route to (permanent) whole body-gene therapy [89].

To establish an *in vivo* model of RDEB, dogs expressing a mutated collagen type VII were analysed. Isolation and analysis of the 9 kb dog collagen type VII cDNA identified the pathogenic mutation G1906S. Highly efficient transfer of the wild-type collagen type VII cDNA to both dog and human primary RDEB collagen type VII-null keratinocytes using recombinant retroviral vectors achieved permanent expression of the transgene product. The expression and post-translational modification profile of the recombinant collagen type VII was comparable to that of the wild-type counterpart. The recombinant canine collagen type VII in human RDEB keratinocytes and dog cells corrected the observable defects caused by RDEB keratinocytes in cell cultures and in *in vitro* reconstructed skin. Hypermotility was fully reverted in human RDEB keratinocytes and strongly reduced in the dog RDEB cells [4].

Animal models

Because most gene therapy protocols require preclinical validation in animals, animal models of EB are of special interest. Most of them suffer from the fact that the majority of the transgenic mice mutated in various adhesion molecules die neonatally, precluding sufficient evaluation for gene therapy purposes. These mouse models include mice deficient for keratin 5 [63], keratin 14 [46], laminin alpha3 [70], laminin gamma2 [54], integrin a6 [26], integrin β4 [80], COL7A1 [30] and COL17A1 [22]. Animal models with clinical symptoms of EB, but presenting a surviving phenotype, include a Belgian horse suffering from a blistering condition. Here, Spirito et al. used immunofluorescence analysis to uncover absent expression of the gamma2 chain of laminin-332, pointing to LAMC2 as the candidate gene. Comparative analysis of the nucleotide sequence of the full-length gamma2 cDNA obtained from skin and tongue epithelium of a JEB foal disclosed the homozygous base pair insertion 1368insC. This mutation results in a downstream premature termination codon and is predicted to cause absent expression of the laminin gamma2 polypeptide [76].

In another model, dogs featuring dystrophic EB have been analysed. These dogs showed only a slight reduction of type XVII collagen (BPAG2) immunoreactivity and the clinical features were resembling those of the human DEB variant named transient bullous dermolysis of the newborn. This variant is associated with mutations in the COL7A1 gene causing temporary blistering of the skin [60].

Although progress in experimental models for correction of EB using viral and non-viral vectors has been ample (for a comprehensive review see [36]), only one approach hitherto has made it into the human system (see above [49]). Therefore,

it is useful to analyse alternative ways for re-establishing the integrity of the dermo-epidermal junction in EB, especially in autosomal dominant variants.

Other approaches to gene correction

"*Natural gene therapy*"

Analysis of naturally occurring phenomena of gene correction could pave the way for alternative approaches to medical gene correction. Reverse mutations (i. e. true back mutations leading to the original wild-type sequence and thus wild-type protein or additional second site mutations that compensate for the effect of the primary inherited mutation) in germline or somatic cells bearing an inherited disease-causing mutation can change the phenotype from affected to normal by reexpression of the involved protein [62]. Revertant mosaicism in EB due to *in vivo* reversion of somatic cells has been described for COL17A1 and KRT14 [18, 37, 72, 75]. In 1997, Jonkman et al. [37] reported a JEB-nH patient with patches of normal appearing skin in a symmetrical leaf-like pattern on the upper extremities. The underlying mutations in the COL17A1 gene have been identified to be R1226X paternally, and 1706delA maternally. In the clinically unaffected areas of skin, ~50% of the basal cells were expressing type XVII collagen protein at a reduced level. The reason for the reexpression of type XVII collagen was a mitotic gene conversion surrounding the maternal mutation, thus replacing the heterozygous mutation. These observations suggest that <50% of full-length type XVII collagen is necessary to correct the phenotypic expression of JEB-nH, which would be in the range of the current viral transduction efficacy. We have described a second JEB-nH patient, in whom the homozygous mutation 4003delTC was partially corrected by the somatic mosaic back mutation 4080insGG. However, no phenotypic correction, that is, clinically normal skin, could be observed in our patient. It is probable that the 25 incorrect amino acids between deletion and insertion prevented functional correction [18]. A surprising variant of the 'natural gene therapy' theme has been presented by Smith et al. [75]. These authors reported on a patient with a 20-year history of EBS Dowling-Meara. As has been noticed with some other patients suffering from EBS, the disease improved over the years. The reason in this patient was that the heterozygous genomic mutation R125C in the keratin 14 gene had been knocked out by a preceding somatic stop codon mutation in keratinocytes. This stop codon prevented the expression of the dominant-negative mutation R125C.

Recently, the group of Marcel Jonkman further demonstrated the occurrence of multiple correcting COL17A1 mutations in distinct type XVII collagen-positive skin patches in 2 unrelated probands [61]. Moreover they reported on 2 JEB-nH patients reflecting revertant mosaics, in which mutation analysis in addition to the germline mutation in the LAMB3 gene also revealed the presence of at least 5

different second-site LAMB3 mutations. The latter all corrected the same inherited mutation *in vivo*, implying that there is not a single preferred mechanism for the correction of a specific mutation [58]. Revertant areas became progressively clinically healthy with normal expression of laminin-332.

The cause of such correcting DNA events remains unclear. It has been suggested that revertant mosaicism is evoked by mutational hot sports such as direct repeats of homonucleotide tracts. The recent data furthermore implies that it occurs at a higher frequency than expected. This would open the possibility of applying revertant cell therapy in mosaic EB of LAMB3 gene by using autologous naturally corrected keratinocytes for transplantation, thereby bypassing the recombinant gene correction phase (Personal communication, Marcel F. Jonkman).

Modulation of splicing

In recent years, data have been accumulating indicating that the spliceosome can be a target for attempts of gene correction. The spliceosome of a cell is the place where small nuclear ribonucleoproteins facilitate the removal of introns from the pre-mRNA (the so-called splicing reaction) and, therefore, control the correct assembly of exons forming the mature mRNA. If mutations disrupt the normal sequence of a splice site, the spliceosome uses other intronic or exonic splice sites (so-called cryptic splice sites) usually leading to out-of-frame transcripts or rarely to in-frame transcripts. In this case, antisense oligonucleotide technology can be used to suppress the use of cryptic splice sites. This approach has been already tested successfully for the suppression of aberrant splicing in erythroid cells from peripheral blood of thalassemic patients [41]. The technology could also be applied in EB patients, who have a splice site mutation producing at least one alternative in-frame transcript. In this case, suppression of splice sites leading to out-of frame transcripts could redirect the splicing machinery to the in-frame splice reactions, therefore producing polypeptides with only small changes in protein composition. A partly successful natural attempt of gene correction by modulation of splicing reactions has been described in patients with the homozygous mutation R785X in the COL17A1 gene [69]. In these patients, the exclusion of exon 33 harbouring this mutation leads to an unusual mild phenotype, although there is only 5% of detectable type XVII collagen protein. This in-frame skipping of exons has also been reported for patients with mutations in the COL7A1 and LAMB3 genes [51].

