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Cicatrising Conjunctivitis

Including a Foreword by John Forrester, Aberdeen

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Developments in Ophthalmology

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Foreword

In this book Wolfgang Bernauer, John Dart and Mark Elder synthesise our current state of knowledge in disorders causing cicatrisation of the conjunctiva. For many years now there has been a gap in the ophthalmic literature with regard to this field, and this volume, in a very timely fashion, fills this need exactly.

Cicatrising conjunctival disease can occur in many conditions and for the jobbing ophthalmologist the diagnosis, but especially the treatment, can be daunting. Most of us throw up our hands in despair when faced with such a case and are only too glad to refer these very difficult problems to specialists in the field.

These three authors undoubtedly have a wide experience in the field and inherited much of it from the work of Peter Wright who set up a pioneering tertiary referral clinic at Moorfields Eye Hospital to deal with these special problems.

The book addresses all aspects of the disease from its clinical manifestation through the path of physiology and immunopathology to current management approaches. Newer knowledge in the field of immunology allows us ever increasing insight into the mechanisms of the disease and more and more targets of auto-immune attack are being identified. However, it is likely that from a treatment point of view knowledge of the cells that cause the damage are likely to provide pointers to future therapies. The notion indeed that an exaggerated T cell response with its associated fibrosis may underly the pathogenesis of some cases of cicatrisation is indeed intriguing and quite plausible. The information on fibrosis and fibrotic mechanisms is therefore very appropriately placed in this text.

The medical and surgical management of cicatrising conjunctival disease is of course extremely difficult. The latter sections of the book address the investigation and management of these conditions and point the way forward to new approaches. There is little doubt that this succinct and excellent volume should provide the bench mark for future studies in this field and should encourage interested ophthalmologists to take up the challenge of how to deal with these problems. For the sake of our patients we have no option.

<div align="right">Prof. John Forrester, Aberdeen</div>

Preface

This handbook is designed to help clinicians faced with the difficulties of treating patients with cicatrising conjunctivitis at both the practical and theoretical levels. Cicatrising conjunctivitis is one of the most challenging causes of ocular surface disease faced by patients and their ophthalmologists because it affects all the components of the peri-ocular system of lids, tears and epithelia, responsible for the maintenance of a clear cornea. Without appropriate intervention, corneal blindness or loss of sight from supervening infection is common. There have been steady advances in the understanding of the pathogenesis of the underlying diseases and in clinical studies validating treatment strategies directed at controlling their inflammatory components as well as some of their sequelae. With this knowledge, they should no longer be associated with an a dismal prognosis, and active intervention can be planned to prevent and treat them and their complications. Many of these diseases are uncommon so that few ophthalmologists have access to enough patients either to accumulate clinical experience in management or to carry out research into treatment and pathogenesis. This book is arranged both to allow easy access to management advice for the dermatologist or ophthalmologist, who has a few affected patients in his care, and to the clinical scientist who wants a summary of the knowledge on pathogenesis, the rationale for different treatment strategies and the potential for new research technologies to understand and treat these disorders.

The impetus for writing this book was given by Peter Wright, who both stimulated our interest in this group of conditions in his clinics at Moorfields Eye Hospital and encouraged us to start research into ocular cicatricial pemphigoid. Although this disease encompasses all the most difficult management

problems that can be encountered in external eye disease – acute and chronic conjunctival inflammation, blepharitis, entropion, trichiasis, tear deficiency, corneal ulceration, infection and iatrogenic disease – he showed us that with recognition of these problems and an understanding of their pathogenesis, many of their worst sequelae could be prevented.

The book has been written now because we have come near the end of a period of research into ocular cicatricial pemphigoid and have a circle of very active colleagues who have been able to contribute to our understanding of both the clinical and fundamental aspects of cicatrising conjunctivitis. Non-progressive causes including trachoma, Stevens-Johnson syndrome and ligneous conjunctivitis are covered in one part. The further chapters are devoted to chronic progressive conjunctival cicatrisation, the diseases which cause it, their clinical and laboratory evaluation, immunopathogenesis, sequelae and management. Manipulation of the wound-healing process, discussed in the last part, relates the advances in the understanding of this topic to the problem of conjunctival cicatrisation.

We hope that this book will both help improve the outcome for patients developing one of these diseases as well as provide a foundation for the clinical scientist wishing to contribute to our knowledge of this fascinating and challenging group of disorders.

Wolfgang Bernauer
John K.G. Dart
Mark J. Elder

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Acknowledgements

This book is the result of the collaboration of ophthalmologists, dermatologists and scientists from several countries. We wish to express our appreciation to each contributor. As a result of their excellent work, this book has become the interdisciplinary update on the problem of conjunctival cicatrisation that we intended.

This book is largely based on our experience with the patients we have examined and treated at Moorfields Eye Hospital in London. We would like to thank them, and the staff of this unique institution, for their support of our projects. We would like to acknowledge the unfailingly cheerful support given by Isabel Moldon for so much of our clinical research included here.

We are indebted to the following experts who reviewed chapters that were outside our scope: Prof. Dr. L. Bruckner-Tuderman, PD Dr. P. Itin, Mr. P. Khaw and Prof. Dr. R. Zinkernagel.

Our interest in cicatrising conjunctivitis could not have developed without the generous support of research grants from the Swiss National Science Foundation, the Guide Dogs for the Blind, the Special Trustees of Moorfields Eye Hospital and Moorfields Eye Hospital Locally Organised Research. Dr. Jonathan Leonard, Prof. Sue Lightman, Mr. Richard Collin, Dr. Paul Hiscott and Mr. Bob Alexander, together with the Corneal and External Disease Fellows at Moorfields Eye Hospital, have all made a substantial contribution to the development of our ideas on this topic: we are very grateful to them.

Our particular thanks to Christa Hug who, over the months that it took to write this text, kept everything in excellent order, typed dozens of letters, and aided in the archiving process. Thanks are due to Prof. Dr. K. Bernauer for his meticulous proofreading. Also to Susanna Ludwig, René Müller and the many helpful hands at Karger Publishers for their support and expertise.

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Last, but not least, we thank our wives and our children, who gave us the time to work on this project.

April 1997   *Wolfgang Bernauer, John Dart* and *Mark Elder*

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To Peter Wright and to our wives Helen, Rae and Alison

1 Introduction

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Introduction to Cicatrising Conjunctivitis

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Conjunctival inflammation may follow a large number of stimuli, including physical and chemical irritants, infective agents, such as bacteria, viruses and other organisms, and may be associated with cutaneous and other systemic conditions that are often immune-mediated. Minor degrees of conjunctival irritation cause hyperaemia and may be associated with transudation of fluid and cells into the conjunctival sac to produce the discharge which is a characteristic feature of conjunctivitis of all types. Involvement of the deeper layers of the conjunctiva, subconjunctival tissue and tarsus produces a different picture of inflammation which tends to be more chronic and is often associated with *cicatrisation*.

The understanding of the factors which initiate and control the inflammatory response and the repair processes is incomplete. These mechanisms are the subject of ongoing research and will be discussed later in this book. It is the persistence of fibroblastic repair processes which results in scar formation and subsequent contracture that produces the *clinical entity of cicatrising conjunctivitis* (table 1).

Preservation of a normal clear cornea is essential for vision and the physical environment of the cornea is of prime importance for the maintenance of physiological function. Lids, tears, mucus and epithelial surfaces all interact to produce a complex physico-chemical system that ensures an optimal corneal environment (table 2). Chronic inflammation and cicatrisation may cause *imbalance of this system*, resulting in loss of the normal conjunctival surface morphology and associated structures, alteration of lid/globe interaction and reduction of tear flow by scarring around lacrimal ductules. These changes, often combined with secondary infection [1], add up to a progressive and powerful insult to the outer eye leading to corneal scarring, vascularisation, perforation, endophthalmitis and eventually loss of the eye. The most striking

1

	Shrinkage		Keratinisation
	Superficial scarring/synechia		Punctate keratitis
	Subepithelial fibrosis		

1

2a *2b*

3a *3b*
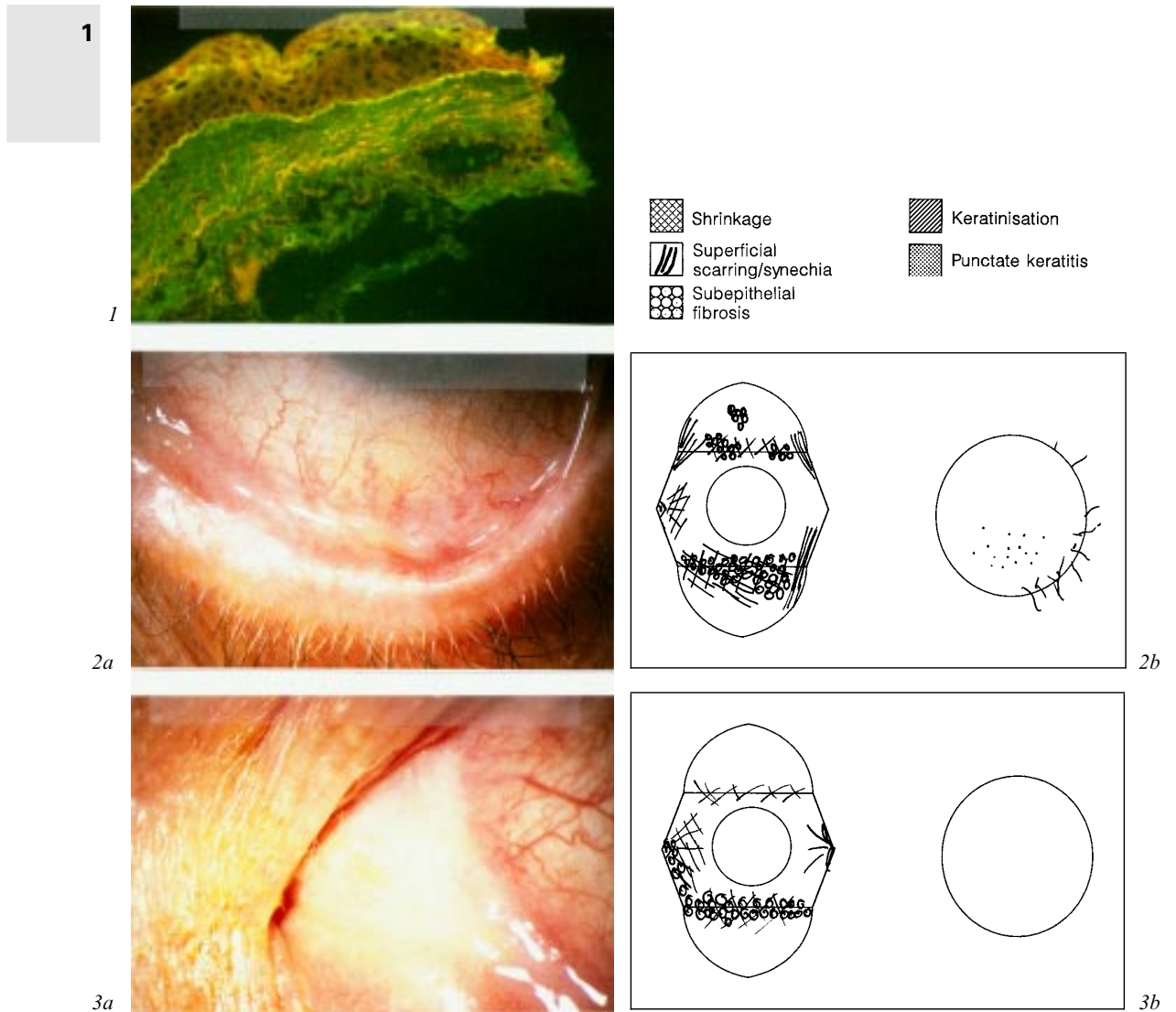
Fig. 1. Ocular cicatricial pemphigoid: immunofluorescence photograph of a conjunctival biopsy specimen. The snap-frozen tissue was cryostat-sectioned and stained with fluorescein-labelled anti-human IgA. Note the line of homogeneous fluorescence of the epithelial basement membrane zone, signifying the presence of deposits of IgA in this region. Original magnification × 100.

Fig. 2. a Ocular disease with moderate inflammation in cicatricial pemphigoid. Note fornix foreshortening, conjunctival thickening and white lines with dense subepithelial fibrosis. *b* Schematic drawing of the eye as shown in the photograph. These standardised drawings are presently used by our group for the prospective monitoring of conjunctival fibrosis and keratopathy in patients with chronic progressive cicatrising conjunctivitis (see also 'Monitoring of Disease Activity and Progression').

feature at the end of such a saga may be the scarred and damaged cornea which is the main impediment to vision. Attempts to relieve the blindness surgically, however, without first dealing with the abnormalities of the corneal surroundings have doomed many corneal grafts to rapid failure. The awareness of the nature of the changes in the conjunctiva, the special features in some disease processes and the situations in which surgical or other treatment can help, are fundamental to an understanding of one of the main pathways to blindness. Surgical treatment of the lids, for instance, can greatly improve the prospects in carefully chosen cases, such as in Stevens-Johnson syndrome, once the acute phase of illness has passed [2], but can have a disastrous effect when carried out in patients with active cicatricial pemphigoid [3–5]. Each form of cicatrising conjunctival response differs, and it is crucial to distinguish them. Of utmost importance therefore is the distinction between *temporally limited* and *chronic progressive conjunctival cicatrisation.*

Most types of cicatrising conjunctivitis are characterised by an acute phase of tissue injury with subsequent scarring. Cicatrisation is thus temporally limited after withdrawl of the noxious factors, leading to a *static* fibrous scar. Alteratively *chronic progressive* cicatrisation is found in cicatricial pemphigoid, linear IgA disease, as part of certain paraneoplastic syndromes and after long-term treatment with systemic and topical medications. These chronic progressive forms of cicatrising conjunctivitis share a common pathogenesis in that they are *potentially anti-basement membrane antibody mediated* [4, 6–15].

The therapeutic approaches to temporally limited conjunctival cicatrisation and the chronic forms of cicatrising conjunctivitis differ greatly:

Withdrawal of the noxious factors with elimination of infection, if present, and symptomatic treatment are the main principles in temporally limited conjunctival cicatrisation.

Prevention of disease progression is the principal aim in the management of chronic progressive conjunctival cicatrisation. This is because once the key features of advanced disease, namely extensive scarring and keratopathy, have occurred, the prognosis for visual rehabilitation is poor. To prevent disease progression, aggressive management, often systemic immunosuppression in addition to symptomatic therapy, may be required.

Fig. 3. a Ocular disease without clinically manifest inflammation in cicatricial pemphigoid. Note the loss of inner canthal architecture with flattening and obliteration of the normal conjunctival folds, plica and caruncle. *b* Schematic drawing of the eye as shown in the photograph.

1

4a

4b

5a

Keratin!

5b

6a

6b
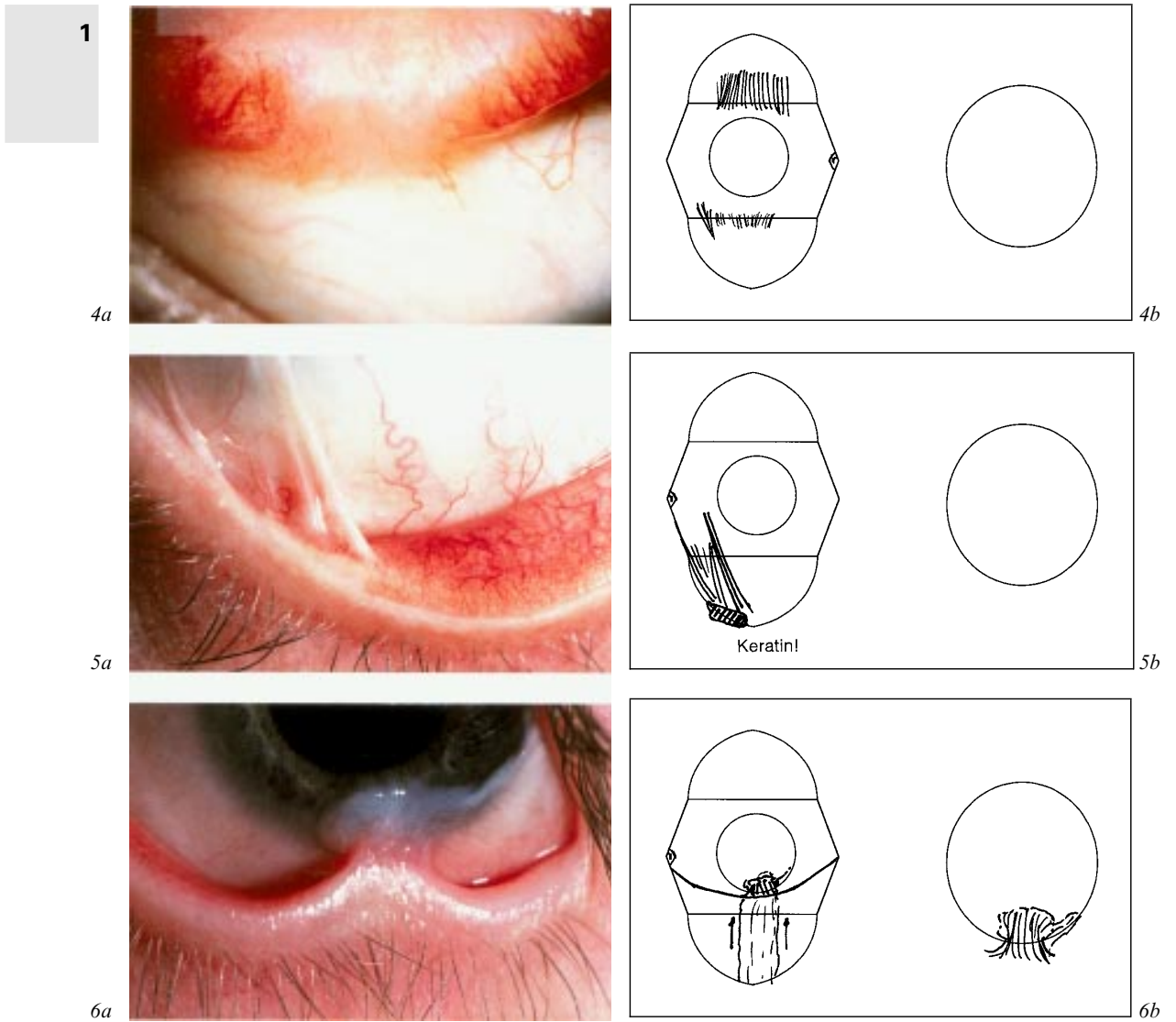
Fig. 4. a Conjunctival scarring following membranous conjunctivitis due to adenovirus infection. Note localised symblepharon formation that does not involve the palpebral conjunctiva or tarsus close to the lid margin, so entropion rarely ensues. *b* Schematic drawing of the eye as shown in the photograph.