Another mechanism of 'corrective' splicing is the activation of naturally occurring splice variants to exclude faulty exons (see above) or to include missing exons. This approach has been described for inclusion of exon 7 in the survival motor neuron 2 gene *in vitro*. By this mechanism, spinal muscular atrophy patients could be protected from the effects of loss of function in the survival motor neuron 1 gene [32]. Modulation of splicing activities can also be attempted

by *trans*-splicing. *Trans*-splicing refers to a process whereby an intron of one pre-mRNA interacts with an intron of a second pre-mRNA, enhancing the recombination of splice sites between these two pre-mRNAs. A practical application of targeted *trans*-splicing to modify specific target RNAs has been used in group I ribozyme-based mechanisms. For example, using the Tetrahymena group I ribozyme, targeted *trans*-splicing has been achieved in human erythrocyte precursors to alter mutant β-globin transcripts from individuals with sickle cell disease [43]. A related approach is spliceosome-mediated-RNA *trans*-splicing (SMaRT). SMaRT uses the introns preceding or following an exon harbouring a mutation as a hinge to introduce a corrected species of RNA into a given pre-mRNA. Therefore, this technique reduces the size of a corrective insert into a viral vector depending on the position of the mutation. This fact is of particular interest in genes coding for large proteins involved in EB, i.e. type VII collagen (9.2 kB mRNA) and plectin (14.2 kB mRNA). Furthermore, endogenous regulation of transgene expression is targeting the pre-mRNA of interest. Proof-of-principle for SMaRT was first demonstrated in the β-human chorionic gonadotropin gene [66]. Its applicability in the correction of genetic diseases has been expanded by work showing correction of the predominant cystic fibrosis transmembrane conductance regulator (CFTR) mutation deltaF508 in a CFTR mouse model and a factor VIII-deficient mouse model *in vivo* [12, 45]. Moreover, it has been demonstrated that keratinocytes also apt for *trans*-splicing reactions in the COL17A1 gene [17] and are, therefore, a potential target for SMaRT.

Recently, our group demonstrated that in EBS-MD fibroblasts harbouring a heterozygous insertion mutation within exon 9 of the plectin gene (PLEC1), transient transfection with a 5′ pre-*trans*-splicing molecule encoding wild-type exons 2–9 led to specific replacement of the mutated 5' portion of the endogenous PLEC1 transcript through *trans*-splicing [84]. Treatment reduced levels of mutant mRNA and restored a wild-type pattern of plectin expression. When EBS-MD fibroblasts were transfected with retroviral constructs, the level of full-length plectin protein in the corrected fibroblasts increased by 58.7%. These data give the perspective that, in the field of splicing reactions, specificity and efficiency may be high enough to allow for high fidelity gene correction.

Aminoglycosides

Aminoglycosides belong to a group of antibiotics that cause extensive misreading of the RNA code [74] and subsequently have been used to suppress stop codons in cultured muscle cells from the mdx mouse, an animal model for Duchenne muscular dystrophy that possesses a premature stop codon in the dystrophin gene. Exposure of mdx myotubes to gentamicin led to the expression and localisation of dystrophin to the cell membrane. The effects of different dosages of gentamicin on expression and functional protection of the muscles of mdx mice were then evaluated. A treatment regimen was identified that resulted

in the presence of dystrophin in the cell membrane in all striated muscles examined and that provided functional protection against muscular injury [6].

These pilot studies led to the initiation of clinical trials. However, conflicting results have been obtained *in vivo* in patients suffering from Duchenne muscular dystrophy [65, 83]. Part of the problem encountered in these studies may stem from the sequence specificity of the stop codon read-through mechanism induced by gentamicin [33]. For cystic fibrosis it has been shown that aminoglycoside treatment has a measurable effect on the basal potential difference and chloride-free isoprotenerol response in selected patients [85]. In addition, other antibiotics with less side effects have been tested *in vivo* to generate stop codon read-through [3]. In EB, the aminoglycoside approach would only be applicable in patients with stop codon mutations.

Inhibition of matrix metalloproteinases

A large number of COL7A1 mutations have been reported in RDEB patients, and the analysis of phenotype–genotype correlations showed evidence for interfamilial and intrafamilial phenotypic variability occurring for the same mutation. Bodemer et al. investigated if tissue destruction in the disease process might result from an imbalance of matrix metalloproteases (MMPs) over tissue inhibitor of metalloproteases (TIMPs) secondary to the genetic defect. Increased amounts of metalloproteases suggested to be operative in the blistering process and tumor invasion was observed in the skin of RDEB-affected siblings, both in lesional and in non-lesional skin as compared with controls. The amounts of MMP-1, -2, -3 and -9 increased particularly in the skin of the more clinically affected patients. Furthermore, for this patient higher amounts of MMP-1 and also lower amounts of TIMP-1 in the unaffected and affected skin have been detected compared with the other two affected patients and with healthy control donors [7]. In addition, while analyzing the protein composition of junctional EB keratinocytes, Gagnoux et al. [20] noticed that they lacked plasminogen activator inhibitor type 1 (PAI-1), which inhibits the conversion of plasminogen to plasmin by urokinase-type plasminogen activator (uPA). Notably, plasmin also degrades the basement membrane and overactivation of the uPA enzymatic cascade produces a junctional EB-like phenotype in transgenic mice. Therefore, although inhibition of MMPs does not compensate the genetic defect in EB, it might lead to an amelioration of the symptoms. Clinical practice shows that this is a desirable aim in many patients with severe types of EB.

Perspectives

Since the first cutaneous *in vitro* gene therapy experiments in the mid-1990s, substantial experimental and preclinical advances have been made in the field of

molecular treatment. The refined knowledge about the genetic basis of alterations within the basement membrane zone as well as the identification of disease-modulating factors have laid the basis for mutation-specific gene correction. In addition, a permanent progress in vector design, administration, immune modulation and techniques like clonal analyses to verify stem-like cell targeting for prolonged *and* regulated transgene expression through multiple cycles of epidermal renewal provides encouraging means to bring us much nearer to broad clinical application of molecular therapy.

Although this has not yet been translated into ultimate therapeutic benefit for numerous patients, and several issues of therapeutic molecular intervention like immunogenicity or biosafety remain still problematic, a promising variety of new biotechnological applications is arising to validate gene therapy as an effective therapeutic modality in humans.

In cutaneous gene therapy the main focus of research is on EB and the most popular strategy is the *ex vivo* approach. A considerable number of necessary steps to a safe and effective gene therapy have been already been taken, including a clinical phase I/II trial in junctional EB. For this group of genodermatoses, two additional EU-funded efforts (Skintherapy and Therapeuskin) started in 2005. In both projects recessive dystrophic EB is proposed to be corrected by a retroviral approach. Given the success of these efforts we can expect several trials for selected EB patients within the next five years.

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4. LIVING WITH EB

4.1 Surveillance for extracutaneous complications

Jo-David Fine

As has been discussed in great detail throughout this monograph, a variety of extracutaneous complications may arise in the setting of hereditary EB, since essentially any organ having an epithelial surface or lining is at risk for mechanical instability and at least microscopic blister formation. These include the external eye (Chapter 2.2.1), oral cavity (Chapter 2.2.3), esophagus, small and large intestine, and anus (Chapter 2.2.4), tracheolaryngeal tree (Chapter 2.2.2), kidney (Chapter 2.2.6) and other portions of the genitourinary tract (Chapter 2.2.6), to include the vagina. In addition, some patients are at risk of developing acral musculoskeletal deformities (Chapter 2.2.5), muscular dystrophy, or dilated cardiomyopathy (Chapter 2.2.6). Others may later develop skin-derived cancers (Chapter 2.1.3), most notably squamous cell carcinomas, many of which may become life-threatening. Although there is a considerable range in the extent and severity of each of these complications, many may be extremely severe or even disabling.

It is also known that the risk of for each of these many complications varies by both age and EB type or subtype. For example, the onset and cumulative risk of developing strictures within the esophagus is different from that of stenosis or stricture formation within the upper respiratory tree; they also differ markedly by EB subtype. Similarly, squamous cell carcinomas, a frequent event in patients with RDEB-HS, do not arise until at least early in the second decade of life, whereas malignant melanomas, a far more rare complication within the same EB subtype, appear to occur only during earlier childhood.

It is clear, therefore, that meticulous surveillance must be performed on patients having those EB subtypes that most are at risk for developing specific complications. At the same time, unnecessary surveillance will only add to the

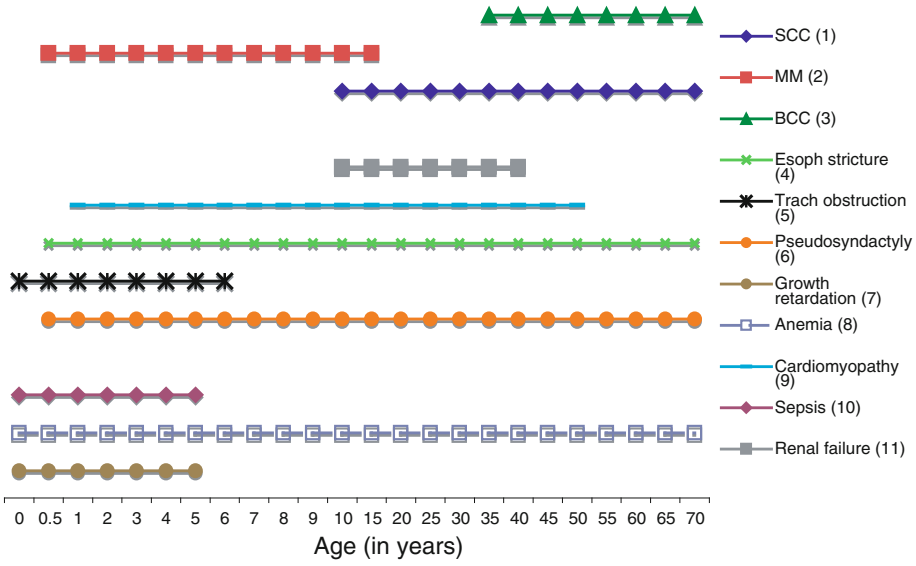

Fig. 4.1-1. Surveillance timetable figure. Pertinent EB types or subtypes: *1* RDEB, JEB (uncommon); *2* RDEB-HS; *3* EBS-DM; *4* RDEB > JEB > DDEB; *5* JEB; EBS-DM (rare); *6* RDEB > DDEB > JEB (rare); *7* RDEB-HS; JEB-H; EBS-DM; *8* RDEB; JEB; *9* RDEB-HS > JEB-nH > RDEB-nHS; *10* JEB > EBS-DM and RDEB-HS; *11* RDEB-HS > RDEB-nHS > JEB-nH

anxiety of an EB patient or his or her family. When, then, is it appropriate to begin to counsel the parents of an EB child about the risk of specific extracutaneous outcomes and then to proactively screen for their earliest presence?

Figure 4.1-1 graphically illustrates the ages at which many of the more common extracutaneous complications arise. The relative frequency for each, as stratified by EB subtype, is summarized within its legends. More precise data, stratified by each major EB subtype and based on lifetable analyses performed on the large cohort of patients enrolled within the National (USA) EB Registry, may be found elsewhere in this monograph.

4.2 Living with EB – impact on daily life

Anja Diem

Introduction

EB is a lifelong challenge, varying not only in the severity of the disease but also by whether secondary complications are present; for example, musculoskeletal deformities or problems with food intake. Speaking of patients as having mild forms of EB is not meant to trivialize their disease. Rather it means only that those children or adults have problems that are oftentimes easier to solve. Problems caused by the disease can be small or very stressful, starting with anxious looks which are not meant to be mean or judgmental, but still are very hurtful and at times can be malicious. Such unintentional behavior by others can result in an EB patient becoming more and more subdued, avoiding contact with others. Information and explanation about the disease and problems associated with it can lead in many cases to respect and understanding for the person, which he or she rightfully deserves.

Living with a special condition like EB, yet still trying to live a normal life with its highs and lows, success and failures, wishes and dreams, strength and weaknesses, has its difficulties. Every form of EB has its own special problems. Just as every person has a different personality, one patient's disease cannot necessarily be compared to another's. In this chapter I will attempt to summarize the duties and challenges that we are confronted with in our clinic, including those that are not necessarily directly related to our patients' underlying disease.