Fig. 5. a The conjunctiva after Stevens-Johnson syndrome. Thin conjunctival adhesions that lack the dense fibrous subconjunctival component seen in cicatricial pemphigoid are characteristic for this condition. Stevens-Johnson syndrome is associated with temporally limited conjunctival scar tissue formation. *b* Schematic drawing of the eye as shown in the photograph.

Fig. 6. a Localised and non-progressive cicatricial changes in dystrophic epidermolysis bullosa. *b* Schematic drawing of the eye as shown in the photograph.
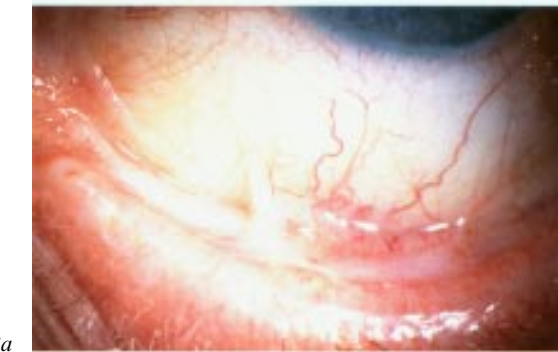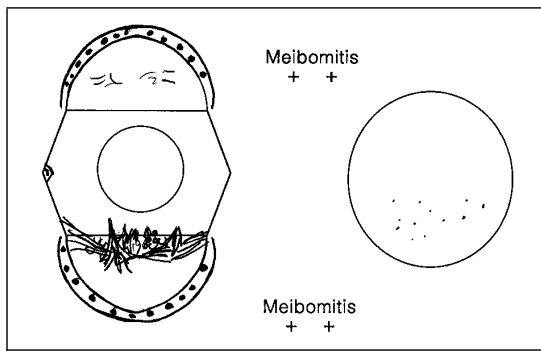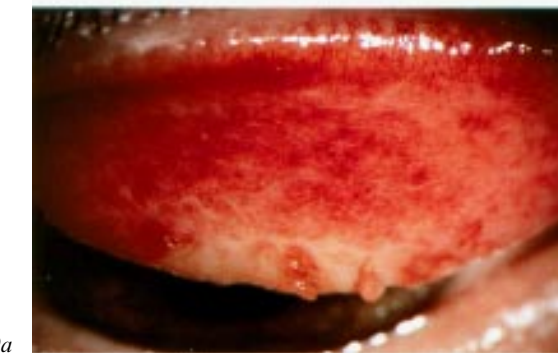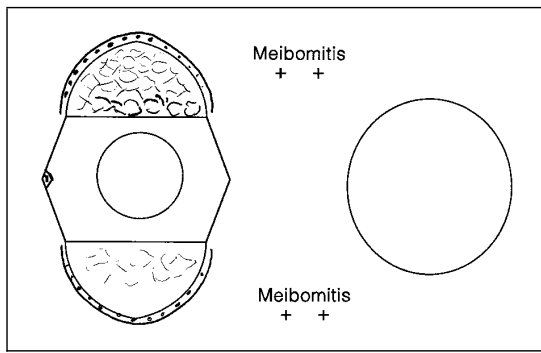
Fig. 7. Scarring of limbal follicles in trachoma (Herbert's pits). Conjunctival fibrosis in trachoma involves mainly the upper lids and the associated corneal signs usually occur in the upper part of the cornea.

Fig. 8. *a* Chronic meibomitis. Note the abnormal gland openings, the rounded posterior lid margin and the scarring in the lower fornix. *b* Schematic drawing of the eye as shown in the photograph.

Fig. 9. *a* Atopic keratoconjunctivitis with papillary hypertrophy of the upper tarsal conjunctiva. A reticular pattern of scarring can be seen. *b* Schematic drawing of the eye as shown in the photograph.

Temporally Limited Conjunctival Scarring or Chronic Progressive Form of Cicatrising Conjunctivitis?

The demonstration of immunoglobulin and/or complement deposition at the conjunctival basement membrane zone by direct immunofluorescence is the classical investigation to establish the diagnosis of ocular cicatricial pemphigoid and some other forms of chronic progressive conjunctival cicatrisation. This *immunopathological method* is *helpful if it is positive* (fig. 1). Positive results, however, can only be expected in about 50% of the patients [4, 6, 7, 10, 16–21]. Thus, a conjunctival biopsy *alone* is frequently not sufficient to establish this diagnosis [21]. Currently, it is the combination of medical history, clinical signs and immunopathological findings in the conjunctiva that is used to classify cicatrising conjunctivitis.

The Medical History

The *medical history* is critical to the differentiation of cicatrising conjunctivitis. Acute tissue injury after chemical and thermal burns, membranous conjunctivitis (in Europe most commonly adenovirus), acute oculomucocutaneous disorders, such as erythema multiforme and Stevens-Johnson syndrome, are all associated with temporally limited conjunctival scar tissue formation. In most cases, the history of these conditions is obvious and on this basis the diagnosis easy. Patients with cicatrising conjunctivitis due to chronic graft-versus-host disease following transplantation can be easily identified. The diagnosis of chronic atopic conjunctivitis can be made based on a personal history of other atopic diseases. Trachoma must be suspected in a population known to be at risk of contracting the disease. The history and type of extraocular lesions, with a dermatology assessment, help in the differentiation of oculomucocutaneous disorders. The history of ocular medications is important for the diagnosis of drug-induced conjunctival cicatrisation.

Pattern of Conjunctival Fibrosis

The *pattern of conjunctival fibrosis* can provide helpful information in the assessment of cicatrising conjunctivitis. Conjunctival fibrosis can be classified as superficial (involving the epithelial structures) or as deep (involving the subepithelial layers). It can be found in a focal or linear pattern or in diffuse sheets. A distinction between deep and superficial scarring is not always possible on clinical grounds alone, and most patients show a combination of

Table 1. Conditions associated with cicatrising conjunctivitis

Physical
  Heat
  Ionising radiation

Chemical

Infection
  Trachoma
  Membranous conjunctivitis (bacterial and viral)

Oculocutaneous disorders
  Erythema Multiforme
  Stevens-Johnson syndrome
  Toxic epidermal necrolysis
  Cicatricial (=mucous membrane) pemphigoid
  Linear IgA disease
  Bullous pemphigoid
  Epidermolysis bullosa
  Dermatitis herpetiformis
  Pemphigus group
  Lichen planus
  Chronic atopic keratoconjunctivitis

Other associated systemic disorders
  Rosacea
  Sjögren's syndrome
  Inflammatory bowel disease
  Graft-versus-host disease
  Immune complex diseases
  Paraneoplastic syndromes

Drug-induced
  Systemic
  Topical (pseudopemphigoid)

superficial and deep scar tissue formation. Subepithelial fibrosis, without obvious involvement of the conjunctival epithelium, however, is characteristic of ocular cicatricial pemphigoid (fig. 2, 3), whereas membranous conjunctivitis is typically followed by superficial scarring (fig. 4). Diffuse subepithelial conjunctival fibrosis may become manifest as 'shrinkage', and conjunctival shrinkage with involvement of canthal structures is an important *early* clinical sign of ocular cicatricial pemphigoid [22, 23]. It may lead to shallow canthal recesses and, in the medial canthus, to a loss of architecture with flattening or oblitera-

Table 2. Physico-chemical protection mechanisms of the outer eye

Physical protection
    Effective lid function and closure (neurological mechanisms control
        palpebral aperture, basal blink rate and induce reflex blinking)
    Tears (washing and dilution)
    Mucus from goblet cells ('sticky trap')
    Lipids from lid margin glands (spreading effect, conserve moisture)
    Corneal and conjunctival epithelium (barrier)

Cellular protection
    Neutrophils
    Macrophages
    Langerhans/dendritic cells
    Lymphocytes

Humoral protection
    Lactic and fatty acids (from sebaceous glands), pH
    Immunoglobulins
    Complement
    Lysozyme
    Lactoferrin
    β-Lysin
    Other antibacterial factors
    Interferons
    Tumour necrosis factor

tion of the normal conjunctival folds, plica and caruncle (fig. 3). Drug-induced conjunctival cicatrisation initially involves the lower fornix [24] which contrasts with these early changes of cicatricial pemphigoid. The fact that drug-induced conjunctival cicatrisation may be unilateral can be also of diagnostic value. Thin conjunctival adhesions that lack the dense fibrous subconjunctival component seen in cicatricial pemphigoid can result from erythema multiforme and its severe forms, Stevens-Johnson syndrome and toxic epidermal necrolysis (fig. 5). Localised and non-progressive cicatricial changes can be found in epidermolysis bullosa [3, 25–27] (fig. 6). Other mucocutaneous disorders that can be associated with cicatricial changes in the conjunctiva include pemphigus vulgaris, bullous pemphigoid and dermatitis herpetiformis [20, 27–29]. In these conditions, superficial scarring occurs which does not show the chronic progressive course characteristic of cicatricial pemphigoid or linear IgA disease. Conjunctival scarring following severe adenovirus infection can lead to localised symblepharon formation resulting in pockets of conjunctiva in the fornices. This fibrosis rarely involves the palpebral conjunctiva or tarsus close

to the lid margin and is usually not intense enough to cause entropion (fig. 4). Trachoma can lead to conjunctival fibrosis that mainly involves the upper lids, and the associated corneal signs usually occur in the upper part of the cornea with scarring of limbal follicles (Herbert's pits) (fig. 7). Chronic posterior lid margin disease (meibomitis) and ocular rosacea that may be followed in severe cases by conjunctival scarring can be differentiated from other chronic forms of cicatrising conjunctivitis on the basis of the typical lid and skin signs. These lid signs include hyperaemia and telangiectasia of the skin of the intermarginal strip, abnormal meibomian gland openings, loss of the sharp posterior lid margin which becomes rounded (fig. 8). Papillary conjunctival changes are seen in atopic keratoconjunctivitis, sometimes together with a distinctive pattern of chicken-wire-like scarring of the superficial tarsal conjunctiva (fig. 9). In Sjögren's syndrome, fibrosis is, if present at all, uncharacteristic and of a modest degree.

Immunopathological Methods

Conjunctival biopsies have an important place in the assessment of cicatrising conjunctivitis. If cicatricial pemphigoid is suspected, the conjunctival tissue should be taken from the bulbar conjunctiva only, since biopsies of the fornix conjunctiva may increase the disease activity with subsequent scarring. Conventional histology of such biopsies does not yield any diagnostic information in most cases. Only *immunopathological methods* with demonstration of immunoglobulins and/or complement deposition in a linear pattern at the epithelial basement membrane of affected conjunctiva allow the condition to be classified as chronic progressive conjunctival cicatrisation (fig. 1). These methods will be discussed in detail in a separate chapter.

References

1   Omerod LD, Fong LP, Foster CS: Corneal infections in mucosal scarring disorders and Sjögren's syndrome. Am J Ophthalmol 1988;105:512–518.
2   Wright P, Collin JRO: The ocular complications of erythema multiforme (Stevens-Johnson syndrome) and their management. Trans Ophthalmol Soc UK 1983;103:338–341.
3   Wright P: Cicatrizing conjunctivitis. Trans Ophthalmol Soc UK 1986;105:1–17.
4   Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
5   De la Maza MS, Tauber J, Foster CS: Cataract surgery in ocular cicatricial pemphigoid. Ophthalmology 1988;95:481–486.
6   Mondino BJ, Ross AN, Rabin BS, Brown SI: Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 1977;83:443–450.
7   Bean SF, Waisman M, Michel B, Thomas SI, Knox JM, Levine M: Cicatricial pemphigiod. Arch Dermatol 1972;106:195–199.

8   Roat MI, Alstadt S, Carpenter AB, SundarRaj N, Thoft A: Antibasement membrane antibody-mediated experimental conjunctivitis. Invest Ophthalmol Vis Sci 1990;31:168–175.

9   Leonard JN, Haffenden GP, Ring NP, McMinn RMH, Sidgwick A, Mowbray JF, Unsworth DJ, Holborow EJ, Blenkinsopp WK, Swain AF, Fry L: Linear IgA disease in adults. Br J Dermatol 1982;107:301–316.

10 Leonard JN, Hobday CM, Haffenden GP, Griffiths CEM, Powles AV, Wright P, Fry L: Immunofluorescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.

11 Anhalt GJ, Kim S, Stanley JR, Korman NJ, Jabs DA, Kory M, Izumi H, Ratrie III H, Mutasim D, Ariss-Abdo L, Iabib RS: Paraneoplastic pemphigus: An autoimmune mucocutaneous disease associated with neoplasia. N Engl J Med 1990;323:1729–1735.

12 Helm TN, Camisa C, Valenzuela R, Allen CM: Paraneoplastic pemphigus: A distinct autoimmune vesiculobullous disorder associated with neoplasia. Oral Surg Oral Med Oral Pathol 1993;75:209–213.

13 Bystrin JC, Hodak E, Gao S-Q, Chuba VJ, Amorosi EL: A para-neoplastic mixed bullous skin disease associated with anti-skin antibodies and a B-cell lymphoma. Arch Dermatol 1993;129:870–875.

14 Pouliquen Y, Patey A, Foster CS, Goichot L, Savodelli M: Drug-induced cicatricial pemphigoid affecting the conjunctiva. Light and electron microscopic features. Ophthalmology 1986;93:775–783.

15 Fiore PM, Jacobs IH, Goldberg DB: Drug-induced pemphigoid. A spectrum of diseases. Arch Ophthalmol 1987;105:1660–1663.

16 Bean SF: Cicatricial pemphigoid – Immunofluorescent studies. Arch Dermatol 1974;110:552–555.

17 Griffiths M, Fukuyama K, Tuffanelli D, Silverman S: Immunofluorescent studies in mucous membrane pemphigoid. Arch Dermatol 1974;109:195–199.

18 Furey N, West C, Andrewa T, Paul PD, Bean SF: Immunofluorescent studies of ocular cicatricial pemphigoid. Am J Ophthalmol 1975;80:825–831.

19 Mondino BJ, Brown SI, Rabin BS: Autoimmune phenomena of the external eye. Ophthalmology 1978;85:801–817.

20 Frith PA, Venning VA, Wojnarowska F, Millard PR, Bron AJ: Conjunctival involvement in cicatricial and bullous pemphigoid: A clinical and immunopathalogical study. Br. J Ophthalmol 1989;73:52–56.

21 Bernauer W, Elder MJ, Leonard J, Wright P, Dark JK: The value of biopsies in the evaluation of chronic progressive conjunctival cicatrisation. Graefe's Arch Clin Exp Ophthalmol 1994;232:533–537.

22 Wright P: Enigma of ocular cicatricial pemphigoid. A comparative study of clinical and immunological findings. Trans Ophthalmol Soc UK 1979:99:141–145.

23 Wright P: External diseases; in Miller ST (ed): Clinical Ophthalmology. Bristol, Wright, 1987, pp 107–128.

24 Schwab IR, Linber JV, Gioia VM, Benson WH, Chao GM: Foreshortening of the inferior conjunctival fornix associated with chronic glaucoma medications. Ophthalmology 1992;99:197–202.

25 Mondino BJ: Bullous diseases of the skin and mucous membranes; in Duane TD, Jaeger EA (eds): Clinical Ophthalmology. Philadelphia, Lippincott, 1991, vol 4, chapt 12, pp 1–19.

26 Richter BJ, McNutt S: The spectrum of epidermiolysis bullosa acquisita. Arch Dermatol 1971;115:1325–1328.

27 Lin AN, Murphy F, Brodie SE, Carter DM: Review of ophthalmic findings in 204 patients with epidermolysis bullosa. Am J Ophthalmol 1994;118:384–390.

28 Duke-Elder S, MacFaul PA: System of Ophthalmology. London, Henry & Kimpton, 1965, vol 8, pp 496–527.

29 Bean SF, Houbar K, Gillett RB: Pemphigus involving the eyes. Arch Dermatol 1975;111:1484–1486.

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Aspects of Trachoma

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Trachoma may be the commonest eye infection in the world with about 150 million people affected by it. Some 6 million of them are blind [1]. Trachoma has ravaged man throughout history with records of treatment of trichiasis in China in the 27th century BC, and the use of papyrus and forceps for trichiasis in Ancient Egypt dates its effects back to the 19th century BC. In more recent times, soldiers returning from Egypt in the Napoleonic wars brought trachoma to Europe, and the resulting pandemic that swept England led to the establishment of specialist eye hospitals [2]. With improvements in the general level of hygiene, trachoma has been eradicated in Europe, but remains a scourge in the underdeveloped nations, particularly in areas where personal and community hygiene are difficult to maintain. Trachoma is still the leading infectious cause of preventable blindness in the world.

Clinical Features

Trachoma is initially seen as an acute, self-limiting follicular conjunctivitis, characterised by follicles of the upper tarsus and/or limbus in response to an infection with *Chlamydia trachomatis.* There is usually an associated papillary response. Repeated inoculation with ongoing reinfection leads to a chronic conjunctivitis with a more severe inflammatory component and the risk of cicatricial changes to the conjunctiva. As the follicles resolve there is appearance of the characteristic stellate scarring in the upper tarsal conjunctiva. With time, this forms dense bands of scars that eventually distort the tarsal plate, causing entropion and trichiasis. Although in rare cases primary corneal
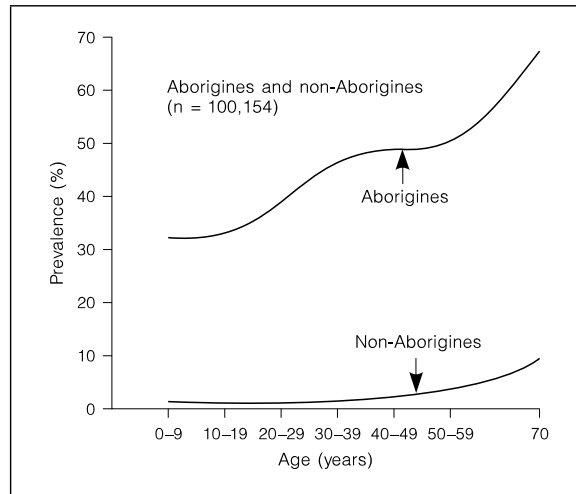
Fig. 1. Prevalence of trachoma in rural Australia, comparing Aborigines and non-Aborigines. From the National Trachoma and Eye Health Programme, 1980.

changes such as pannus may obscure vision, blindness in trachoma is usually the result of corneal opacification from either trichiasis or associated bacterial keratitis [3].