EB patients have unexpected medical and surgical problems, as does everyone else. The presence of such illnesses, though, at times may exacerbate their

underlying genodermatosis. Patients with EB, even those with relatively localized skin involvement, may experience moderate to severe pain, caused simply by standing, walking, lying down or even sitting. Symptomatic involvement within the oral cavity can impair eating, negatively impacting on overall nutritional status. EB patients can develop very painful blisters on their eyes which may prevent them from seeing for days. Of course this medical condition and its side effects have a great influence on daily life, preventing patients from participating in many activities that are usually taken for granted. Only being able to participate with limitations and often being absent from school or work can have a negative outcome. Another important factor is the length of time needed for life's daily activities, wound care, therapy and the preparation of meals, eating and bathing. Patients, therefore, must find the right balance between therapy and the care that is really needed. A daily time plan needs to be made to account for necessary therapy and hygiene, but oftentimes there is not enough time to accomplish everything that is needed. As a result, these patients and their caregivers need to learn to cut down on unnecessary actions and yet still perform the important tasks. Of course, parents and caretakers often find it difficult to make these decisions. Time needs to be found for dressing changes, as well as time for school, job, leisure time and friends, and all the little things that make one's daily life full. This is far easier said than done, but most EB families somehow find a way to meet these many needs, once they establish routines and prioritize accordingly.

General information

Adaptation of the home and the work place should be planned differently. In every case a quiet private space is desirable for dressing changes. This should ideally be in a bathroom with a bathtub, or shower. It is much easier and less painful for an EB patient to remove the bandages by first pre-moistening them with water (Fig. 4.2-1). It can also reduce the amount of time required to perform

Fig. 4.2-1. The daily change of dressings by an EB school boy

this necessary daily task. The place where the person can lie down for bandage changes should be well cushioned. It also needs to be adjusted to the right height for the caregiver. To help distract the person from this often painful and time-consuming procedure, a TV, radio or CD player is often very helpful. Expensive adaptations in the home may be needed so as to accommodate the use of a wheelchair. Seating must be cushioned and skid proof padding is recommended. Other adaptative measures will need to be made according to individual circumstances.

Clothing (Fig. 4.2-2) and footwear

A very important issue is clothing. It must be very soft and have no pressure points from buttons or seams. Clothing that is too warm or too tightly fitted should definitely be avoided. Special materials that are particularly well tolerated by most EB patients are cotton, rayon and silk. Protective dressings can be placed under the clothing and over those areas that are easily irritated or traumatized, to prevent further rubbing and the formation of blisters. Unfortunately, with children it is often difficult to find clothing that is functional and yet also "cool" and in style.

It is particularly important to find shoes that fit properly; otherwise blisters will arise. Shoes should be neither too large nor too small, and they need to be

Fig. 4.2-2. Appropriate clothing for the Austrian winter

made from a material that is both soft and sturdy. Sweating should be avoided at all costs, as it contributes to the development of blisters, especially in patients with Weber-Cockayne EB simplex. The sole of the shoe should be flat. Unfortunately, leather soles may transmit heat from pavement to the soles; they are also rather stiff until worn for prolonged periods of time. As such, they may facilitate blister formation in some children. It is oftentimes a financial burden for EB parents to find the correct type of shoes, since they are frequently quite expensive. Orthopaedic shoes are particularly good at providing support, and may be beneficial for those EB patients needing shoes which have specially designed heels and inserts.

EB and sports

Sports and mobility are necessary for building muscle and bone, for the maintenance and efficiency of the body's functions, and for building self confidence and a positive body image. It is known that participation in sports is good for general health and has been proven to influence other coexisting illnesses. Naturally, this is also evident in people with EB. However, sports participation must be judiciously restricted, due to the risk of easy injury to the skin. Still, though, EB patients need to get enough daily exercise. This should begin in childhood, when natural mobility should be supported and encouraged. Bandages for the especially vulnerable areas should be applied, as they can be especially helpful at this time. The children should be allowed to play without too many restrictions, so that they can learn their boundaries and at the same time improve their coordination (Fig. 4.2-3). The latter is important, since poor coordination leads to restricted mobility and can lead to insecurity and increased risk of injury.

Participation in sports is important not only in childhood. Exercise and sports should continue to be important throughout life. Which types of exercise are appropriate for someone with EB? This will depend on the extent and severity of skin involvement, as well as the child's overall health. Most types of sport can be practiced (sometimes even playing soccer and skiing), and it is appropriate to allow the child to try such an activity if it is physically reasonable and if the child can learn the limitations imposed by his or her disease. According to the severity of the disease an individual consultation is important and should be carried out.

House pets

When someone has EB there is no reason not to have a house pet (unless there is a co-existing allergy). On the contrary, the relationship with a house pet can have an extremely pleasant effect on one's overall self-esteem. The

Fig. 4.2-3. a) and **b**) Emanuel "Catching butterflies"; **c**) Jenny on a rocking horse; **d**) Valentin during outdoor activities

Fig. 4.2-4. a–c Alois with guide dog Sunny

friendship, faithfulness, and love that an animal gives to his owner can help in making the difficulties of every day life more bearable. When time, money, space and patience, love and readiness for the responsibility are all available, consideration for having a house pet should seriously be considered. Particularly good is a trained guide dog (Fig. 4.2-4), but almost every animal is a possibility.

Vacation planning

When planning a vacation it is essential to organise it properly. There are many possibilities, but ultimately one really needs to think about what would be best for both the EB child and his or her family. An ideal vacation might be one that offers something different from every day life and yet is also relaxing and stress-free. Of course this is also dependent on the financial resources available, the age of the children (both affected and unaffected), and other pertinent factors.

Of great importance is the weather. Conditions such as extreme dry heat or bitter cold (Fig. 4.2-2) can have a negative effect on people with EB. If the decision is made to spend the vacation in the southern regions or in warmer climates it is best not to go in the height of summer, when it is hottest, and to make sure that the

room has climate control. A neutral climate is preferable for most EB patients although a visit with the sun, sand and ocean can help improve the health of the skin.

Fig. 4.2-5. **a)** Dominik and his computer; **b)–d)** Drawing is important for the use of fingers **(c)** Serina **d)** Martin; **e)** "The village with snow" = Christmas card

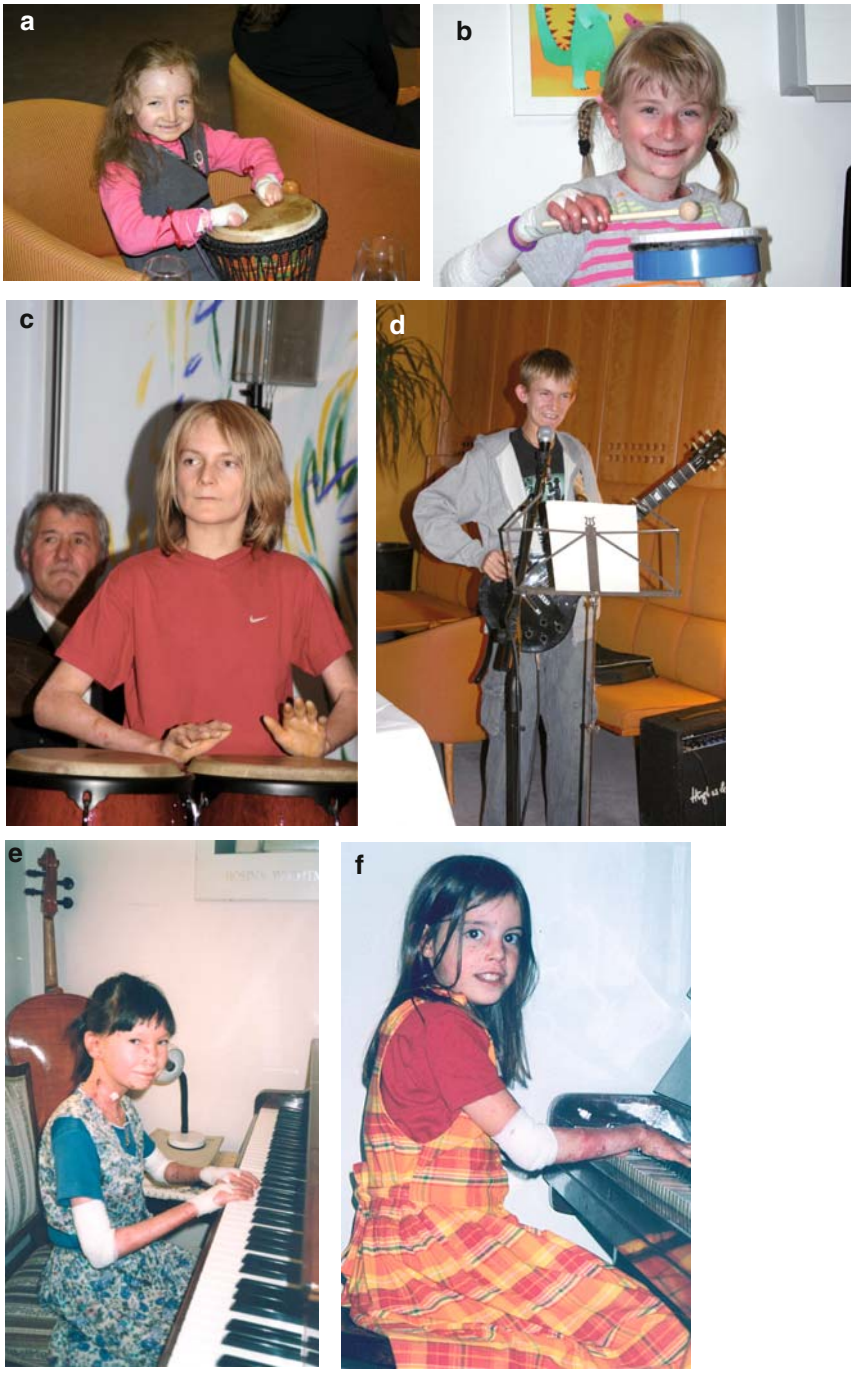

Fig. 4.2-6. Violetta **(a)** and Nina **(b)** as drummer girls; **c)** The drummer boy – Valentin could become a professional! **d)** Or better the guitar? Ianina **(e)** and Lena **(f)** on the piano

During city visits it is essential to find the correct transportation. Long walks are not advisable, which is why city and museum tours should be carried out only after careful planning. The time and extra help needed for the use of a wheelchair is unavoidable.

Hobbies

For those with EB whose life decisions and possibilities are restricted, hobbies may be especially important. Because of the need of a strictly organised day, a hobby offers a balance and a chance to relax. Art, drawing and painting (Fig. 4.2-5), collecting, reading, playing an instrument (Fig. 4.2-6), singing, and other recreational activities are all possible and are enjoyed with great pleasure.

Alternative medicine

As with other chronic illnesses that have no true cure, parents and relatives of an EB child may at one time or another look into "alternative" types of medicine, in the hope that they might help change the course of their relative's illness. There are indeed many unconventional approaches which have achieved beneficial outcomes, both subjective and objective, in individual patients. In the absence of concerns over patient safety, medical and nursing personnel should be open to hearing of new therapies, including those which are currently viewed as alternative ones, to be willing to share with their patients and their families whatever information they may have regarding those modalities, and to be available to assist them in comparing the risks and potential benefits of new therapies with those currently being employed. Unfortunately, although many anecdotal reports of benefit exist, most are based on observations made in only one or a few patients, making it impossible to critically assess their validity, efficacy, and safety. Whenever concerns arise, however, it still behooves the physician to follow the physician's creed of "first do no harm." Hopefully someday it may be possible to more rigorously compare data derived from much larger numbers of patients, to see whether any of these "alternative treatments" might significantly help in enriching the quality of life for patients with EB.

Costs

A sometimes overwhelming problem with a chronic illness is the financial burden that it brings. What resources are available to cover the costs related to the adaptation of living space, supplies, and the support given by care personnel? Where does the money come from to pay for the essential things in life or for the things that just make life easier and more pleasant? Fortunately health care by obligatory insurances is provided in Austria and is available for patients with EB.

In other countries, however, this is where the problem begins, since the costs for dressing materials, frequent treatments and operations may not be fully covered by the respective health care systems. In many countries medical health coverage is not even available or obtainable. Even in countries in which the insurance system includes coverage for chronic illness, it may not cover all of the costs, forcing the family to finance at least part of it themselves. The special healthcare needs of EB patients, given how variable they may be, are not easy to incorporate into an overly structured insurance system. Therefore it is essential that insurance companies are willing to help the families in developing individualized solutions concerning both care and coverage. Costs related to enhancing the quality of life of an EB patient, as opposed to covering essential therapies, may not be covered by healthcare insurers. For instance, additional equipment needed for the household, suitable furniture, or special food and extra hours of therapy, will need to be financed by other means. Involved families need assistance in their search for all possible sources of financial help. These options may vary considerably, not only from country to country but also between one city and the other. Fortunately, in some countries, including Austria, financial help is available to EB patients and their families from local or national EB support groups. However, these organizations often still lack sufficient funds of their own to fully support the financial needs of all of their patients. It is, therefore, absolutely essential that a caring and highly trained social worker be integrated into the overall management plan of every EB patient.