Epidemiology of Trachoma

Although largely eradicated from industrialized nations, trachoma remains the world's leading infectious cause of blindness, with an estimated 150 million people affected by it and some 6 million blind world wide as a result of its effects. Trachoma is responsible for 15% of the global burden of severe visual loss. Estimates of global incidence in 1992 was of over 20 million new cases of active trachoma, implying it is an ongoing problem of significant magnitude [1].

The disease is particularly common and severe in countries with hot, dry and dusty climates and poor hygiene conditions. One of the largest surveys of the prevalence of trachoma was the National Trachoma and Eye Health Programme in Australia. The prevalence of trachoma in rural Australia among Aborigines was 38% overall compared to 1.7% in non-Aborigines [4] (fig. 1).

Acute follicular trachoma is initially seen in childhood, with most of the disease in the pre-school children being in this form. As age increases, the
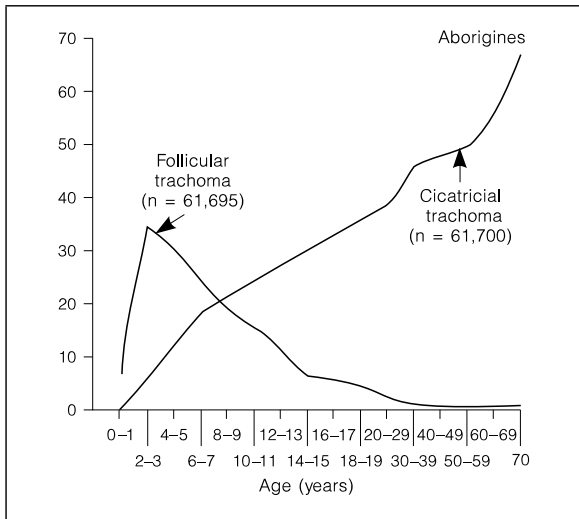
Fig. 2. Prevalence of follicular and cicatricial trachoma in Australian Aborigines. From the National Trachoma and Eye Health Programme, 1980.

proportion of children with follicular and cicatricial disease increases. In the 10–19 age group 19% of aborigines with trachoma had both follicular and cicatricial disease. In the older age group, cicatricial disease predominated (fig. 2).

There were some areas in Australia with a particularly high prevalence of trachoma such as the Red Centre, the Western desert and the cattle country of the Kimberleys. In these areas more than two in every three people seen had trachoma, with a very high incidence among the young and with up to 95% of the elderly bearing the scars of trachoma [4]. Other surveys confirm hyperendemic trachoma in many areas of Africa and Central America [5–8]. In Chiapas, Mexico, approximately 25% of those less than 10 had significant inflammatory trachoma and almost 100% of those aged over 40 had cicatricial trachoma [9].

Some general risk factors associated with trachoma include poor personal and family hygiene with intimate family contact, poor community hygiene, poverty, and a general lack of industrial development. More specific factors increasing risk of trachoma include crowding in sleeping quarters, lack of piped water and the sharing of towels and the presence of flies [10, 11].

However, the major risk factor associated with occurrence and severity of inflammatory trachoma in children is the lack of facial cleanliness or a low frequency of face washing [11]. This finding has been confirmed in subsequent

studies of the impact of face washing on the incidence of severe trachoma in Tanzania. The odds of having severe trachoma in a village with a face-washing programme was 0.62 compared with control villages [12].

Several risk factors for cicatricial trachoma have been identified, particularly crowded living conditions and poor water supply. The high correlation of the severe manifestations of the disease with poor housing and an inadequate water supply suggest that the most severely affected individuals are probably the poorest members of society [5].

Women are at a greater risk of trichiasis than men in hyperendemic areas. For example in Tanzania, 8% of people older than 55 had trichiasis, and the risk for women was four-fold. In areas where 25–30% of women have trachomatous scarring, 4–5% will have trichiasis and an additional 4–5% will have corneal opacities. Women's close contact with children, who are the main source of active chlamydial infection, is the most likely risk factor for repeated infection and so severe disease and trichiasis. In Tanzania women with trichiasis were almost four times more likely to have had a mother with trichiasis. This may imply some genetic predisposition or factor related to the immune responsiveness to chlamydial infection, or simply the sharing of a common environment and hygiene practices [13].

Other factors related to a higher incidence of trichiasis in women include sleeping close to the cooking fire possibly associated with further irritation from smoke and dust. Also the pathogenic response to infection with *C. trachomatis* may be more profound during pregnancy since the cornea undergoes changes in physiology such as a relative tear deficiency. There appears to be a complex of associations related to poor living conditions during the child-bearing years. Intense exposure to *C. trachomatis*, past infection inferred from a mother with trichiasis and repeated re-infection from her children lead to increased risk of severe disease and blindness [12, 13].

Pathophysiology

Acute infection with *C. trachomatis*, usually of serovars A, B, Ba or C, leads to acute conjunctival inflammation. In the early stages of the disease there is hyperplasia of the conjunctival epithelium with infiltration of both the epithelial and subepithelial tissues, primarily by polymorphs and some lymphocytes. Later, as the lymphocytes aggregate to form germinal centres, a follicular conjunctivitis develops which primarily involves the upper tarsal plate [14]. Follicles, as well as their sequelae, Herbert's pits, may be seen at the limbus. Superficial keratitis with pannus may also occur. A single episode usually resolves completely, leaving minimal scarring. A quantitative associ-

ation of *C. trachomatis* and the intensity of inflammation has been shown in Tanzanian children, with the highest isolation rate and greatest number of chlamydial inclusions being present in those eyes with the most severe disease [15, 16].

Chronic infection, which is presumed to be predominantly due to reinfection, leads to intense conjunctival inflammation with papillary hypertrophy and marked lymphocyte infiltration. This may result in conjunctival cicatrisation and lead to entropion and trichiasis. Active inflammation, as characterised by follicle formation, may reappear in eyes with established cicatrisation. In trachoma there is a prodromal period when active organism is present, but before clinical signs of disease are present. Conversely, the duration of active conjunctival disease far exceeds the time that infective organisms can be detected. Cultures performed while the disease is on the wane are found to be negative, implying that the persistent inflammation has an immune basis [17].

The exact mechanisms involved in the initiation of scarring are unclear, but recent work using animal models and immunohistochemical studies in human conjunctiva has given valuable clues as to the immunopathology. The current concept is that repeated exposure to *C. trachomatis* leads to an immune-mediated scarring response that may be best understood as a hypersensitivity to the continued presence of chlamydial antigen.

Animal models of trachoma confirm the role of recurrent infection in the production of chronic disease. Cynomolgus monkeys inoculated with a single dose of *C. trachomatis* serovar A, B or E develop an acute, self-limiting follicular conjunctivitis that is intense for 4–6 weeks and then slowly subsides. The non-specific signs of inflammation resolve within a month whereas the follicular response may take up to 3 months, after which all animals have fully recovered.

In contrast, animals receiving weekly inoculations develop a chronic, progressive follicular conjunctivitis for as long as the inoculations are continued. With time, these animals develop typical stellate scars in the superior tarsal conjunctiva that are recognisable both clinically and histologically. Once the weekly reinoculation is ceased the follicular response slowly resolves. Similar findings were obtained with *C. trachomatis* serovars A, B or E [18, 19].

Thus, a single inoculation causes inclusion conjunctivitis whereas repeated inoculation causes trachoma. It appears that it is the frequency or persistence of exposure that determines the clinical course of the disease.

Histological Studies of Human Trachoma

Although other inflammatory cells will be numerous in the conjunctiva infected with chlamydia, the overwhelming cells are lymphocytes arranged as islands of B cells and surrounded in a sea of subepithelial T cells. The upper

palpebral conjunctiva of individuals with trachomatous conjunctivitis has been biopsied and shows an epithelial infiltrate of polymorphs, macrophages, T cells and dendritic cells. In the underlying stroma the inflammatory infiltrate was organised as B cell lymphoid follicles and there was a diffuse infiltrate of plasma cells and scattered B lymphoid cells, dendritic cells, T cells macrophages and polymorphs. Plasma cells were located in a subepithelial band, and as a dense infiltrate around the acini of the accessory lachrymal glands. The plasma cells are predominately IgA type [14, 20].

In active trachoma, T helper/inducer lymphocytes predominate in the substantia propria whereas in inactive trachoma it is the T suppressor/cytotoxic cells that are more commonly found [14]. This was confirmed in frozen section studies, where inflamed conjunctiva showed increased CD4+ lymphocytes compared to predominately CD8+ lymphocytes in non-inflamed biopsies. In the cicatricial stage, the inflammatory infiltrate is comprised mainly of T cells, outnumbering B cells and plasma cells by between 2 and 17 times. The inference is that the T cells are involved in the genesis of tarsal thickening and conjunctival scarring seen in the latter stages of trachoma [21].

Immunopathogenesis

Activated T cells appear to form the main inflammatory cell response in chronic cicatrising trachoma in the conjunctiva and tarsus in the absence of identifiable chlamydial infection. Thus the scarring is due to chronic delayed-type hypersensitivity in response to the continued presence of chlamydial antigen. The identification of this antigen has been important in understanding the pathogenesis of cicatrising conjunctivitis.

C. trachomatis has two cell types in its life cycle, although only one of these, the elementary body, exists outside cells. The elementary body is highly antigenic and expresses a number of different antigens on its surface. Major outer membrane protein (MOMP) is the major structural protein of *Chlamydia* and makes up 20% of its dry weight and 60% of the cell wall and so has been regarded as a good candidate molecule. MOMP contains antigenic domains for species, subspecies and type-specific specificities [22]. Lipopolysaccaride (LPS) also has been suggested as an antigen that could mediate the hypersensitivity response to chlamydial infection. However, in animal models, neither MOMP nor LPS inoculation leads to inflammation [23].

In contrast, a single inoculation of the chlamydial heat shock protein 60 (hsp60) leads to marked inflammation in animal models. Heat shock proteins are strong antigens expressed during *C. trachomatis* infection and hsp60 remains the most likely candidate antigen for generating the major immunopathic response. Immune responses to chlamydial hsp60 are correlated with disease sequelae in humans and seem to be genetically controlled in part by genes in

Table 1. Simplified WHO diagnostic criteria

TF, trachomatous inflammation, follicular: greater than five follicles (>0.5 mm in size) on the central upper tarsal conjunctiva

TI, trachomatous inflammation, intense: inflammatory thickening of the upper tarsal conjunctiva with more than 50% of deep tarsal vessels obscured.

TS, trachomatous scarring: presence of easily visible scarring in the upper tarsal conjunctiva

TT, trachomatous trichiasis: presence of at least one eyelash rubbing the eyeball

CO, corneal opacity: presence of easily visible central corneal opacity, sufficiently dense to obscure the pupil margin (thought to be consistent with a visual acuity of 6/18 or less)

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the MHC locus. This would suggest that the persistent inflammation characteristic of trachoma may be maintained by an antigenic substance released by either transient episodes of actual infection or by continuing low levels of infection, which are maintained by reinfection [24]. It has been proposed that chlamydial hsp60 may break tolerance and induce auto-immune inflammatory damage or be the focus of cell-mediated immune damage during chronic infection and this immune response is at least partially determined by HLA type [25].

Clinical Diagnosis

Clinical diagnosis is generally straightforward in florid cases of trachoma. In follicular trachoma the characteristic signs of tarsal and/or limbal follicles are usually associated with papillae. Evidence of previous infection such as pannus, conjunctival scarring, Herbert's pits and eventually trichiasis and corneal scarring imply a diagnosis of cicatricial trachoma. Most surveys now use simplified WHO definitions of disease for standardisation [26] (table 1).

The simplified WHO trachoma grading scheme is designed to be used with the grader examining the patients with a $2.5 \times$ loupe (fig. 3).

In milder cases, the diagnosis may be more difficult, and specimens for laboratory testing can be considered. In the past, Giemsa cytology has been performed on conjunctival scrapings. Although Giemsa cytology has a high specificity for trachoma, it has a low sensitivity and is no longer used. The more definitive test is culture, which is highly specific. Chlamydial culture was the gold standard, but because of the logistic complexity involved with its use in the field, it is not often used and has been replaced with newer immunologic

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Fig. 3. Clinical features of trachoma. *A* Trachomatous inflammation-follicles (TF). *B* Trachomatous scarring of upper palpebral conjunctiva (TS). *C* Trachomatous trichiasis (TT).

Table 2. Comparison in the severity of trachoma in children aged 10 years or less between face washing 0–6 times per week and 7 or more times per week (both communities) [9]

Frequency of face washing per week	Severity of trachoma			Percentage with trachoma
	none	moderate	severe	
0	11	8	2	48
1–2	77	36	7	36
3–6	207	35	5	27
7 or more	154	17	1	10

and molecular tests. Immunofluorescent staining of cytology using a direct fluorescent antibody technique is a useful method for field diagnosis because of its speed and simplicity. It is a highly sensitive test (94%) and has good specificity. However, even with sensitive tests, only 70–80% of those with severe disease and 20–30% of those with mild disease are likely to have a positive test.

Polymerase chain reaction (PCR)/enzyme immunoassay (EIA) offers improved sensitivity, especially in milder cases of trachoma. PCR also offers the advantages of less stringent requirements for specimen collection and transport, making this technique well suited to use in the field. Also the semi-quantitative aspect of PCR-EIA may be useful in monitoring response to therapy [27, 28].

In summary, clinical diagnosis remains the best means of assessing and diagnosing trachoma together with a combination of DFA cytology and PCR/EIA in difficult cases.

Management of Trachoma

Prevention

In hyperendemic areas, epidemiological studies have shown the most important parameter associated with the occurrence and severity of trachoma in children was the presence of clean faces.

Children who washed their faces 7 or more times a week had significantly less trachoma than those who washed less. These effects were independent of age, family hygiene or socio-economic status (table 2).

The impact of facial cleanliness was confirmed in a randomised controlled intervention trial in Tanzania where an extensive community-based health

education campaign that increased the number of children with clean faces reduced the prevalence and severity of trachoma in those children [10].

This points to an intervention strategy that is practically achievable. A campaign emphasising the benefits of personal hygiene, combined with provision of water to desert areas is a very effective measure to significantly reduce the prevalence of inflammatory trachoma.

Treatment

The recommended treatment of trachoma in children has been topical tetracycline ointment for a period of 6 weeks or on 5 consecutive days each month for 6 months [29]. Patients with severe disease should be considered for systemic therapy [30]. In children with active trachoma, topical tetracycline can be difficult to apply and compliance can be poor. This can be compounded by the discomfort and burning caused by the ointment and the need for treatment long after the eyes are asymptomatic.

Recent studies have shown a single dose of azithromycin (1 g for adults or 20 mg/kg for children) to be at least as effective, and may be much more practical. A single dose leads to the cure of trachoma without significant side effects in 78% of cases compared with 72% who were treated conventionally. Azithromycin is now widely used for trachoma control in some endemic areas [31–33].

Management of Trachomatous Trichiasis

Trichiasis is caused by entropion, misdirected lashes or both. Severe trichiasis is associated with severe visual loss that follows corneal damage secondary to abrasions from the lashes combined with tear film defects from disturbed lachrymal and lid function. Macallan estimated that 30% of individuals who had trachoma of any marked extent would develop trichiasis [2]. Clearly, correction of trichiasis and lid deformity is a key strategy for preventing blindness from trachoma and leads to a significant improvement in vision as well as stopping further visual loss.

Epilation can be performed by the patients themselves or by their relatives, but it has only a transient effect. Timed cryoablation for trichiasis without marked entropion is reasonably effective. Success appears to be higher with treatment to the whole lid margin. Skin depigmentation and alopecia of normal lashes can occur and may be unacceptable to patients. A controlled trial of cryoablation revealed a 56% success rate in cases of trichiasis without entropion [34].

Trichiasis with entropion is best managed with a tarsal rotation operation with a complete incision of the scarred tarsal conjunctiva and tarsus parallel to the lid margin (a modified Trabut operation). A large prospective randomised

controlled trial in Oman proved this to be the operation of choice for mild and severe trichiasis in the absence of lid closure defects. It is the preferred procedure recommended by WHO. The marginal tarsus is relieved of the inturning force from progressive conjunctival scarring and contraction, and the lashes are diverted outwards and away from the globe. The key is to avoid lid shortening or tarsal excision and so avoid corneal complications from inadequate closure [35].

Eyes with defective lid closure require more complex procedures involving incision of the tarsal insertion of the levator and tarsal inlay grafts. These procedures may well be beyond the expertise of the local health workers who can't be trained to perform entirely satisfactory entropion repair in the villages [36].

Future Advances

Vaccination

Since the successful cultivation of *C. trachomatis* in the 1950s, an effective vaccine has been looked for as a way of reducing the incidence of trachoma. However, it is not clear whether humoral immunity is important in resistance to ocular infection. It is possible to produce serum antibodies to *C. trachomatis* with a systemic vaccine, but tear antibodies are not induced in the absence of prior ocular exposure except in maximally immunised groups. It is possible to stimulate mucosal immunity and induce tear antibodies by priming the conjunctiva to produce a brisk antibody response to infection. This is done best by the topical instillation of the vaccine antigen to the eye. This may be sufficient to induce protection against infection, although results using whole EB have been disappointing. It is possible that not only the protective but also the destructive immune responses may be stimulated. Despite evidence of vigorous MOMP-specific and other chlamydia-specific serological and cell-mediated immunity, the use of MOMP as a vaccine leads to only partial immunity from subsequent chlamydial infection. The failure of MOMP extract as a trachoma vaccine suggests that further work looking at other antigens is needed to induce more effective protective immunity [37].

References

1   WHO: Global Scientific Meeting on Future Approaches to Trachoma Control. The Magnitude of the Problem. Geneva, World Health Organization, 1996.
2   Duke-Elder S (ed): System of Ophthalmology. St. Louis, Mosby, 1965, vol 8/1, pp 254–299.
3   National Trachoma and Eye Health Program. Trachoma, The Ocular Condition. Sydney, Royal Australian College of Ophthalmologists, 1980, pp 24–28.

4   National Trachoma and Eye Health Program. The Prevalence of Trachoma in Australia. Sydney, Royal Australian College of Ophthalmologists, 1980, pp 37–53.

5   Dawson CR, Daghfous T, Messadi M, Hoshiwara I, Schachter J: Severe endemic trachoma in Tunisia. Br J Ophthalmol 1976;60:245–252.

6   West SK, Rapoza P, Munoz B, Katala S, Taylor HR: Epidemiology of ocular chlamydial infection in a trachoma hyperendemic area. J Infect Dis 1991;163:752–756.

7   Bailey RL, Hayes L, Pickett M, Whittle HC, Ward ME, Mabey DCW: Molecular epidemiology of trachoma in a Gambian village. Br J Ophthalmol 1994;78:813–817.

8   West S, Munoz B, Turner V, Mmbaga BBO, Taylor HR: The epidemiology of trachoma in Central Tanzania. Int J Epidemiol 1991;20:1088–1092.

9   Taylor HR, Millan-Velasco F, Sommer A: The ecology of trachoma: An epidemiological study of trachoma in Southern Mexico. Bull World Health Organ 1985;63:559–567.

10   West S, Munoz B, Lynch M, Kayongoya A, Chilangwa Z, Mmbaga BBO, Taylor HR: Impact of face-washing on trachoma in Kongwa, Tanzania. Lancet 1995;345:155–158.

11   Taylor HR, West S, Mmbago BBO, Katala SJ, Turner V, Lynch M, Munoz B, Rapoza PA: Hygiene factors and increased risk of trachoma in Central Tanzania. Arch Ophthalmol 1989;107:1821–1825.

12   West SK, Congdon N, Katala S, Mele L: Facial cleanliness and risk of trachoma in families. Arch Ophthalmol 1991;109:855–857.

13   Turner VM, West SK, Munoz B, Katala S, Taylor HR, Halsey N, Mmbaga BBO: Risk factors for trichiasis in women in Kongwa, Tanzania: A case-control study. Int J Epidemiol 1993;22:341–347.

14   Burd EM, Tabbara KF, Nasr AM, Taylor PB: Conjunctival lymphocyte subsets in trachoma. Int Ophthalmol 1988;12:53–57.

15   Dawson CR: Immunology of ocular chlamydial infections. Int Ophthalmol Clin 1985;25:95–106.

16   Treharne JD: The microbial epidemiology of trachoma. Int Ophthalmol 1988;12:25–29.

17   Taylor HR, Rapoza PA, West SK, Johnson S, Munoz B, Katala S, Mmbaga BBO: The epidemiology of infection in trachoma. Invest Ophthal Vis Sci 1989;30:1823–1833.

18   Taylor HR, Prendergast RA, Dawson CR, Scachter J, Silverstein AM: An animal model of cicatrizing trachoma. Invest Ophthalmol Vis Sci 1981;20:422–433.

19   Taylor HR, Johnson SL, Prendergast RA, Scachter J, Dawson CR, Silverstein AM: An animal model of trachoma. II. The importance of repeated infection. Invest Ophthalmol Vis Sci 1982;23:507–515.

20   El-Asar AM, Van den Oord JJ, Geboes K, Missotten L, Emarah MH, Desmet V: Immunopathology of trachomatous conjunctivitis. Br J Ophthalmol 1989;73:276–282.

21   Reacher MH, Pe'er J, Rapoza PA, Whittum-Hudson JA, Taylor HR: T cells and trachoma: Their role in cicatricial disease. Ophthalmology 1991;98:334–341.

22   Caldwell HD, Kroumhout J, Schlachter J: Purification and partial characterisation of the major outer membrane protein of *Chlamydia trachomatis*. Infect Immun 1981;31:1161–1176.

23   Grayston JT, Wang S: New knowledge of chlamydiae and the diseases they cause. J Infect Dis 1975;132:87–105.

24   Taylor HR: Trachoma research. Report to the International Organisation against Trachoma, Rome, 1986, pp 25–58.

25   Brunham RC, Peeling RW: *Chlamydia trachomatis* antigens: Role in immunity and pathogenesis. Infect Agents Dis 1994;3:218–233.

26   Thylefors B, Dawson CR, Jones BR, West SK, Taylor HR: A simple system for the assessment of trachoma and its complications. Bull World Health Organ 1987;65(4):477–483.

27   Bobo L, Munoz B, Viscidi R, Quinn T, Mkocha H, West SK: Diagnosis of *Chlamydia trachomatis* eye infection in Tanzania by polymerase chain reaction/enzyme immunoassay. Lancet 1991;338:847–850.

28   Bailey RL, Hampton TJ, Hayes LJ, Ward ME, Whittle HC, Mabey DCW: Polymerase chain reaction for the detection of ocular chlamydial infection in trachoma-endemic communities. J Infect Dis 1994;170:709–712.

29   Dawson CR, Jones BR, Tarizzo ML: A guide to trachoma control. Geneva, World Health Organization, 1981.

2

30 World Health Organization: Strategies for the prevention of blindness in national programmes: A primary health care approach. Geneva, World Health Organization, 1984.

31 Taylor HR: Treatment of trachoma. Geneva, World Health Organization, 1996.

32 Bailey RL, Arullendran P, Whittle HC, Mabey DCW: Randomised-controlled trial of single dose azithromycin in treatment of trachoma. Lancet 1993;342:453–456.

33 Tabbara KF, El-Asar AMA, Al-Omar O, Choudhury AH, Al-Faisal Z: Single-dose azithromycin in the treatment of trachoma: A randomised controlled study. Ophthalmology 1996;103:842–846.

34 Rice DR, Kersten RC, Al Hazzaa S: Cryotherapy for trichiasis in trachoma. Arch Ophthalmol 1989;107:1180–1182.

35 Reacher MH, Munoz B, Alghassany A, Daar AS, Elbualy M, Taylor HR: A controlled trial of surgery for trachomatous trichiasis of the upper lid. Arch Ophthalmol 1992;110:667–674.

36 Reacher MH, Huber MJE, Canagaratnam R, Alghassany A: A trial of surgery for trichiasis of the upper lid from trachoma. Br J Ophthalmol 1990;74:109–113.

37 Campos M, Pal S, O'Brien TP, Taylor HR, Prendergast RA, Whittum-Hudson JA: A chlamydial major outer membrane protein extract as a trachoma vaccine candidate. Invest Ophthalmol Vis Sci 1995;36:1447–1491.

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Stevens–Johnson Syndrome

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Stevens–Johnson syndrome (syn. erythema multiforme major) was first described in 1922 [1]. It is a rare condition, but when it does occur it poses a serious threat to vision. It is part of a spectrum of acute blistering diseases which affect the skin and mucous membranes. The precise pathology is not well understood, but the central process is considered to be an immune-complex-induced vasculitis affecting the superficial cutaneous and mucosal vasculature. Exposure to drugs and to micro-organisms is known to initiate the process, but a precipitating event cannot always be identified.

It is the long-term sequelae of the acute necrotising conjunctivitis which constitute the major threat to vision. These chronic changes pose a serious threat to the ocular surface and therefore to vision. Because the initial episode is so short-lived, and generally affects young people, and because the subsequent chronic changes are so persistent, it is with the chronic form of the disease that ophthalmologists spend most time.

Definitions and Classification

The term 'erythema multiforme' was proposed by Hebra [2] in 1866 for patients with erythematous conditions of the skin, and associated stomatitis and conjunctivitis. Stevens and Johnson [1] rediscovered the disease in 1911, describing 2 cases. Since then, eruptive fever with stomatitis and ocular inflammation has been known as Stevens–Johnson syndrome.

It has been accepted for some time now that there are three related conditions: erythema multiforme, sometimes called erythema multiforme minor, in which there is skin involvement but the mucous membranes are spared; erythema multiforme major, which tends to be referred to as 'Stevens–Johnson syndrome', in which the skin and the mucous membranes are involved, and a more severe

Table 1. Drug preparations reported to have caused Stevens-Johnson syndrome

Systemic medications
- Sulfonamides
- Phenytoin
- Barbiturates
- Non-steroidal anti-inflammatory agents
- Penicillins
- Salicylates

Topical medications
- Scopolamine
- Sulfonamide
- Tropicamide

expression of the same process which has been termed 'toxic epidermal necrolysis' and which is sometimes referred to as 'Lyell syndrome' [3, 4]. A more formal classification has recently been proposed by Chan et al. [5].

External Risk Factors

In many cases, an association with medication, infections, malignancy, and collagen-vascular disease is apparent. Exposure to drugs and infections is the usual association. When there is a drug association, the disease typically manifests 7–21 days after exposure to the suspected agent. Sulfonamides are the most often recorded agents causing erythema multiforme [5], but the condition has also been reported after exposure to topical ophthalmic preparations. A list of drugs reported to have caused Stevens–Johnson syndrome is set out in table 1.

Cutaneous staphylococcal infection often precedes toxic epidermal necrolysis [6], and herpes virus infection, mycoplasma pneumonia, measles and infectious enteritis have been associated with Stevens–Johnson syndrome.

Pathology

Although drugs and micro-organisms have been implicated in the pathogenesis of the conditions, it is the host response which is damaging. There is an aberrant reaction by the immune system which damages tissues, but the precise nature of this aberrant response is unfortunately unclear. Circulating immune complexes and deposition in the mucosal microvasculature have been reported in patients with Stevens–Johnson syndrome.

Histological examination of the eyes of patients with erythema multiforme major in the acute phase reveals a non-specific inflammatory response with a prominent vasculitis and immune complexes deposited in the superficial mucosal vasculature [7, 8]. In the skin there is inflammation and separation of the epidermis and dermis. There is also a disturbance of CD4:CD8 lymphocyte ratios, but how these changes are related to histological changes is not clear [9–11].

Clinical Features – General

Stevens–Johnson syndrome (erythema multiforme major) is a systemic disease with acute and chronic phases. In the acute phase, it is characterised by fever, skin lesions, and inflammatory changes in at least two mucous membranes.

The skin lesions are generally similar in a particular case, unlike in erythema multiforme minor where there is considerable variability. The lesions have a characteristic target morphology, are generally less than 3 cm in diameter and cover between 10 and 20% of the skin surface. These bullous eruptions appear suddenly and last for up to 4 weeks.

The mucous membranes which can be involved include the oropharynx, lips, genito-urinary tract, conjunctiva and viscera. These surfaces become inflamed, blister and ulcerate [12, 13].

In addition, the patient tends to be unwell with fever, joint and muscle aches, perhaps a sore throat, or even nausea and vomiting.

During the acute phase, even apparently unaffected areas of the skin may be inclined to blister when rubbed. This has been termed the 'Nikolsky sign'.

Clinical Features – Ocular

In the acute phase of the disease, patients develop a non-specific conjunctivitis which may progress to bulla formation and ulceration. The conjunctivitis, which may precede the skin disease, is acute, usually symmetrical, with a mucopurulent discharge [14–17]. Corneal ulceration can occur in severe cases and may be complicated by infection [18]. This occurs concurrently with the skin lesions. Uveitis may also occur but this is less common. Eye involvement is usually bilateral, and tends to be symmetrical. Uniocular involvement is rare [15]. The ocular, oral, and skin manifestations of the acute condition are seen in figure 1.

The chronic sequelae of Stevens-Johnson syndrome are more varied in their expression, and represent what can be considered a common pathway
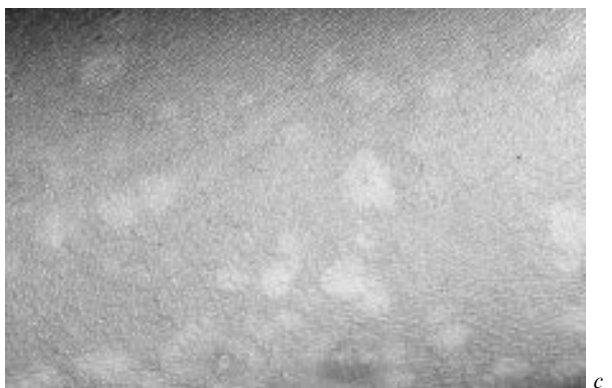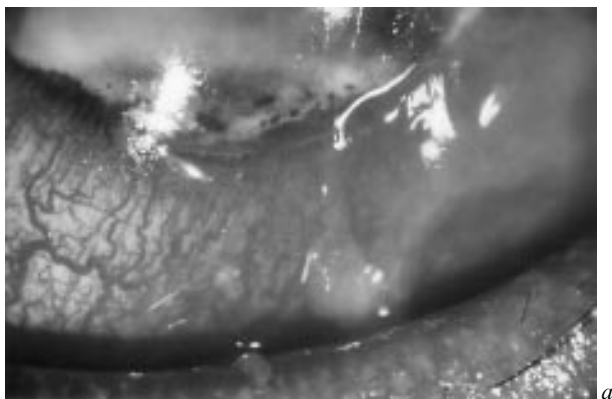
Fig. 1. Stevens–Johnson syndrome typical case 3–4 weeks after onset. *a* Purulent conjunctivitis with corneal ulceration. *b* Involvement of the mucus membrane of the mouth – note resolving target lesion in periocular skin. *c* Devolving target lesions in the skin.

ACUTE OCULAR DISEASE	corneal ulceration		conjunctival inflammation	
	↓		↓	
CHRONIC OCULAR DISEASE	corneal scarring	submucosal fibrosis	loss of glandular elements	loss of limbal stem cells
		↓	↓	↓
		trichiasis	tear film deficiency	epithelial metaplasia and superficial corneal vascularisation
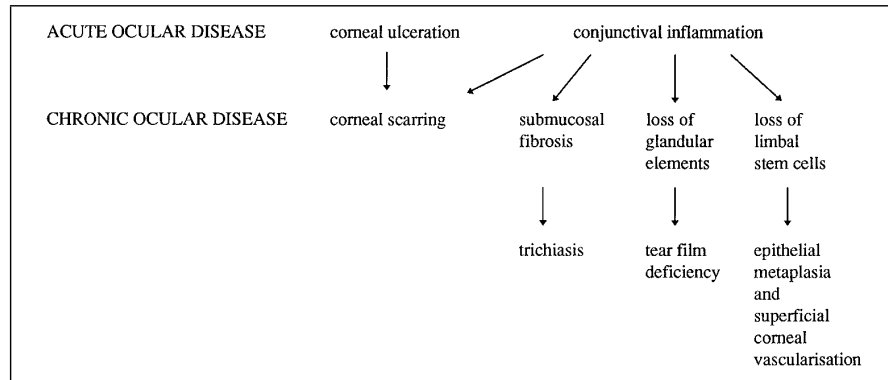
Fig. 2. The pattern of ocular pathology and clinical expression of ocular surface diseases including Stevens–Johnson syndrome.

for the eyes of patients with severe inflammatory disease of the ocular surface. The route of evolvement of the acute to the chronic form is set out in figure 2.

Submucosal inflammation leads to sub-mucosal fibrosis. This distorts the topography of the ocular surface and, in severe cases, may create symblepharon, and even ankyloblepharon.

The epithelium of the ocular surface is also altered. There is a loss of glandular structures and mucous-secreting goblet cells resulting in a dry ocular surface and an increase in the proportion of corneal epithelial cells derived from conjunctival origins. This suggests limbal stem cell damage. Distortion of the lid margin and dysplastic changes in the follicles result in trichiasis.

Corneal scarring and vascularisation may occur as a result of corneal involvement in the acute phase of the disease, or as a result of the various processes described above. These corneal changes and the abnormal tear film can combine to affect vision. Impaired vision and chronic discomfort are common in patients who have had Stevens–Johnson syndrome [19, 20]. The typical chronic phase of the disease is seen in figure 3.

Management

Diagnosis

In both the acute and the chronic phase, the diagnosis of Stevens–Johnson syndrome is based on clinical findings. Few other conditions can be confused with the acute illness with its cutaneous and mucosal sloughing.
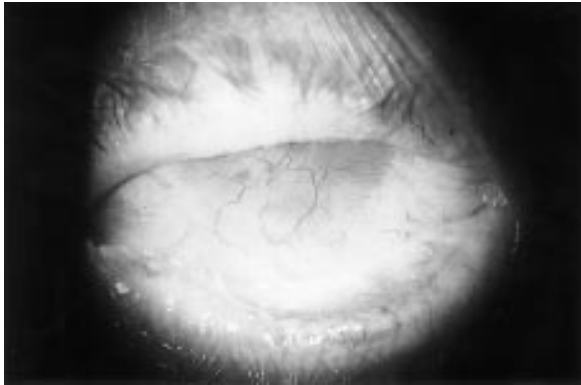
Fig. 3. Stevens–Johnson syndrome. Chronic phase – tear film deficiency, epithelial metaplasia, symblepharon and conjunctivilisation of the corneal epithelium.

Although no conditions are very similar, Kawasaki disease, Liener disease, toxic shock syndrome and staphylococcal scalded skin syndrome and other conditions producing erythema, such as contact dermatitis or burns, should be considered.

A number of conditions involving submucosal fibrosis can produce a clinical picture similar to that seen in the chronic phase of Stevens–Johnson syndrome. These include ocular cicatricial pemphigoid, and various conditions in which chronic inflammation has resulted from infection, as occurs in trachoma, as a result of allergies in several atopic individuals, or damage from chemicals or irradiation. Stevens-Johnson syndrome is usually differentiated from these conditions on the basis of the initial acute illness and perhaps exposure to a known trigger.

Ocular cicatricial pemphigoid is the most common problem needing to be differentiated from the chronic phase of Stevens–Johnson syndrome. In many cases of ocular cicatricial pemphigoid, linear IgG or IgA and complement can be demonstrated along the epithelial basement membrane in conjunctival biopsies.

Treatment

Acute Phase. Patients in the acute phase of Stevens–Johnson syndrome are critically ill and are generally managed in an intensive-care setting. Management of the ocular problems tends to be dominated by the need to manage the severely ill patient.

There are no treatment imperatives other than to treat any established infections vigorously. The use of corticosteroids either systemically or locally

is controversial and of no proven value. Similarly, the value of topical prophylactic antibiotics remains unproven and controversial. Various procedures have been advocated for limiting symblepharon formation, including glass-rodding and the use of stents or shells, but the value of these approaches has not been proven either. It is important to maintain good ocular hygiene. Regular eye toilets and the use of sterile irrigation can increase the patients' comfort and decrease ocular inflammation.

Chronic Phase. More treatment options are available to those suffering from the chronic phase of Stevens–Johnson syndrome. There is generally widespread disturbance in many elements of the ecosystem of the ocular eye. The tear film is deficient, the epithelium metaplastic or dysplastic, the conjunctiva scarred and shortened into symblephara, the lids malpositioned and usually the lashes are deranged with distichiasis and trichiasis [20].

The tear film can be replaced or added to with tear film supplements. If the tear film shortage is severe and the puncta are patent, occlusion will be beneficial.