Aged based stages

The newborn

When a baby is born and there is a suspicion that EB exists, it is a normal reaction that many questions will be asked. In the first days of the baby's life the parents will go through many "hot and cold" emotional feelings. From the time the parents realize that "something is not right" with their baby until the time that the definitive diagnosis is made, feelings of uncertainty and anxiety will show, yet everyone reacts differently, according to the circumstances in which the child was born. During this particularly stressful period of time, the parents need to be given emotional support from everyone involved in the infant's care. As soon as the exact diagnosis is made the parents need to be informed about the special needs that the child is going to have as a result of having EB. The parents have a right to know what is going on with their baby, and details of the diagnosis should be given without delay, even if it is serious.

A proper place should be chosen by the physician to discuss the diagnosis, one in which there can be undisturbed discussion with the family. Enough time has to be planned so that all the questions that come up from the family can be answered

in as thorough a manner as possible. It is likely, though, that this will most likely not happen after a single meeting. At the time when the parents first learn about the diagnosis, it is understandable that they will likely have a rather short attention span and be unable to absorb and understand all of the information that is being presented, even after they have asked and re-asked many questions. For this reason, it is very important to have more than one meeting with the family. The diagnosis that has been given to the baby, what the cause of it is, the symptoms that he or she will have and the problems that EB will likely create, should be given to the parents with empathy and honesty. At the same time it is crucial that the parents be told that it is never possible to accurately predict exactly how life will be for the particular baby, given the great variability in clinical course which is known to occur within a single EB subtype, let alone within even the same family. There should always be hope expressed! The parents must be treated with respect and given the emotional support that they will need in order to accept their child as he or she is. Comprehensive information is needed for the parents, to develop trust in their physicians, so that they can cope with the surprisingly new situation they are now in.

The baby that was born with EB or is suspected to have EB has needs like every other newborn. Feelings of closeness and security and a stimulating environment (color; pictures; mobile) will help. Due to the vulnerability of their skin they also need meticulous medical and nursing care. Trained therapist and nursing care need to be organised. The parents need to learn as soon as possible how to care for the baby themselves, learning to give the baby physical closeness without inadvertently causing harm. An EB baby should never be lifted up from underneath the arms, as there the skin is very easily injured. It is best to use a pillow or a thick blanket to pick up or carry the baby. It is also important to avoid adherent materials (see also Chapter 3.3). Parents will need to be educated about the most appropriate dressing materials (Chapter 3.1.1) that be used on EB skin. Adhesive electrodes should be avoided unless there is a serious need for their use. Usually, though, there are other ways to apply them without injuring EB skin (see Fig. 3.3-2d).

Breast feeding

When the mother of an EB baby is able to breast feed it is really beneficial for the nutritional value to the child (Chapter 3.4). At the same time it can also facilitate a positive bond between mother and child, which is otherwise often hindered by the stressful situation. With more severe forms of EB that are accompanied by involvement of the oral mucosa, breast feeding is the gentlest form for nutritional intake. If there is any reason that breast feeding is not possible, however, then the mother should not get stressed about not being able to directly provide breast milk to her child. A baby with EB can be fed with milk pumped mechanically from the breast. Alternatively, baby formula can be used.

If the baby has symptomatic oral cavity involvement, then a special feeding nipple (Habermann Sauger®) can be employed, to reduce pain and trauma during feeding.

Discharge from the hospital

Discharge to the home is possible as soon as the parents feel confident in caring for the baby. It is necessary to organise transitional arrangements for them well in advance of the actual day of discharge. In the beginning the parents will need a contact person who is readily available for them at any time, should questions or problems arise. They will also need to establish a relationship with a family doctor or pediatrician in their hometown, who is willing to take over the day-to-day care of the infant. It is important to remember that the care

Fig. 4.2-7. a) The Austrian eb – family; **b)** EB families visit the zoo together

of a small baby with EB is labor-intensive and very time consuming. A proper place to provide optimal wound care and bandaging needs to be arranged. Whenever possible it would be helpful to have a home care nurse available to assist the parents at that particularly difficult time. Even if a nurse is available to the parents, though, close friends and relatives should all be familiar with the care needed by the child.

It is helpful for the parents to be able to contact and meet other parents of children who have EB (Fig. 4.2-7), as this is a good way to find support, both physical and emotional, during the first days at home. We have found that in these first days the greatest challenge that the parents encounter is trying to decide which problems are due to the special effects and characteristics of EB and which are simply a baby's normal needs. Good monitoring and assessment of the baby by the parents and caretakers, and communication between the parents and the doctor taking care of the baby, are essential in providing the most proper care.

The baby's bed should have a soft pad, like lambskin, to lie on, and the sides of the crib should be covered in a soft material, to prevent injury. The bed should not be used as a table for changing dressings, as this may be associated with painful, unpleasant procedures. The bed should instead become a safe restful place for the baby where later, as a small child, he or she can retreat to as a safe haven.

Preschooler

Motor skills development

When the child is of preschool age the parents and caregivers are in for a challenge. Early on he or she will be beginning to sit and trying to crawl. It is normal in this phase for minor or sometimes even more major accidents to occur. When an EB child has an accident it often involves the development of blisters and it is then followed by pain. Parents understandably want to protect their children against all accidents and pain, as they do not want to see them suffer. It is also important for the child to find out for himself what his physical abilities are and thus learn his or her own boundaries. A balance needs to be found between the parents' protection and the child's will, whereby they protect the child and yet allow him or her to explore new things. This is a major challenge for both sides of the family. The parents need to be advised that the relative vulnerability of their child's skin is often related to the type of EB they have and that all EB children should still be allowed some measure of freedom. Parents need to be informed about protective measures they need to enforce, such as bandaging techniques for the knees and elbows, so that the

child can crawl without serious injury, and for the feet, when their child is first learning how to walk.

Early consultations with a physical and occupational therapist are recommended, to evaluate the kinetic motion problems that often lead to motor functional disabilities. Early actions can modify the restrictions and help with the physical development of the child (Chapter 3.5).

In those children who have a type of EB that increasingly restricts the movements in the fingers, hands, and even the tongue and opening of the mouth, practice fun exercises should be pursued in a playful way, to help to delay or prevent the development of constrictions. Unfortunately, practice shows that it is not completely possible to stop this restrictive process in every severely affected child, yet from the exercises it is possible to maintain the movement they already have. Mouth exercises, for example, can be done by making frowns and smiling in front of a mirror, blowing bubbles and best of all, singing, which most children enjoy doing.