Epithelial metaplasia, particularly if it has reached the point of keratinisation, can often be reversed or reduced by the use of topical vitamin A [21, 22].

Conjunctival shrinkage needing treatment can be managed with z-plasty or mucous membrane grafting. The mucous membrane can be harvested from the nose, labia or palate. More recently, conjunctival autografts or allografts have been used for this purpose. Conjunctival autografts have been preferred to mucous membrane for patients who have unilateral disease. Unfortunately, the most deserving cases are usually bilateral and there is no satisfactory source of conjunctiva. For these patients, conjunctival allografts have been employed or conjunctiva has been harvested from living related donors.

Some patients with Stevens–Johnson syndrome show signs of limbal stem cell deficiency. The epithelium is unstable, at worst with chronic epithelial defects and in its milder form with keratinisation and widespread staining with fluorescein or Bengal rose. There is usually superficial corneal vascularisation and invariably disturbance of the limbal vascular palisades. In these patients, the state of the corneal epithelium and overlying tear film is critical to vision. In recent times attempts at achieving normal corneal epithelium have included autografts of the limbus when the contralateral eye has been normal [23]. However, this is usually not the case in patients with Stevens–Johnson syndrome and it is necessary to use limbal allografts. This approach has been very successful in the short term. Long-term results have not yet been reported [24, 25].

References

1 Stevens AM, Johnson FC: A new eruptive fever associated with stomatitis and ophthalmia. Am J Dis Child 1922;24:526–533.
2 Hebra F: On Diseases of the Skin, Including the Exanthematha, translat and ed by CH Fagge. London, New Sydenham Society, 1866, vol 1.
3 Lyell A: Toxic epidermal necrolysis: An eruption resembling scalding of the skin. Br J Dermatol 1956;68:355–361.
4 Lyell A: Requiem for toxic epidermal necrolysis. Br J Dermatol 1990;122:837–838.
5 Chan HL, Stern RS, Arndt KA, Langlois J, Jick SS, Jick H, Walker AM: The incidence of erythema multiforme, Stevens–Johnson syndrome, and toxic epidermal necrolysis: A population-based study with particular reference to reactions caused by drugs among outpatients. Arch Dermatol 1990; 126:43–47.
6 Lyell A: A review of toxic epidermal necrolysis in Britain. Br J Dermatol 1967;79:662–671.
7 Ginandes GJ: Eruptive fever with stomatitis and ophthalmia: Atypical erythema Exudativum multi-forme (Stevens–Johnson). Am J Dis Child 1935;49:1148–1160.
8 Alexander MK, Cope S: Erythema multiforme exudativum major (Stevens-Johnson syndrome). J Pathol Bacteriol 1954;68:373–380.
9 Kazmierowski JA, Wuepper KD: Erythema multiforme: Immune complex vasculitis of the superficial cutaneous microvasculature. J Invest Dermatol 1978;71:366.
10 Safai B, Good RA, Day NK: Erythema multiforme: Report of two cases and speculation on immune mechanisms involved in the pathogenesis. Clin Immunol Immunopathol 1977;7:379–385.
11 Wuepper KD, Watson PA, Kazmierowski JA: Immune complexes in erythema multiforme and the Stevens–Johnson Syndrome. J Invest Dermatol 1980;74:368–371.
12 Araujo OE, Flowers FP: Stevens-Johnson syndrome. J Emerg Med 1984;2:129–135.
13 Elias PM, Fritsch PO: Erythema multiforme; in Fitzpatrick TB et al. (eds): Dermatology in General Medicine. New York, McGraw-Hill, 1987, pp 555–563.
14 Dunagin WG, Millikan LE: Drug eruptions. Med Clin North Am 1980;64:983–1003.
15 Ashby DW, Lazar T: Erythems multiforme exudativum major (Stevens–Johnson syndrome). Lancet 1951;i:1091–1095.
16 Bianchine JR, Macaraeg PVJ Jr, Lasagna L, Azarnoff DL, Brunk SF, Hvidberg EF, Owen JA: Drugs as etiologic factors in the Stevens–Johnson syndrome. Am J Med 1968;44:390–405.
17 Howard GM: The Stevens–Johnson syndrome: Ocular prognosis and treatment. Am J Ophthalmol 1963;55:893–900.
18 Patz A: Ocular involvement in erythema multiforme. Arch Ophthalmol 1950;43:244–256.
19 Arstikaitis MJ: Ocular aftermath of Stevens–Johnson syndrome: Review of 33 cases. Arch Ophthalmol 1973;90:376–379.
20 Wright P, Collin JR: The ocular complications of erythema multiforme (Stevens-Johnson syndrome) and their management. Trans Ophthalmol Soc UK 1983;103:338–341.
21 Tseng SC, Maumenee AE, Stark WJ, Maumenee IH, Jensen AD, Green WR, Kenyon KR: Topical retinoid treatment for various dry-eye disorders. Ophthalmology 1985;92:717–727.
22 Wright P: Topical retinoic acid therapy for disorders of the outer eye. Trans Ophthalmol Soc UK 1985;104:869–874.
23 Kenyon KR, Tseng SC: Limbal autograft transplantation for ocular surface disorders. Ophthalmology 1989;96:709–722.
24 Tsai RJF, Tseng SCG: Human allograft limbal transplantation for corneal surface reconstruction. Cornea 1994;13:389–400.
25 Coster DJ, Aggarwal RK, Williams KA: Surgical management of ocular surface disorders using conjunctival and stem cell allografts. Br J Ophthalmol 1995;79:977–982.

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Membranous, Pseudomembranous and Ligneous Conjunctivitis

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Conjunctival cicatrisation that follows membranous conjunctivitis varies considerably in severity but is generally not as pronounced as that seen in ocular cicatricial pemphigoid or trachoma. This chapter considers the main causes of membranous and pseudomembranous conjunctivitis highlighting current recommendations for treatment, with particular emphasis on the difficult problem of ligneous conjunctivitis.

Pathophysiology

The clinical picture of conjunctivitis reflects the pathological processes that occur during inflammation. Vasodilatation resulting in hyperaemia and reduced blood flow is followed by an increase in vascular permeability that allows exudation of plasma containing cells, fibrin and other extracellular mediators of inflammation. Such exudation produces conjunctival oedema and chemosis. As the exudate passes to the surface of the conjunctiva, it mixes with the tears and mucin to form a discharge.

Membranous and pseudomembranous conjunctivitis occurs when the inflammatory discharge rich in fibrin coagulates on the conjunctival surface. A pseudomembrane lies superficially on the surface and can be readily peeled or scraped away without causing bleeding. In contrast, a true membrane includes the conjunctival epithelium within the coagulum leaving a raw, bleeding surface on removal. Such deposition of exudate within the substance of the epithelium results in epithelial necrosis which heals with scar formation. Extracellular matrix proteins such as tenascin and fibronectin have recently

Table 1. Causes of pseudomembranous and membranous conjunctivitis

Infective	Bacterial conjunctivitis
	Beta-haemolytic streptococci
	Neisseria gonorrhoeae
	Neisseria meningitidis
	Corynebacterium diphtheriae
	Viral conjunctivitis
	Adenovirus
	Herpes simplex (primary infection)
	Chlamydia
	Chlamydia ophthalmia neonatorum
Non-infective	Chemical burns
	Stevens-Johnson syndrome [3]
	Ocular cicatricial pemphigoid [3]
	Lyell syndrome [4]
	Factitious conjunctivitis [5]
	Ligneous conjunctivitis

been demonstrated to be present in addition to fibrin [1]. These are adhesion molecules which participate in tissue repair and may be important in cicatrisation.

Specific features of conjunctival anatomy determine certain clinical aspects that may be of help in diagnosis. Over the tarsal plates and at the limbus, papillary conjunctivitis is a non-specific indicator of inflammation, often existing together with conjunctival hyperaemia in those parts where anchoring septa are absent. Chronicity of inflammation can result in the rupture of these septa and cause either confluence of the papillae or formation of giant papillae. The latter are associated with conjunctival inflammation due to allergic eye disease, contact lenses or ocular prostheses and protruding sutures. The presence of follicles due to focal infiltration of the conjunctiva by lymphocytes can also be of diagnostic importance. They are seen in conjunctivitis of viral, chlamydial and toxic origin as well as in cat scratch disease and may be normally present in the inferior tarsal conjunctiva of children and young adults.

It has long been recognised that the formation of a true membrane or pseudomembrane reflects only a difference in the intensity of a single process [2]. Membrane and pseudomembrane can both be present at the same time. The distinction is therefore not as important from a clinical point of view as

establishing the presence of the exudate. The causes of membranous and pseudomembranous conjunctivitis are listed in table 1.

Infective Causes

Bacterial

Streptococci are frequently isolated from patients with conjunctivitis although they are more significant for their association with keratitis and endophthalmitis [6]. *Beta-haemolytic streptococci* are invasive micro-organisms producing exotoxin, which can cause a severe purulent conjunctivitis with membrane or pseudomembrane formation, not uncommonly with corneal involvement. They are sensitive to chloramphenicol, second-generation cephalosporins (cefuroxime), penicillin and erythromycin.

Neisseria gonorrhoeae conjunctivitis in adults is acquired by self-inoculation from the infected genital tract. Neonatal infection occurs during passage through an infected birth canal. Gonoccus typically produces a hyperacute purulent conjunctivitis with massive swelling of the eyelids, conjunctival chemosis, membranes and pseudomembranes, a copious thick discharge and pre-auricular adenopathy. The organism can invade intact corneal epithelium leading to suppurative or non-suppurative keratitis and perforation in inadequately treated cases.

Neisseria meningitidis can cause an identical hyperacute conjunctivitis but occurs far less commonly. It affects children more often than adults and its importance lies in its association with meningitis which may be preceded by the conjunctivitis.

Both gonococcal and meningococcal conjunctivitis require systemic treatment. The sexual partners of patients with *N. gonorrhoeae* also need treatment and this should include cover for chlamydial infection which is often found in association with gonococcus. The current recommendations for treatment are listed in table 2.

Membranous conjunctivitis has classically been associated with *diphtheric conjunctivitis*. Mass inoculation with diptheria toxoid has greatly reduced the incidence of diptheria and this form of conjunctivitis is now rare in developed countries. Among underprivileged groups living in crowded conditions as well as in tropical areas, cutaneous diptheria may remain a source of ocular infection [8]. When suspected on clinical grounds, treatment must be prompt since the severity of systemic involvement (cardiac, peripheral nervous) depends on the amount of exotoxin absorbed prior to the eradication of the organism. After excluding hypersensitivity, diphtheria antitoxin (10,000–30,000 units increased to 40,000–100,000 units in severe cases) is administered systemically

Table 2. Current recommendations for treatment of Neisseria infections

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Treatment for *N. gonorrhoeae* or *N. meningitidis* [7] infection

Systemically
– Ceftriaxone 1 g i.m. in a single dose or Ceftriaxone 1 g i.v. b.d. for 1–2 days
  if keratitis is present *and*
– Doxycycline 100 mg b.d. orally for 7 days

Local measures
– Ocular irrigation with buffered saline until discharge-free
– Topical erythromycin 0.5% or penicillin G (100,000 U/ml)

Treatment of Neisseria ophthalmia neonatorum [7]

Systemically
– Ceftriaxone 25–50 mg/kg/day i.v. or i.m. in a single dose (max. 125 mg) –
  for disseminated infection continue above for 7 days *and*
– Erythromycin syrup 50 mg/kg/day in 4 divided doses 14 days

Local measures
– Ocular irrigation with buffered saline until discharge-free
– Topical penicillin G (100,000 U/ml)

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and topically together with systemic penicillin, erythromycin, tetracycline or vancomycin.

Viral

Adenoviral infection produces several clinical entities which are indistinguishable in the early stages. Acute follicular conjunctivitis with a watery discharge and pre-auricular adenopathy occurs unilaterally, often with the other eye becoming involved after a few days. Pharyngoconjunctival fever is more common in children giving rise to conjunctivitis, fever, headache, pharyngitis but only mild epithelial keratitis. Epidemic keratoconjunctivitis is more frequent in adults half of whom develop pharyngitis and rhinitis but typically causes more severe conjunctival inflammation and corneal involvement. Pseudomembrane and membrane formation occurs in both forms, more often following epidemic keratoconjunctivitis. No effective form of treatment is currently available. Topical interferon has not proved to be effective [9, 10]. Live vaccines to protect against adenoviral respiratory infections use viral strains that do not cause ocular infection. Warm or cold compresses and topical lubricants may provide symptomatic relief. The administration of topical steroids is controversial but may be of use to limit post-membranous conjunctival scarring as well as for significant keratitis. It is essential to exclude

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Table 3. Treatment of chlamydial ophthalmia neonatorum [7, 17, 18]

– Erythromycin 50 mg/kg/day p.o. in 4 divided doses 14 days
or
– Trimethoprim-sulphamethoxazole 0.5 ml/kg/day in 2 divided doses 14 days
– Topical tetracycline 1% q.i.d. 21 days
or
– Topical erythromycin 0.5% q.i.d. 21 days

2

herpes simplex infection before starting steroids; immunoassay kits to detect both adenovirus [11] (Adenoclone, Cambridge Bioscience, Worcester, Mass., USA) and herpes simplex [12] (Herpcheck, Dupont, Wilmington, Del., USA) are available. Viral shedding from the conjunctiva can persist for 7–12 days. Careful hygiene in the clinic and emergency room and advice to patients with regard to isolation and sharing of fomites is an important aspect of management.

Primary infection with *herpes simplex* type I causes a follicular conjunctivitis with watery discharge and ipsilateral pre-auricular adenopathy very similar to adenovirus. Skin vesicles on the lids or face may or may not be present. Rarely, the conjunctivitis can be membranous. Animal studies suggest that differences between viral strains may be important in the pathogenicity of the organism but it remains uncertain whether this applies to ocular infection in humans [13]. Corneal involvement occurs in about 30% of cases of primary herpes simplex infection, and topical acyclovir is generally recommended although the condition is usually self-limiting and without serious sequelae unlike recurrent infection.

Chlamydia

Chlamydia is the most common cause of neonatal conjunctivitis [14] and usually presents towards the end of the first week of life as bilateral mucopurulent conjunctivitis of variable severity with conjunctival membranes or pseudomembranes in severe cases. Earlier presentation can occur with prolonged rupture of membranes [15]. The diagnosis is confirmed by immunoassay [16] (Chlamydiazyme, Abbott Laboratories, North Chicago, Ill., USA, or Chlamydia Microtrak, Syva, Palo Alto, Calif., USA) and isolation of the organism on cell culture. Exclusion of gonococcal infection is essential. Systemic treatment is required given the potential of Chlamydia to cause pneumonitis and otitis media (table 3). Ofloxacin is an effective agent against Chlamydia and

can be used topically and systemically in adults but systemic fluoroquinolones are currently not recommended for children [19].

Non-Infective Causes

Severe *chemical and thermal burns* can lead to marked conjunctival inflammation and epithelial destruction. Alkalis penetrate more deeply into the tissues than acids and thus are more destructive but both can lead to pseudomembrane and membrane formation with symblepharon, dry eye, trichiasis and lagophthalmos resulting from cicatrisation. Immediate copious irrigation with removal of all particles from the conjunctival fornices remains the most important aspect of treatment. Topical citrate 0.5% and potassium ascorbate 10% 0.5–2 hourly has decreased the incidence of corneal perforation in severe alkali burns [20]. Topical corticosteroids may be of use to diminish scarring and symblepharon formation but should not be continued beyond the first week after injury as they increase the risk of corneal melting by enhancing collagenolysis and inhibiting fibroblast activity. Corneal grafting in severely burnt eyes carries a poor prognosis and should be delayed for at least 1 year. Prior reconstructive surgery to the lids or conjunctiva will often be required. Recently, interest has centred on the transplantation of limbal stem cells to maintain the integrity of the corneal epithelium [21, 22].

Stevens-Johnson syndrome and *ocular cicatricial pemphigoid* are discussed in separate chapters.

Ligneous Conjunctivitis

Ligneous conjunctivitis is a rare form of membranous and pseudomembranous conjunctivitis that is particularly resistant to treatment. Borel [23] first introduced the term in 1934 to emphasise the characteristic hard, woody consistency of the affected lids. Similar cases of recurrent conjunctival membrane formation had already been described by Bouisson [24] and Lijo-Pavia [25] who provided the first detailed histology of the condition. Ligneous conjunctivitis is rare and the literature contains only a few reports of more than one or two cases [26–28]. However the clinical features are sufficiently specific to consider the condition a separate clinical entity. Typically infants and young children are affected but it can occur at any age [29, 30] with a slight female preponderance 1.7:1. The concept of ligneous conjunctivitis of young girls [31] is therefore misleading.

Fig. 1. Ligneous conjunctivitis involving upper tarsal conjunctiva in a 4-year-old boy.

Fig. 2. Close-up photograph of same patient as in figure 1.

An acute or subacute conjunctivitis occurs with the formation of membranes and pseudomembranes most commonly on the upper tarsal conjunctiva although the lower tarsal and the bulbar conjunctiva can also be involved (fig. 1). About half of the reported cases are bilateral. The membranes arise from a base attached to the tarsus and assume a flattened, smooth surface from compression and movement of the lids over the globe (fig. 2). Overlying the membrane, there is often a thin fibrinous pseudomembrane that can be wiped away. Although the membranes may persist for many years with little discomfort or visual disability [32, 33], secondary corneal vascularisation, scarring and thinning occur in about 25% of cases. Severe keratopathy, including perforation, appears less commonly in the more recent literature, perhaps as
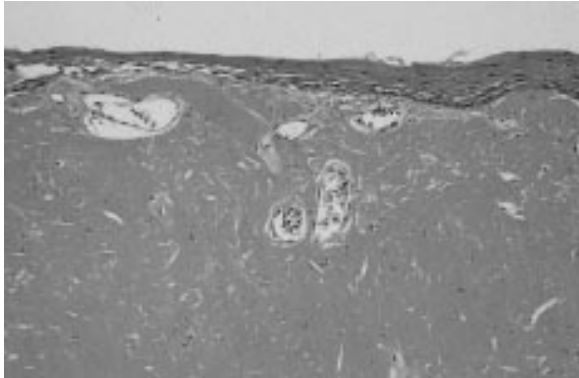
Fig. 3. Histology of ligneous conjunctivitis. Note accumulation of hyaline material with scattered blood vessels under atrophic epithelium. HE. ×40.

a result of increasing availability of topical antibiotics. Spontaneous resolution has been reported in a few cases [26, 34]. Conversely, relapse is possible after years of quiescence [28].

A history of an upper respiratory tract infection or a systemic febrile illness prior to the onset of ligneous conjunctivitis is often obtained. Deposition of membranes, similar to those found in the eye, can occur in other mucosal sites such as the respiratory tract [35], the cervix [36], the middle ear [34], the vocal cords [37] and the gingiva [38]. There is an association with hydrocephalus [39]. Surgical trauma has been a trigger for the condition in several cases [28–30, 40]. Ligneous conjunctivitis has been reported in siblings [26, 39, 41] and in different generations of the same family [42], suggesting that hereditary factors are important though by far the majority of cases are sporadic.

Histologically, the membranes consist of subepithelial deposition of amorphic hyaline material which is vascularised to varying degrees and with a variable cellular component which is predominantly lymphoplasmacytic, but which can also contain neutrophils, eosinophils and mast cells. The surface epithelium is generally atrophic, absent in places, with areas of epithelial downgrowth into the underlying hyaline material forming crypts containing goblet cells and mucus. Fibrin is a consistent feature of the ligneous membranes [28, 34, 43] (fig. 3, 4).

Ligneous conjunctivitis must be distinguished from granulomatous conjunctivitis (as seen in cat scratch disease, tularaemia or tuberculosis) and conjunctival synthetic fibre granuloma [44].

The clinical picture and the histochemical composition of the membranes suggest that the condition represents an abnormally brisk response to conjunc-
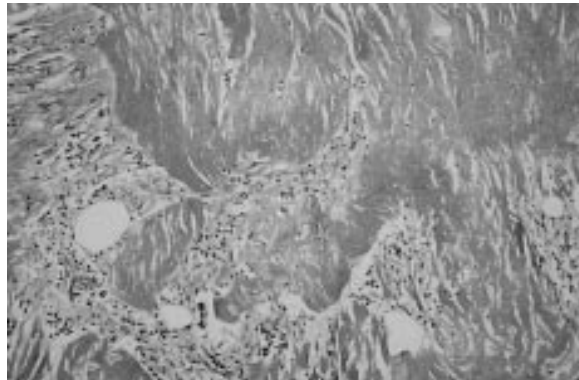
Fig. 4. Histology of ligneous conjunctivitis. The hyaline material stains positively for fibrin. Picro-Mallory stain. ×40.

tival inflammation of any cause leading to massive outpouring of plasma constituents, fibrin in particular, which coagulate to form a thick hard membrane. The underlying cause of such a response remains totally obscure. Hidayat and Riddle [26] reported the presence of abnormal blood vessels with gaps between endothelial lining cells on electron microscopy of recurrent membranes, and Kanai and Polack [45] showed deposition of material around capillaries reminiscent of basement membrane thickening in diabetic vasculopathy. In neither study could these changes be shown to be the primary cause of ligneous conjunctivitis. Cooper et al. [35] suggested that the increased vascular permeability may result from a hypersensitivity reaction, having identified numerous eosinophils and degranulated mast cells in a case of ligneous conjunctivitis which responded to treatment with sodium cromoglycate. Such mast cell infiltration is not consistently present in ligneous conjunctivitis. François et al. [46] emphasised the presence of hyaluronidase-sensitive mucopolysaccharides in the ligneous membranes and postulated a hereditary disorder of mucopolysaccharide metabolism. Again, mucopolysaccharide deposition is not a constant feature of ligneous conjunctivitis [26, 28, 45] and such that there is may be related to the goblet cells, trapped in the hyaline material by epithelial downgrowth. Several micro-organisms have been isolated from affected eyes but none have been recovered consistently, and they probably represent secondary infection.

Treatment of ligneous conjunctivitis remains very difficult. Topical or systemic antibiotics or antivirals have no effect. Excision, whether or not accompanied by cautery, conjunctival graft [47, 48] or ultraviolet irradiation [49] is followed by prompt re-accumulation of the membrane within days.

Fig. 5. Ligneous conjunctivitis of left upper lid in 1-year-old boy.

With repeated excisions, the lesion-free interval appears to get shorter. Paufique and Moreau [50] treated one case successfully with excision followed by multiple doses of beta-irradiation but others have not been able to repeat this [26, 51]. Similarly, excision followed by cryopexy and fibrinolysin was successful in one case [52] but again this was not reproduced [26]. Cohen-Tervaert et al. [37] report a case which responded to systemic azathioprine.

In the absence of an understanding of the pathogenesis of ligneous conjunctivitis, therapeutic strategies have been refined on the basis of histological findings. Several authors have advocated the use of topical hyaluronidase and α-chymotrypsin to break down the mucopolysaccharides that they considered to be the main constituent of the membranes [29, 46, 52]. However mucopolysaccharide deposition is neither a major nor an invariable feature of ligneous conjunctivitis [26, 28, 45] and thus it is not surprising that this treatment regime has not been successful in all cases [26, 28, 45, 53].

The rarity of the condition and the diversity of the histological findings make any formal assessment of treatment protocols difficult. It is clear that any inflammatory stimulus, including surgical excision in the absence of prophylactic measures, is a trigger for rapid recurrence of membrane, a situation roughly parallel to the brisk response to surgery seen in active cicatricial pemphigoid.

The consistent presence of fibrin has led to the use of topical heparin in the treatment of ligneous conjunctivitis at Moorfields Eye Hospital. In a series of 17 patients treated by excision, topical heparin, α-chymotrypsin (in 12 patients) and corticosteroids, 76% were successfully controlled, and in those who suffered recurrent membranes, the lesion-free interval was longer than
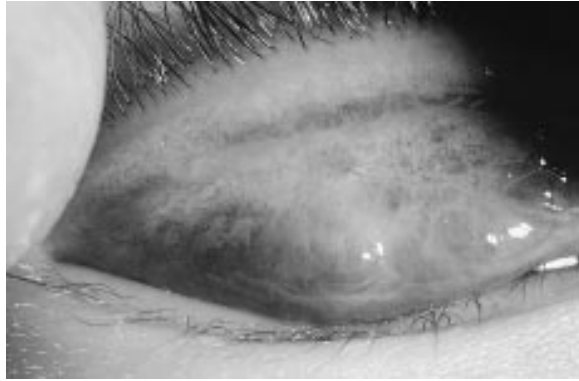
Fig. 6. Same patient as in figure 5, 6 months after treatment with topical heparin and steroids. This patient was followed up for 2 years with no recurrence. Mild conjunctival cicatrisation is evident.

Table 4. Suggested treatment for ligneous conjunctivitis [28]

– Excision of membrane
– Cautery to achieve complete haemostasis
– Immediate (peroperative) topical Heparin (5,000 IU/ml)
  topical Prednisolone 1% (frequency: 0.5–1 hourly)
– When epithelium healed, reduce frequency and concentration of
  Heparin (1,000 IU/ml) and Prednisolone (0.5%, 0.3%, 0.1%)
– Monitor membrane/mucus re-accumulation
– Antibiotic cover until epithelium healed

usually seen in this condition (mean 7.8 months, range 6 days to 6 months) [28]. Standard heparin binds antithrombin III and accelerates the combination of antithrombin with thrombin, thereby creating an inactive complex which prevents the conversion of fibrinogen to fibrin. Obsessive attention to detail is essential for success with this form of treatment. Surgical excision of the membranes must be followed by meticulous cautery of any oozing vessels on the conjunctival surface and topical heparin (1,000 or 5,000 IU/ml) must be started immediately. Heparin and corticosteroid drops are given intensively (0.5–1 hourly) until re-epithelialisation of the conjunctival surface is verified by lack of fluorescein staining. Thereafter, the drops are tapered both in frequency and concentration according to the clinical picture of mucus or membrane re-accumulation. Great care is needed to avoid minor trauma to

the conjunctiva during lid eversion or debridement of superficial mucus; any bleeding and inflammation result in recurrent fibrin deposition. α-Chymotrypsin in concentration suitable for topical use is no longer available. Its action is to break down any membrane that does recur. This should also be possible with the use of heparin and corticosteroids alone provided treatment is started sufficiently promptly and intensively. A summary of this regime is given in table 4 (fig. 5, 6).

Other agents which have been useful in selected cases may be indicated on the basis of the histology obtained in individual patients. Thus cyclosporin may be considered when there is marked lymphocytic infiltration [54, 55] or cromoglycate when abundant mast cells are present [35, 56]. Treatment of this enigmatic condition will of necessity remain empirical until a better understanding of its pathogenesis is obtained.

2

References

1 Kivela T, Tervo K, Ravila E, Tarrkanen A, Virtanen I, Tervo T: Pseudomembranous and membranous conjunctivitis. Immunohistochemical features. Acta Ophthalmol (Copenh) 1992;70:534–542.
2 Jessop WH: On membranous conjunctivitis. Trans Ophthalmol Soc UK 1902;22:41–59.
3 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.
4 Prendiville JS, Herbert AA, Greenwald MJ, Easterly NB: Management of Stevens-Johnson syndrome and toxic epidermal necrolysis in children. J Pediatr 1989;115:881–887.
5 Braude L, Sugar J: Chronic unilateral inferior membranous conjunctivitis (factitious conjunctivitis). Arch Ophthalmol 1994;112:1488–1489.
6 Jones S, Cohen EJ, Arentsen JJ, Laibson PR: Ocular streptococcal infections. Cornea 1988;7:295–299.
7 Centers for Disease Control and Prevention: 1993 Sexually transmitted diseases treatment guidelines. MMWR 1993;42(No RR-14):50–64.
8 Brinser JH: Diagnostic Ocular Microbiology; in Tabbara KF, Hyndiuk RA (eds): Infections of the Eye. Boston, Little, Brown, 1986, chapt 10, pp 129–130.
9 Adams CP, Cohen EJ, Albrecht J, Laibson PR: Interferon treatment of adenoviral conjunctivitis. Am J Ophthalmol 1984;98:429–431.
10 Wilhelmus KR, Dunkel EC, Herson J: Topical human fibroblast interferon for acute adenoviral conjunctivitis. Graefes Arch Clin Exp Ophthalmol 1987;225:461–464.
11 Kowalski RP, Gordon YJ: Comparison of direct rapid tests for the detection of adenovirus antigen in routine conjunctival specimens. Ophthalmology 1989;96:1106–1109.
12 Dunkel EC, Pavan-Langston D, Fitzpatrick K, Cukor G: Rapid detection of herpes simplex virus (HSV) antigen in human ocular infections. Curr Eye Res 1988;7:661–666.
13 Rinne JR, Abghani SZ, Stulting RD: The severity of herpes simplex viral keratitis in mice does not reflect the severity of disease in humans. Invest Ophthalmol Vis Sci 1992;33:268–272.
14 Rapoza PA: Epidemiology of neonatal conjunctivitis. Ophthalmology 1986;93:456–461.
15 Armstrong JH, Zacarias F, Rein MF: Ophthalmia neonatorum: A chart review. Pediatrics 1976;57:884–892.
16 Sheppard JD, Kowalski RP, Meyer MP, Amortgui AJ, Slifkin M: Immunodiagnosis of adult chlamydial conjunctivitis. Ophthalmology 1988;95:434–443.
17 Heggie AD, Jaffe AC, Stuart LA: Topical sulfacetamide versus oral erythromycin for neonatal chlamydial conjunctivitis. Am J Dis Child 1985;139:564–566.

18 Rapoza PA, Quinn TC, Keissling LA, Green WR, Taylor HR: Assessment of neonatal conjunctivitis with direct immunofluorescent antibody stain for chlamydia. JAMA 1986;255:3369–3373.

19 Neu HC: Microbiologic aspects of fluoroquinolones. Am J Ophthalmol 1991;112:15S–24S.

20 Pfister RR: Ascorbic acid in the treatment of alkali burns of the eye. Ophthalmology 1980;87: 1050–1057.

21 Kenyon KR, Tseng SCG: Limbal autograft transplantation for ocular surface disorders. Ophthalmology 1989;96:709–723.

22 Tan DTH, Ficker LA, Buckley RJ: Limbal transplantation. Ophthalmology 1996;103:29–36.

23 Borel G: Un nouveau syndrome oculo-palpebral. Ann Ocul 1934;171:207–222.

24 Bouisson M: Ophtalmie suraiguë avec formation de pseudo-membranes à la surface de la conjonctive. Ann Ocul 1847;17–18:100–104.

25 Lijo-Pavia J: Tumor inflamatorio fungoso recidivante de la conjuntiva palpebral. Semana Med 1924;31:326–331.

26 Hidayat AA, Riddle PJ: Ligneous conjunctivitis. A clinicopathologic study of 17 cases. Ophthalmology 1987;94:949–959.

27 Firat T: Ligneous conjunctivitis. Am J Ophthalmol 1974;78:679–688.

28 De Cock R, Ficker LA, Dart JG, Garner A, Wright P: Topical heparin in the treatment of ligneous conjunctivitis. Ophthalmology 1995;102:1654–1659.

29 Weinstock SM, Kielar RA: Bulbar ligneous conjunctivitis after pterygium removal in an elderly man. Am J Ophthalmol 1975;79:913–915.

30 Girard LJ, Veselinovic A, Font RL: Ligneous conjunctivitis after pingueculum removal in an adult. Cornea 1989;8:7–14.

31 Legrand J, Hervouet F, Ertus: La conjonctivite ligneuse des petites filles. Bull Soc Ophtalmol Fr 1973;71:1077–1085.

32 Verhoeff FH: A case of ligneous conjunctivitis now 36 years in duration. Am J Ophthalmol 1958; 45:246–251.

33 Spencer LM, Straatsma BR Foos RY: Ligneous conjunctivitis Arch Ophthalmol 1968;80:365–367.

34 Arcus D, Wanton D, Donshik P, Choo L, Newman RA, Albert D: Ligneous conjunctivitis with ear involvement. Arch Ophthalmol 1990;108:514–519.

35 Cooper TJ, Kadzan JJ, Cutz E: Ligneous conjunctivitis with tracheal obstruction. A case report with light and electron microscopy findings. Can J Ophthalmol 1979;14:57–62.

36 Rubin A, Buck D, MacDonald MR: Ligneous conjunctivitis involving the cervix. Case report. Br J Obstet Gynaecol 1989;96:1228–1230.

37 Cohen-Tervaert D, Cruysberg JRM, Deutman AF, Manschot WA: Ligneous conjunctivitis. Doc Ophthalmol 1986;64:5–11.

38 Gunhan O, Celasun B, Perrini F, Covani U, Perrini N, Ozdemir A, Bostanci H, Finci R: Generalized gingival enlargement due to accumulation of amyloid-like material. J Oral Pathol Med 1994;23: 423–428.

39 Chambers JD, Blodi FC, Golden B, McKee AP: Ligneous conjunctivitis. Trans Am Acad Ophthalmol Otolaryngol 1969;73:996–1004.

40 Bierley JR, Blandford DL, Weeks JA, Baker RS: Ligneous conjunctivitis as a complication following strabismus surgery. J Pediatr Ophthalmol Strabismus 1994;31:99–103.

41 Bateman JB, Isenberg SJ, Pettit TH, Simons KB: Ligneous conjunctivitis: An autosomal recessive disorder. J Pediatr Ophthalmol Strabismus 1986;23:137–140.

42 Goldmann H, Hof W: Familiäre pseudomembranöse Conjunctivitis. Schweiz Med Wochenschr 1954;84:73–75.

43 Eagle RC, Brooks JSJ, Katowitz JA, Weinberg JC, Perrt HD: Fibrin as a major constituent of ligneous conjunctivitis (letter). Am J Ophthalmol 1986;101:493–494.

44 Ferry AP: Synthetic fiber granuloma. 'Teddy Bear' granuloma of the conjunctiva. Arch Ophthalmol 1994;112:1339–1341.

45 Kanai A, Polack FM: Histologic and electron microscope studies of ligneous conjunctivitis. Am J Ophthalmol 1971;72:909–916.

46 François J, Hanssens M, Victoria-Troncoso V: Etude pathologique, histochimique, pathogénique et thérapeutique de la conjonctivite ligneuse. Ophthalmologica 1968;155:169–185.

47 Gartner J: Zur Therapie und Pathogenese der Konjunktivitis lignosa. Albrecht von Graefes Arch Klin Exp Ophthalmol 1974;190:229–245.

48 Schwartz GS, Holland EJ: Induction of ligneous conjunctivitis by conjunctival surgery. Am J Ophthalmol 1995;120:253–254.

49 Kalt E, Autier: La conjonctivite pseudo-membraneuse à streptocoques de durée inaccoutumée. Bull Soc Ophtalmol Paris 1927;77–79.

50 Paufique L, Moreau P-G: La conjonctivite ligneuse. Ann Ocul 1953;186:12–33.

51 Winter FC, Michler RR: Chronic membranous conjunctivitis. Arch Ophthalmol 1953;49:161–163.

52 Firat T, Tinaztepe B: Histochemical investigations on ligneous conjunctivitis and a new method of treatment. Acta Ophthalmol (Copenh) 1970;48:3–13.

53 Melikian HE: Treatment of ligneous conjunctivitis. Ann Ophthalmol 1985;17:763–765.

54 Holland EJ, Chan C-C, Kuwabara T, Palestine AG, Rowsey JJ, Nussenblatt RB: Immunohistologic findings and results of treatment with cyclosporin in ligneous conjunctivitis. Am J Ophthalmol 1989;107:160–166.

55 Rubin BI, Holland EJ, de Smet MD, Belfort R Jr, Nussenblatt RB: Response of reactivated ligneous conjunctivitis to topical cyclosporine (letter). Am J Ophthalmol 1991;112:95–96.

56 Friedlaender MH, Ostler HB: Treatment of ligneous conjunctivitis with cromolyn: A case report. Proctor Bull 1978;1:3.

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Cicatricial Pemphigoid

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Definition

Cicatricial pemphigoid (CP) is an immune-mediated subepithelial and subepidermal disease, characterised by blisters or bullae of the mucous membranes and, to a lesser extent, of the skin. These lesions typically heal with scar formation, thus the term *cicatricial* pemphigoid.

Involvement of the ocular mucous membrane, i.e. the conjunctiva, is frequent and for the conjunctival disease manifestations, the term *ocular cicatricial pemphigoid* is used. In the dermatologic literature, CP is widely described as 'benign mucous membrane pemphigoid' [1]. Historic terms for this condition include 'ocular pemphigus', 'scarring pemphigoid' and 'essential shrinkage of the conjunctiva'.