Promotion of intellectual abilities

Making important decisions for the child's future should help in the development of all of his or her senses and in the enhancement of physical and intellectual

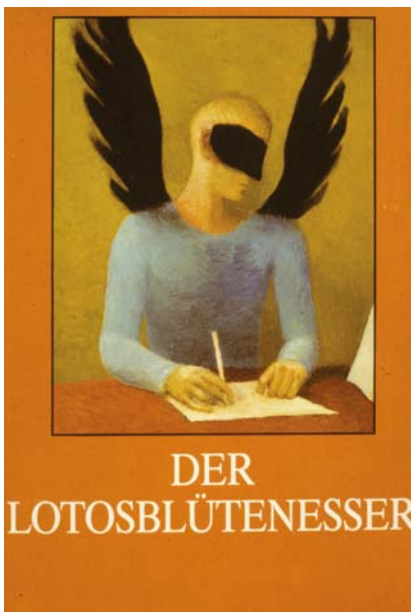

Fig. 4.2-8. The EB patient as an author

abilities (Fig. 4.2-8). In later years the child's talents and special abilities can at least partially offset the limitations caused by physical disabilities that may develop.

Information about the disease

It is helpful to teach the child in easy words about the vulnerability of skin. In kindergarten the child will be confronted with frequent questions and a lack of understanding. The more confidence he or she has in knowing about the disease, the easier it will be to cope in unaccustomed situations. At this stage it is important to have playmates of the same age, and they should be informed about this rare skin disease, so that parents and child can learn to deal with both the positive and negative reactions that they may receive. A basic requirement for life with a severe chronic disease like EB is positive self esteem. It is important for everyone involved in caring for EB children to give them the support they need to achieve this.

Dressing changes (Chapter 3.1.1)

During early childhood difficulties with bandage changes will undoubtedly develop, as it often hurts and takes a long time. Pain and discomfort can cause anxiety for the child and the parents. The feelings of helplessness and being at the mercy of someone can anger an EB child and drive him or her into a rage which may be very difficult to manage. Advice and support may be necessary during this particularly difficult period of life. It may not be physically possible for parents to always provide the most optimal wound care, following a sophisticated care plan, every day of the week, given the other issues and conflicts that may at times unexpectedly arise. Despite that, the parents need to be assured that they are doing the right thing. For the years ahead they need self assurance, since their child will need to have the bandages changed fairly often in the week. All the tricks to divert the child should be used. Proven successful techniques include telling stories or listening to them on CD or tape, or watching videos and DVDs. Moreover, the child needs to learn relaxation techniques (and parents too!) and there are many ways available to learn them.

School days

Beginning school

Numerous fears accompany the child (and the parents) in the first days of school, and they are not only in relation to the learning needs. Will their classmates accept them, will they find a friend, and is the school structurally safe? Will the child have a long way to walk between classrooms? Are there dangerous areas in the

class where the child might get injured? Is there a quiet place for the child to rest? Will the child have a teacher who is empathetic, caring, and patient? Is there enough time in the teacher's day, along with her/his normal teaching duties and commitments, to invest energy into the care of the special needs of an EB child? These questions and concerns can be answered only when there is a good relationship developed between the school and the parents. For their part in this partnership, the parents need to provide information about EB to the school's teachers and staff, and about their child's extra needs while at school to both their classmates and the teachers. Because of the rareness of EB it is not unusual that an EB child is the first one with this disease to have entered the school.

Prior to that, the parents will also need to get sufficient information from school administration and other parents of EB children who reside in their hometown, so as to identify the best possible school for a child with this particular disease. And undoubtedly, more questions will still arise. For example, which school will be most appropriate for the child? Does the type of school wanted even exist within the community? Whenever possible, it is preferable that the child be integrated into a normal school. Unfortunately, for some children this is not possible. In that situation, a special needs school is an option.

Some children with EB can cope very well. Others need a little help, while some need to have a fulltime assistant with them, to cope with their daily and special medical needs. This is often related to which type of EB the child has. It will also be influenced by the child's own level of development, and lastly what type of personality the child has. It should also be ascertained whether the child will be able to eat at school. An EB child with mucous membrane involvement will need to have special meals provided. This may not be available in every school, as it involves the need for special equipment and individualized preparation of the meal. However, quite often even the children who have more severe forms of EB can still be integrated into a regular school without major problems arising.

Finding friends is not always easy, due to the obvious effect of the illness and the child's restrictions at recreational activities. Being labelled as a "special needs" child can also be an obstacle. How easy school life becomes for an EB child often relates to the personality of the child, and certainly whether other children try to avoid rather than accept them. The better other children are informed about the illness, the more success the EB child will have. As a result of great efforts on behalf of the child by both the parents and the teachers, most children with EB do manage to build good friendships.

Despite the fact that society is better informed, EB children cannot avoid receiving prying looks by others who are ignorant of the underlying disorder. In

rare situations, EB wounds may be confused with child abuse, even by pediatricians who are unaware of the underlying diagnosis. Classmates and teachers can play a very important role in providing support and encouragement to the EB child.

Even after a successful school start, parents still need to pay attention to the problems that may later arise. Most of these problems are easy to take care of, others require a lot of effort to resolve. There are many ideas and answers to help to solve them. On a day where the student is having a lot of pain or is feeling too exhausted to attend school, a helpful teacher might be willing to organise a web camera in the classroom so that the child can also see what is being taught in class that day. A computer with voice recognition is helpful for the student who has mobility problems in his or her fingers and is unable to type fast enough to finish assignments or papers for school. Little things like special scissors or pencil adapters can make it much easier for the EB child to participate in the activities of the classroom.

Allowing the EB child to leave the classroom a few minutes earlier than the other children will provide an opportunity to avoid the rush of students during break, or when heading home, thus avoiding the chance of injury. The use of a lambskin padding on the child's seat will make it more comfortable for sitting for prolonged periods of time. Whenever reasonable the EB child should be allowed to participate in gym class activities, but with special attention paid to the extra needs and disease-mandated boundaries that the child may have. An EB child can become easily exhausted, for example, due to pain or anemia. Such a child should be able to decide what can and cannot be done. As a correlate, we have repeatedly seen that children who are allowed to make their own choices often make very good ones.

Small accidents are unavoidable in the school setting. It is important that the school has a person trained in first aid (and with sufficient knowledge about EB), and that first aid kits, stocked with "EB-friendly dressings," be available for use. Most teachers and classmates learn quickly the "do's and don'ts" related to having EB. As a consequence, the EB child experiences a normal school day with all of its highs and lows!

Adolescence

In this oftentimes difficult phase of life, friends become very important. All adolescents want to fit in, and at the same time they want to develop their independence and experience things on their own. Thoughts about physical contact with the opposite sex start to form, especially during puberty, and are accompanied by body changes and mood swings. The family, parents, and child have much to cope with. Teenagers with EB go through this phase just like any

other child, yet those with EB have the additional problems and burdens that accompany their disease. The problem of looking good becomes very important, physical limitations bother them more, and the constant appearance of new blisters, wounds, and scars come to the forefront. At this time, establishing contact with other teenagers who have the same illness can help, since it gives them a chance to see that they are not the only ones in the world with EB. But sometimes at this phase of development they may prefer to avoid contact with other affected teenagers, since being different has always been a part of their lives and they want to show that they have a right to be different and unique, and are yet still seen as a normal person. Parents need to help them gain their independence and autonomy and to become responsible for themselves and their actions. This is not only in reference to wound care, which has been taken care of by their parents up until then. This becomes increasingly important for their future development of friendships and for the pursuit of a satisfying way of life. Even though it may be difficult for parents of an EB child to distance themselves as the child becomes older and more self-sufficient, responsible teenagers need to be able to have progressive control over their lives.

The question of a future profession is also an issue at this time. The teenager should be advised, according to his or her impairment, as to which jobs might be most suitable, realistic, or attainable. It is important to get the assistance and expertise of job counsellors who help the teenager to identify all of the available possibilities, both locally and at more distant locations. Choices of the types of employment may be limited, due to the teenager's illness. Abilities, talents and special interests of the teenager also need to be taken into account and can play a large role in what is finally chosen.

Adulthood

The most important issue for someone with EB at this stage is to be self-reliant and independent. Most patients normally wish to have his or her own apartment, a driver's license, a job, a partner and a family. Wishes vary and there are different outcomes. There are many obstacles to overcome to fully achieve all of their dreams.

Choice of profession

When looking for a profession an adult with EB should not focus on the deficiencies that are present as a result of the disease, but instead look at all the possibilities that are available. The choice of profession will often depend on the type and severity of EB. For most forms of EB the potential range of choices may be unlimited, yet not all professions may be realistically possible. This, of course, applies for everyone, not just those affected by a rare disease such as EB. Factors influencing the choice of

profession also include the educational level achieved, the personality of the EB person, and whether the individual has special skills or talents.

Once vocational training has been completed, a decision must be made as to whether full time or part time work will be pursued. If it becomes impossible to work part time or to work at all, due to the extent and severity of disabilities present, a disability pension should be taken into consideration. It is worth remembering that for most adults, their feeling of self-esteem is influenced by the choice and success of their careers. In the setting of a severely disabling disease like EB, however, even a relatively insignificant job might be satisfying. When employment is not realistic, a hobby can at least provide some balance in their live.

Operating motor vehicles

Having a disease such as EB does not exclude one's obtaining a driver's license. On purchasing a car several important adaptations may need to be made, particularly for those with severe generalized forms of EB. A large mirror should be installed so that the driver does not have to make excessive spontaneous movements while driving. A knob on the steering wheel will make it easier to turn the wheel, or to turn it using one hand. It would also be beneficial to have power windows, an automatic transmission, soft seats, automatic door locks and a well padded safety belt.

Relationships and sexuality

Very difficult and sensitive topics are personal relationships and sexuality. If in today's world, finding a partner who is "rich, young and beautiful" is difficult, then it must be even harder for an EB adult to fulfill such a dream.

Society norms are luckily not the major issue of one's whole life. It would be ideal if it were possible for people to look and see that there is a normal person in front of them, rather than one affected by EB, and that an individual with EB has the same strengths and weaknesses as others do. The way would then be freed to be candid with each other if and when the conditions fit, and then a relationship could be built based on equal footing. There are many encouraging examples (Fig. 4.2-9) of this that we have seen among our patients, although it is all too often that the wish for a serious relationship is not met. The need is always there and the longing for sexual contact is not so easy to put aside. Everyday answers are not always available and a sensitive discussion of these issues is necessary. It is important for both the adult with EB and the caretaker to take the person serious about his or her most personal needs. With creativity and an open mind, other ways might be found that can satisfy the need for sex and tenderness.

Fig. 4.2-9. A mother and wife who also has EB

General physical examinations

As EB patients age, the more severely affected ones may develop increasing physical limitations, as the result of a worsening of extracutaneous complications (see Chapter 4.1). The most notable complication is squamous cell carcinoma, which can lead to death. For the overall well-being of the EB patient, a visit to a dermatologist at least twice a year is recommended, once adolescence is reached.

It also should not be forgotten that routine, EB independent medical check ups are still necessary, as it is possible for a person with EB to develop other unrelated health problems, which can be more difficult to treat in the setting of such a disabling disease.

Conclusion

An important goal for everyone with EB is to develop strong character and self confidence. This can be achievable with lots of effort and the love and support provided by family and friends (Fig. 4.2-10). All patients with EB should have the opportunity to experience the many things that life has to offer and, even with

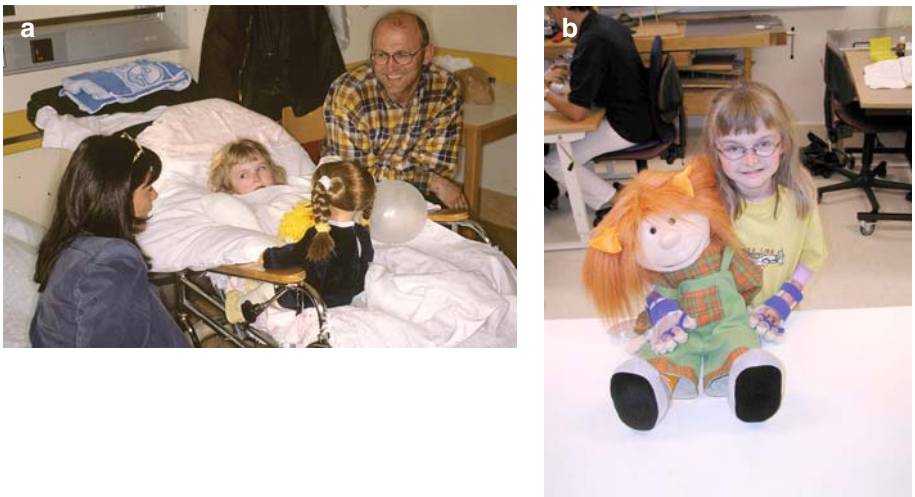

Fig. 4.2-10. **a** and **b** After hand surgery, the puppet was well received

their many disease-associated limitations, to be able to lead to a happy and fruitful life.

Regretably, reality often deviates from idealizations. Expectations may have to be modified on an individual basis, and with the efforts and cooperation of all participants. Every patient has his or her own EB. This means that every individual with EB has a unique history and personality. Therefore, experiences with the illness will differ from one patient to another. Age-dependent individualized care of this heterogeneous group of patients is necessary. Our experience with many EB patients over several decades shows that this approach will pay off.

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