Epidemiology

CP is a relatively rare disease and it is estimated that one in every 15,000 to 40,000 patients treated in an eye clinic may have CP [2–4]. There are no racial or geographic predilections, but a genetic susceptibility seems to be involved, and this is discussed in detail elsewhere in this volume ('What Initiates the Immune Response?', pp. 123–126). CP affects more women than men and usually occurs in elderly people with a peak of presentation in the seventh decade [2, 3, 5]. Children with this condition, however, have been reported [6–8]. Due to the varying constellation of cutaneous, mucosal, and ocular lesions, patients may present first to the general practitioner, ophthalmologist, dermatologist, dentist, otolaryngologist or gastro-enterologist. Data on the relative frequency of the sites involved vary depending on the specialist that examines the patient first and evaluates the data (table 1) [2, 3, 9–17].

Table 1. Reports on the relative frequency of sites involved in CP (from 1970)

Source and references	Patients	Eyes	Mouth	Nose/ sinus	Pharynx/ larynx	Oesophagus	Genitalia	Skin
Ophthalmology [2, 3, 9]	274	274 (100)	88 (32)	27 (10)	34 (12)	30 (11)	12 (4)	39 (14)
Oral surgery/ENT [10–12]	282	150 (55)	265 (94)	44 (16)	42 (15)	8 (3)	34 (12)	42 (15)
Dermatology [13–17]	201	136 (68)	167 (83)	54 (27)	70 (35)	9 (5)	42 (21)	50 (25)
Total, n	757	560 (74)	520 (69)	121 (16)	146 (20)	47 (6)	88 (12)	131 (17)

Figures in parentheses are percentages.

3 A

Clinical Features

The striking features of CP are chronic progressive conjunctival cicatrisation and recurring bullae or erosions of the mucous membranes and the skin which may result in permanent scarring. All mucous membranes including the conjunctiva, the oral mucosa, nose, larynx, pharynx, oesophagus, penis, vulva, vagina and anus may be affected [2, 4, 13].

Ocular Disease
Approximately 65–70% of patients with CP who present to the dermatologist show conjunctival involvement [13, 16–18]. Early ocular symptoms are those of any nonspecific chronic conjunctivitis and may include conjunctival irritation, hyperaemia and discharge [19]. Blisters are rarely seen in the eye, but it is the invasion of the submucosa by newly formed connective tissue with subsequent contraction which is regarded as the essential destructive process in the conjunctiva [20]. An early clinical sign is the involvement of canthal structures [21] leading to shallow canthal recesses and in the medial canthus to a loss of architecture with flattening or obliteration of the normal conjunctival folds, plica and caruncle (fig. 1, and colour plates in 'Cicatrising Conjunctivitis', pp. 1–10 and 'Monitoring of Activity', pp. 111–122) [22]. Other early signs are conjunctival thickening and white lines of subepithelial fibrosis, which usually first involve the lower fornix (fig. 2). Conjunctival involvement in CP typically occurs in both eyes, it may, however, be highly asymmetrical, and subtle signs are easily missed by unexperienced examiners. Progression
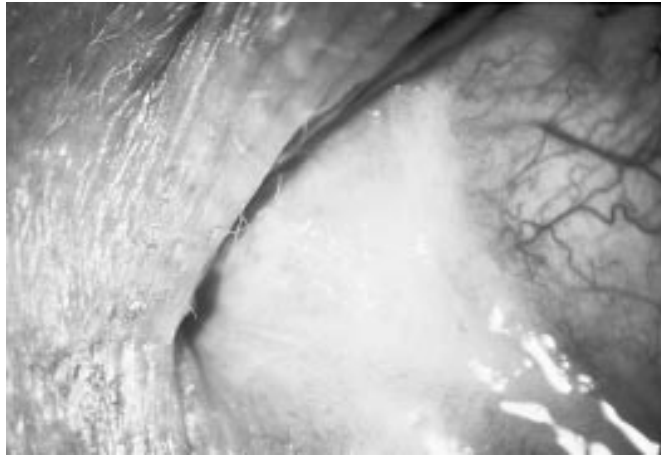
Fig. 1. Ocular disease without clinically manifest inflammation in cicatricial pemphigoid. Note the loss of the inner canthal architecture with flattening and obliteration of the normal conjunctival folds, plica and caruncle.
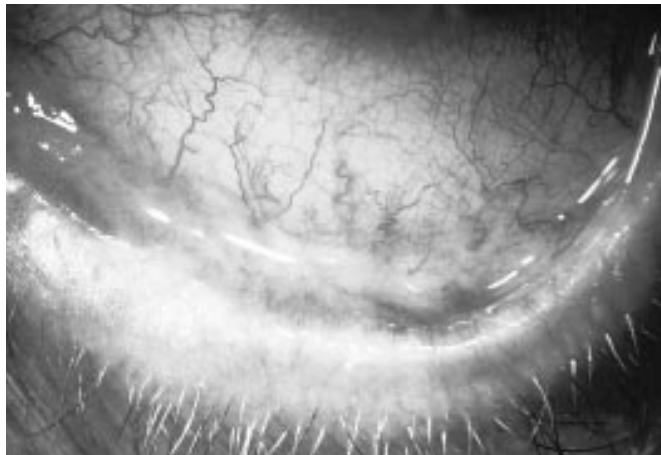
Fig. 2. Ocular disease with moderate inflammation in cicatricial pemphigoid. Note fornix foreshortening, conjunctival thickening and white lines with dense subepithelial fibrosis.

of the subepithelial fibrosis results in shrinkage of the fornices and, as the process continues, in the formation of symblephara (fig. 3) with their sequelae (see section 3D of this book, pp. 176–191) [2]. Conjunctival ulceration (fig. 4) may occur as part of an acute manifestation of the ocular disease and is followed by further, rapid progression of cicatrisation [23].
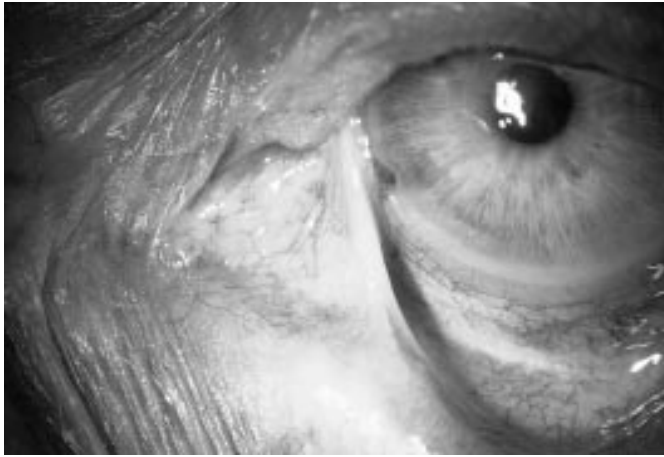
Fig. 3. Chronic ocular disease in cicatricial pemphigoid. Note symblepharon formation and fornix foreshortening.
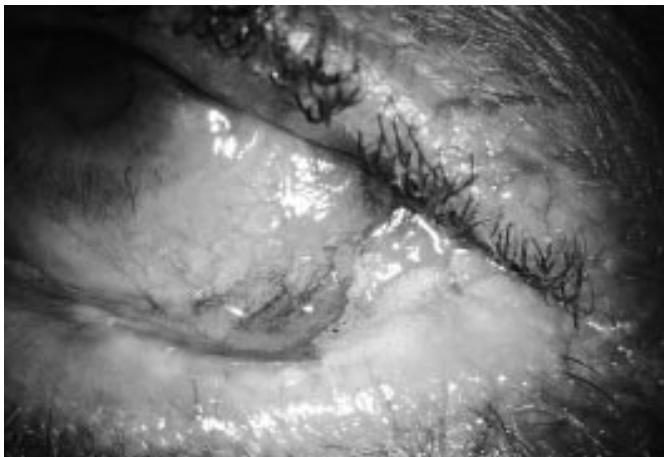
Fig. 4. Ocular cicatricial pemphigoid with severe conjunctival inflammation in a 73-year-old man. Note conjunctival swelling, ulceration and symblepharon formation.

Extra-Ocular Mucous Membrane Lesions

After conjunctival involvement *oral lesions* are the most common manifestation in CP (table 1). They occur in approximately 70% of patients and affect in decreasing frequency the gingiva, buccal mucosa, palate, alveolar ridge, tongue, and lower lip [4, 12]. Depending on the extent of the lesions,
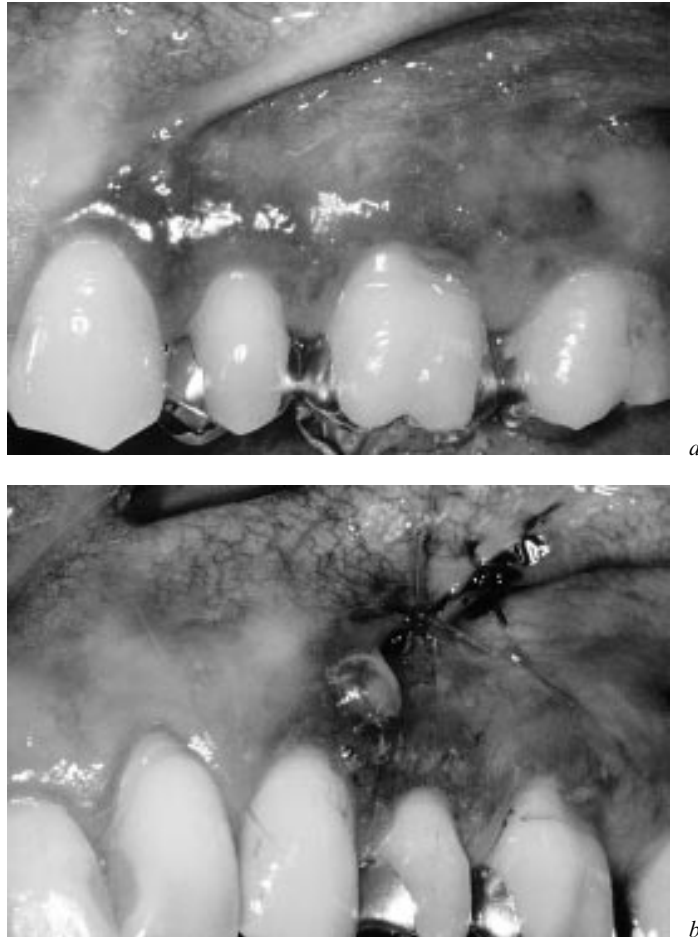
Fig. 5. a Desquamative gingivitis in CP. Note erythema, oedema and desquamation of the mucosa. *b* Same patient as above after mucosal biopsy. The gentle touch by a cotton tip used during the procedure was sufficient to cause the formation of this blister. Courtesy of E. Rateitschak, Institute of Dentistry, University of Basel, Switzerland.

oral CP may cause little discomfort. On examination, two types of lesions may be found: a desquamative gingivitis and vesiculo-bullous eruptions [3, 10]. In mild cases, only erythema and oedema may be seen, whereas in moderate to severe cases, paraesthesia, desquamation of the mucosa, and frank blister formation resulting in erosions or ulcers can be observed [10, 24, 25] (fig. 5a, b). The gingivitis may be diffuse or localised with little tendency of healing, and may persist for years without significant change. Lesions in other sites within

Fig. 6. Erosive lesions affecting the palate and alveolar ridge in a 76-year-old woman who was diagnosed to suffer from CP.

the oral cavity appear as vesicles and bullae that may stay intact for a few days [12, 26]. Their rupture and coalescence result in erosions of the mucous membranes which often heal with scarring [13] (fig. 6). Adhesions may develop between the buccal mucosa and the alveolar process as well as around the uvula and tonsillar fossae. They are very rarely severe enough, however, to interfere with function [13].

Manifestations of CP are less common in the *nose* than in the mouth [13] and usually occur on the septum. Involvement of the nasal mucosa presents with persistent crusting, obstruction, discharge and epistaxis. On examination, erosions (ruptured vesicles) with scarring and stenotic changes may be seen [12, 13].

In the *pharynx* CP may lead to a sore throat and difficulties in swallowing. In severe cases, progressive scar formation occurs that may lead to a stenotic web between the soft palate and the nasopharynx. Surgical intervention is required to maintain a nasal airway in these cases [12, 13].

Laryngo-tracheal involvement is rare but when present can be life-threatening. Symptoms are intermittent hoarseness or dysphonia when the vocal cords are affected. Dyspnoea and sputum production may indicate severe laryngeal involvement with scarring and stenosis. On laryngoscopy an erosion of the epiglottis is the most frequent finding [13] (fig. 7). With severe scarring, tracheostomy may be required [13].

Oesophageal involvement is frequently overlooked, but may be found in up to 27% of patients with CP [9]. Patients sometimes complain of heartburn,
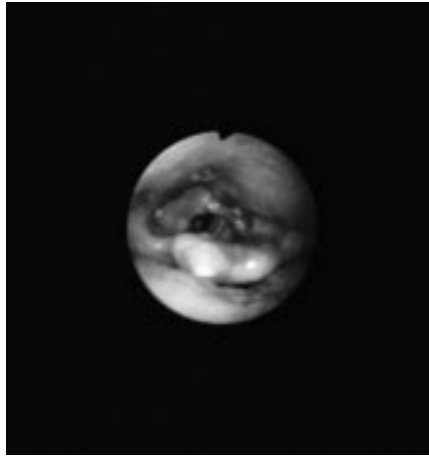
Fig. 7. Laryngoscopy in a CP patient shows a large area of erosions on the epiglottis. Courtesy of J.N. Leonard, Department of Dermatology, St. Mary's Hospital, London, UK.

Fig. 8. A barium swallow demonstrates oesophageal strictures in a 76-year-old woman. The diagnosis of CP had only been established a few weeks before she became unable to eat solid food.

dysphagia, and phagodynia, but usually the physician has to highlight a possible involvement in order to elicit a history of progressive inability to swallow large pieces of food [27]. Ruptured blisters leave erosions that heal with scarring and may lead to stenosis. To confirm oesophageal involvement a barium swallow (fig. 8) and endoscopy may be necessary [12].

Fig. 9. Blisters and erosions on erythematous skin in CP.

Fig. 10. Same patient as above 3 months later. Note the development of scar tissue.

Erosions of *anal and rectal tissue* may cause spasm and pain on defecation with intermittent bleeding. Severe involvement may lead to scarring that results in narrowing of the anal canal and functional impairment [13].

Genito-urinary lesions cause pain on urination and hamper sexual function. With severe scar formation urethral or vaginal stenosis may occur [13].

Fig. 11. Atrophic scar of a scalp lesion in CP.

Skin Lesions

Involvement of the skin is less frequent than mucous membrane disease and is reported in 5–34% of patients [2, 3, 9, 12, 13, 16, 17]. When skin lesions arise in CP they appear as tense vesicles and bullae on normal or erythematous skin, indistinguishable from those observed in bullous pemphigoid. Approximately one third are reported to heal subsequently with scarring [28]. In contrast to CP, scarring is never seen in bullous pemphigoid.

Clinical Subtypes of Cicatricial Pemphigoid

Clinically CP may be divided into four types: (1) Most patients have predominant mucous membrane involvement with few skin lesions (fig. 9, 10). The auto-antigens described are heterogeneous. (2) Pure ocular CP may be regarded as a distinct subgroup. These patients have neither lesions of other mucous membranes nor skin lesions; there may be a distinct target antigen involved [29, 30]. (3) Generalised skin lesions are described in disseminated CP. This is a rare disease and not clearly classified; there may be some 'hidden' cases of epidermolysis bullosa acquisita [31–37]. (4) A fourth distinct clinical variant is the localised CP of the Brunsting-Perry type. Chronic recurrent vesiculo-bullous eruptions are localised on the head and neck leaving behind atrophic scars (fig. 11). The target antigen was determined in one case and
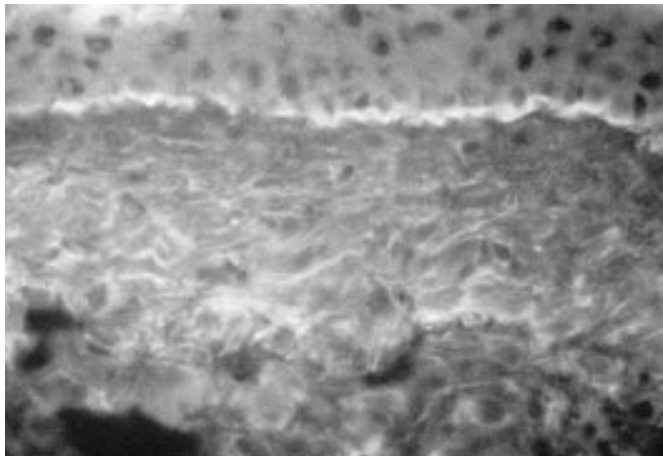
Fig. 12. Chronic CP: direct immunofluorescence photograph of a conjunctival biopsy specimen. The snap-frozen tissue was cryostat-sectioned and stained with fluorescein-labelled anti-human IgG. Note the line of homogeneous fluorescence of the epithelial BMZ demonstrating the presence of IgG deposits in this region. Original magnification × 250.

identified as the 180-kD bullous pemphigoid antigen; immuno-electron-microscopic studies localised the autoantibody binding to the anchoring filament region [37–39].

Pathogenesis and Classification

The auto-immune character of subepidermal bullous diseases has been suggested by circumstantial evidence [2, 14, 15, 40, 41–47] and is further supported by the recent introduction of animal models for CP, bullous pemphigoid and epidermolysis bullosa acquisita [48–50]. Based on these findings, it is believed that the following sequence of events leads to the mucous membrane and skin lesions in CP: Circulating antibodies bind to the antigen of the basement membrane zone (BMZ), which may be demonstrated as linear deposits at the BMZ by direct and indirect immunofluorescence (fig. 12). The complement system gets activated, and complement components are deposited predominantly at the lamina lucida. Chemo-attractant properties of these components give rise to an inflammatory reaction in the subepithelial tissue with invasion of polymorphonuclear leucocytes, macrophages, lymphocytes, plasma cells and mast cells [2, 27, 47, 51–53]. The digestion of the lamina lucida by hydrolytic enzymes may lead to subepithelial blister formation.

Subsequent activation and hyperproliferation of fibroblasts may result in the formation of scar tissue [54].

The antigens for antibody binding in CP have not been fully clarified yet. Traditionally sera of patients with this disorder have a low prevalence of circulating anti-BMZ antibodies when routine diagnostic techniques are used [42, 30, 56, 57]. There is growing evidence, however, that anti-BMZ auto-antibodies play the essential role in CP [29, 30, 57, 58]. Several potential target antigens have recently been described by immunoblotting and immunoprecipitation (see also 'Immunological investigations', pp. 102–110). (1) IgG-auto-antibodies to the alpha-subunit of laminin 5 are found in high titres in a distinct group of CP [58, 59]. Laminin 5, previously called epiligrin, kalinin, nicein, or BM600, is a large glycoprotein of about 600 kD associated with anchoring filaments in the human epidermal BMZ. Auto-antibodies immunoblot the alpha-3-subunit under reduced conditions as a keratinocyte-associated precusor of 200 kD or the processed molecule of 165 kD in human keratinocyte medium and are detected at the dermal side of salt split skin by indirect immunofluorescence. (2) The 180-kD bullous pemphigoid antigen (BPAg2), collagen XVII, is detected by IgG and IgA auto-antibodies in a second group of CP patients [57, 60, 61]. This antigen is associated with hemidesmosomes of basal keratinocytes. In CP the auto-antibodies are localised to the extracellular part of the BPAg2, indistinguishable from the localisation of antibodies to laminin 5 by immuno-electron microscopy. This is in contrast to bullous pemphigoid with auto-antibody binding close to the basal keratinocyte membrane. On salt split skin, these auto-antibodies bind to the epidermal side as shown by indirect immunofluorescence. (3) A 168-kD antigen in extracts of buccal mucosa is described in a third group of CP patients [62]. This antigen is distinct from the alpha-subunit of laminin 5 and the BPAg2. The IgG auto-antibodies bind to the epidermal side of salt split skin. (4) In pure ocular CP IgA auto-antibodies to a 45-kD antigen are described. These IgA antibodies bind to the epidermal side of EDTA-separated skin [29]. There are sporadic reports of several other antigens in CP such as the 230-kD BP antigen (BPAg1) and antigens of 90, 120, 130, 140 and 205 kD [57, 63]. This heterogeneity of target antigens may be due to a complex functional structure of multiple polypeptides at the BMZ, targeted by distinct auto-antibodies in subgroups of CP leading to the same clinical picture.

Until recently, the classification of immune-mediated subepidermal/subepithelial bullous disorders was largely based on clinical and immunofluorescence patterns [1] (table 2). With the introduction of newer techniques, such as immunoblotting, immunoprecipitation and immuno-electron microscopy, some immune-mediated subepidermal/subepithelial bullous disorders could be further characterised. New efforts in disease characterisation take into

account the clinical aspect as well as the target antigen. As mentioned above, identical clinical pictures may be associated with auto-antibody binding to different target antigens. This phenomenon may be explained by auto-antibody binding to several different but structurally related polypeptides that represent a functional unit of molecules. Disturbance of this unit at different but closely related epitopes may lead to the same clinical aspect. With this background, several diseases may be classified and regarded as distinct clinicopathological entities: in bullous pemphigoid, the hemidesmosomal polypeptides of 230 and 180 kD are found inside or close to the external membrane of the basal keratinocyte. In epidermolysis bullosa acquisita, a 290-kD polypeptide, collagen VII, the major component of anchoring fibrils of the BMZ is the only recognised antigen yet [64]. In linear IgA disease, the target antigens are heterogeneous, and considerable overlap of target antigens of other subepidermal bullous diseases is suspected. Besides a 97-kD antigen [65], a 285-kD antigen [66] and a 125-kD antigen [67], the epidermolysis bullosa acquisita (EBA) antigen [68] and the 180-kD BPAg [69] are described. In this context the auto-antigens in CP seem to be heterogeneous, however, they are in part distinct, compared to the auto-antigens in other subepidermal bullous diseases.

Histopathology

Conventional histology of mucous membrane and skin biopsies are not diagnostic for CP [70]. Similar findings as in CP were reported in bullous pemphigoid, herpes gestationis, linear IgA dermatosis/chronic bullous disease of childhood and epidermolysis bullosa acquisita [71]. Histologic findings in CP vary, depending on the tissue biopsied and on the timing of the biopsy in relation to disease duration. Early skin biopsies may demonstrate vacuolisation beneath basal epithelial cells with minimal unspecific inflammatory cell infiltration. In later stages, formation of subepidermal bullae in the absence of acantholysis is seen [1, 71]. Biopsies from extraocular mucosal lesions may demonstrate separation of epithelium from underlying connective tissue at the level of the basement membrane and an unspecific inflammatory infiltrate of eosinophils or neutrophils. The biopsy findings in the conjunctiva are described in 'The Cellular Response in the Conjunctiva' (pp. 149–158).

Immunopathology

Immunopathological methods with analysis of biopsy specimens are the only diagnostic techniques currently available for the evaluation of patients with suspected CP. These immunological investigations are described in detail

Table 2. Immune-mediated bullous dermatoses [70, 71; modified]

Disorder	Incidence of clinical features			Laboratory features
	skin	mucosal lesions	conjunctival disease	direct immunofluorescence
Intra-epidermal				
Pemphigus	100%[1]	oral lesions very common	uncommon	intercellular IgG
Subepidermal/subepithelial				
Bullous pemphigoid	100%	oral lesions in up to 58%	uncommon	IgG, C3, linear pattern at BMZ
Herpes gestationis	100%	oral lesions relatively rare	not described	IgG, C3, linear pattern at BMZ
Cicatricial pemphigoid	5–34%	80–90%	chronic progressive cicatrisation common	immunoglobulins, complement, linear pattern at BMZ
Epidermolysis bullosa acquisita	100%	variable	occasionally	
Linear IgA disease and chronic bullous disease of childhood	100%	common	cicatrising conjunctivitis in <50%	IgA in linear pattern at BMZ
Dermatitis herpetiformis	100%	uncommon	uncommon	Dermal papillary granular IgA

Ab=Antibody; Ag=antigen.
[1] The initial disease, particularly in pemphigus vulgaris, often presents with oral lesions only.

elsewhere (pp. 102–110). The finding of *linear* deposits of immunoglobulins and/or complement at the conjunctival basement membrane by direct immuno-fluorescence or the immune peroxidase technique is generally regarded as diagnostic of CP [40, 42, 44] (fig. 12). This finding is characteristic but non-specific. The findings are identical in other bullous disorders (table 2), in lupus erythematosus and occasionally in the conjunctivae of patients with progressive drug-induced conjunctival cicatrisation [45, 72, 73] (table 2).

serum by indirect immunofluorescence	indirect immunofluorescence on salt-split skin	antibody binding
IgG anti-intercellular Ab	negative	desmosomal Ag
IgG anti-BMZ Ab	epidermal	230-kD (BPAg1) and 180-kD (BPAg2) component of hemidesmosomes
'HG factor' (complement-fixing IgG anti-BMZ Ab)	epidermal	180-kD BPAg2
routine testing rarely shows anti-BMZ Ab	epidermal and dermal (both sides?)	180-kD BPAg2 (and 230-kD BPAg1); 168-kD epidermal Ag; 45-kD Ag; laminin 5 (dermal); 90-kD, 130-kD, 140-kD, 205-kD epidermal components
IgG-anti BMZ Ab	dermal	290-kD major component and 145-kD collagenase-resistant fragment of type VII collagen
IgA anti-BMZ Ab (minority of patients and low titer)	epidermal, dermal?, both sides?	97-kD, 120-kD, 180-kD, 285-kD, 290-kD proteins
IgA anti-endomysial Ab, IgA antigliadin Ab	negative	

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Diagnosis

The diagnosis of CP is made on clinical grounds and supported by the immunopathological findings of a biopsy. When a patient presents with cicatrising conjunctivitis or a chronic recurrent blistering or erosive process of the extra-ocular mucous membranes, the diagnosis of CP should be suspected, especially if scarring is present. Biopsy specimens (conjunctiva, extra-ocular

mucous membranes, skin) should be obtained to confirm the clinical diagnosis. This is particularly important with regard to systemic immunosuppressive therapy.

Differential Diagnosis

The differential diagnosis of cicatrising conjunctivitis is discussed in 'Introduction to Cicatrising Conjunctivitis' (pp. 1–10) and is summarised in table 1 of that chapter (p. 7). The chronic progressive course of conjunctival scarring is a feature of CP, linear IgA disease and epidermolysis bullosa acquisita but not of the other bullous disorders. As in CP, extra-ocular mucosal lesions are common in bullous pemphigoid, linear IgA disease and epidermolysis bullosa acquisita. In contrast to bullous pemphigoid and linear IgA disease, mucosal and skin lesions tend to be more scarring in CP.

The demonstration of linear immune deposits at the BMZ by direct immunofluorescence confirms the diagnosis of CP, bullous pemphigoid, linear IgA disease and epidermolysis bullosa acquisita (table 2). CP, bullous pemphigoid and epidermolysis bullosa acquisita cannot be distinguished by direct immunofluorescence since in all three conditions deposits of IgG and C3 and occasionally other immunoglobulins are detected. If IgA deposits predominate or only IgA is seen, the diagnosis of linear IgA disease should be suspected [71]. Circulating auto-antibodies may be detected by indirect immunofluorescence in all these conditions. However, in bullous pemphigoid they are observed with higher frequency and at higher titres than in CP and linear IgA disease. Perilesional split skin or indirect immunofluorescence on salt split skin may help to differentiate CP from epidermolysis bullosa acquisita in most cases: if the immune deposits are limited to the floor of the induced cleavage, the diagnosis is most likely epidermolysis bullosa acquisita; whereas deposits on the roof or both the roof and floor of the cleavage are in favour of CP [70, 71]. To further distinguish these conditions, immunoblotting, immunoprecipitation or immuno-electron microscopy studies are required.

References

1 Pye RJ: Bullous eruptions; in Rook A, Wilkinson DS, Ebling FJG: Textbook of Dermatology edited by Champion RH, Burton JL, Ebling FJG: Oxford, Blackwell Scientific Publications, 1992, vol 3, pp 1623–1673.
2 Foster CS: Cicatricial pemphigoid. Trans An Ophthalmol Soc 1986;84:527–663.
3 Mondino BJ, Brown SI: Ocular cicatricial pemphigoid. Ophthalmology 1981;88:95–100.
4 Ahmed AR, Kurgis BS, Rogers RS III: Cicatricial pemphigoid. J Am Acad Dermatol 1991;24: 987–1001.

5   Tauber J, Foster CS: Cicatricial pemphigoid; in Mannis MJ, Macsai MS, Huntley AC: Eye and Skin Disease. Philadelphia, Lippincott-Raven, 1996, pp 261–271.

6   Iglesias LS, Quismorio FP, McDonnell PJ: Ocular cicatricial pemphigoid in a twelve-year-old boy. Cornea 1992;11:365–367.

7   Rosenbaum MM, Esterly NB, Greenwald MJ, Gerson CR: Cicatricial pemphigoid in a 6-year-old child: Report of a case and review of the literature. Pediatr Dermatol 1984;2:13–22.

8   Jolliffe DS, Sim-Davis D: Cicatricial pemphigoid in a yound girl: Report of a case. Clin Exp Dermatol 1977;2:281–284.

9   Elder MJ, Bernauer W, Leonard J, Dart JKG: Progression of disease in ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:292–296.

10 Shklar G, McCarthy PL: Oral lesions of mucous membrane pemphigoid: A study of 85 cases. Arch Otolaryngol 1971;93:354–364.

11 Laskaris G, Sklvounou A, Stratigos A: Bullous pemphigoid, cicatricial pemphigoid and pemphigus vulgaris: A comparative survey in 278 cases. Oral Surg 1982,54:656–662.

12 Hanson RD, Olsen KD, Rogers RS: Upper aerodigestive tract manifestations of cicatricial pemphigoid. Ann Otol Rhinol Laryngol 1988;97:493–499.

13 Hardy KM, Perry HO, Pingree GC, Kirby TJ: Benign mucous membrane pemphigoid. Arch Dermatol 1971;104:467–475.

14 Griffiths M, Fukuyama K, Tuffanelli D, Silverman S: Immunofluorescent studies in mucous membrane pemphigoid. Arch Dermatol 1974;109:195–199.

15 Bean SF: Cicatricial pemphigoid – Immunofluorescent studies. Arch Dermatol 1974;110:552–555.

16 Person JR, Rogers RS: Bullous pemphigoid responding to sulfapyridine and the sulfones. Arch Dermatol 1977;113:610–615.

17 Leonard JN, Wright P, Williams DM, Gilkes JJH, Haffenden GP, McMinn RMH, Fry L: The relationship between linear IgA disease and benign mucous membrane pemphigoid. Br J Dermatol 1984;110:307–314.

18 Mondino BJ, Linstone FA: Ocular pemphigoid. Clin Dermatol 1987;5:28–35.

19 Wright P: Cicatrizing conjunctivitis. Trans Ophthalmol Soc UK 1986;105:1–17.

20 Duke-Elder S, MacFaul PA: System of Ophthalmology. London, Henry Kimpton, 1965, vol 8, pp 496–527.

21 Wright P: Enigma of ocular cicatricial pemphigoid. A comparative study of clinical and immunological findings. Trans Ophthalmol Soc UK 1979;99:141–145.

22 Wright P: External diseases; in Miller St (ed): Clinical Ophthalmology. Bristol, Wright 1987, pp 107–128.

23 Mondino BJ, Brown SI, Lempert S, Jenkins MS: The acute manifestations of cicatricial pemphigoid: Diagnosis and treatment. Ophthalmology 1979;86:543–552.

24 Nisengard RJ, Neiders M: Desquamative lesions of the gingiva. J Periodontol 1981;52:500–510.

25 Rogers RS, Sheridan PJ, Nightingale SH: Desquamative gingivitis: Clinical, histopathological, immunopathologic and therapeutic observations. J Am Acad Dermatol 1982;7:729–735.

26 Foster ME, Nally FF: Benign mucous membrane pemphigoid (cicatricial mucosal pemphigoid): A reconsideration. Oral Surg 1977;44:697–705.

27 Mutasim DF, Pelc NJ, Anhalt GJ: Cicatricial pemphigoid. Dermatol Clin 1993;11:499–510.

28 Lever WF: Pemphigus conjunctivae with scarring of the skin. Arch Dermatol 1944;49:113–117.

29 Smith EP, Taylor TB, Meyer LJ, Zone JJ: Identification of a basement membrane zone antigen reactive with circulating IgA antibody in ocular cicatricial pemphigoid. J Invest Dermatol 1993;101:619–623.

30 Chan LS, Yancey KB, Hammerberg C, Soong HK, Regezi JA, Johnson K, Cooper KD: Immune-mediated subepithelial blistering diseases of mucous membranes. Pure ocular cicatricial pemphigoid is a unique clinical and immunopathological entity distinct from bullous pemphigoid and other subsets identified by antigenic specificity of autoantibodies. Arch Dermatol 1993;129:448–455.

31 Church RE, Sneddon IB: Ocular pemphigus with generalised bullous eruption. Br J Dermatol 1956; 68:128–131.

32 Behlen CH, Mackey DM: Benign mucous membrane pemphigus with a generalized eruption. Arch Dermatol 1965;92:566–567.

33 Battyani Z, Magyarlaki M, Zombai E, Schneider I: Disseminated cicatricial pemphigoid. Hautarzt 1991;42:307–310.

34 Hausser I, Fartasch M, Schleiermacher E, Anton-Lamprecht I: Disseminated cicatricial pemphigoid in a child and in an adult. Ultrastructural diagnostic criteria and differential diagnosis with special reference to acquired epidermolysis bullosa. Arch Dermatol Res 1987;279:357–365.

35 Braun-Falco O, Wolff HH, Ponce E: Disseminiertes vernarbendes Pemphigoid. Hautarzt 1981;32: 233–239.

36 Provost TT, Maize JC, Ahmed AR, Strauss JS, Dobson RL: Unusual subepidermal bullous diseases with immunologic features of bullous pemphigoid. Arch Dermatol 1979;115:156–160.

37 Kurzhals G, Stolz W, Maciejewski W, Karpati S, Meurer M, Breit R: Localized cicatricial pemphigoid of the Brunsting-Perry type with transition into disseminated cicatricial pemphigoid. Report of a case proved by preembedding immunogold electron microscopy. Arch Dermatol 1995;13:580–585.

38 Brunsting LA, Perry HO: Benign pemphigoid? A report of seven cases with chronic, scarring, herpetiform plaques about the head and neck. Arch Dermatol 1957;75:489–501.

39 Bedane C, Prost C, Bernard P, Catanzano G, Bonnetblanc JM, Dubertret L: Cicatricial pemphigoid antigen differs from bullous pemphigoid antigen by its exclusive extracellular localization; a study by indirect immunoelectron microscopy. J Invest Dermatol 1991;97:3–9.

40 Bean SF, Waisman M, Michel B, Thomas C, Knox JM, Levine M: Cicatricial pemphigoid. Immuno-fluorescent studies. Arch Dermatol 1972;106:195–199.

41 Furey N, West C, Andrews T, Paul PD, Bean SF: Immunofluorescent studies of ocular cicatricial pemphigoid. Am J Ophthalmol 1975;80:825–831.

42 Mondino BJ, Ross AN, Rabin BS, Brown SI: Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 1977;83:443–450.

43 Mondino BJ, Brown SI, Rabin BS: Autoimmune phenomena of the external eye. Ophthalmology 1978;85:801–817.

44 Leonard JN, Hobday CM, Haffenden GP, Griffiths CEM, Powles AV, Wright P, Fry L: Immunfluo-rescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.

45 Frith PA, Venning VA, Wojnarowska F, Millard PR, Bron AJ: Conjunctival involvement in cicatricial and bullous pemphigoid: A clinical and immunopathological study. Br J Ophthalmol 1989;73:52–56.

46 Roat MI, Alstadt S, Carpenter AB, Sundar Raj N, Thoft A: Antibasement membrane antibody-mediated experimental conjunctivitis. Invest Ophthalmol Vis Sci 1990;31:168–175.

47 Bernauer W, Wright P, Dart JK, Leonard J, Lightman S: The conjunctiva in acute and chronic mucous membrane pemphigoid. An immunohistochemical analysis. Ophthalmology 1993;100:339–346.

48 Lazarova Z, Yee C, Darling T, Briggaman RA, Yancey KB: Passive transfer of anti-laminin 5 antibodies induces subepidermal blisters in neonatal mice. J Clin Invest 1996;98:1509–1518.

49 Liu Z, Diaz LA, Troy JL, Taylor AF, Emery DJ, Fairley JA, Guidice GJ: A passive transfer model of the organ-specific autoimmune disease, bullous pemphigoid, using antibodies generated against the hemidesmosomal antigen, BP180. J Clin Invest 1993;92:2480–2488.

50 Borradori L, Caldwell JB, Briggaman RA, Burr CE, Gammon WR, James WD, Yancey KB: Passive transfer of autoantibodies from a patient with mutilating epidermolysis bullosa acquisita induces specific alterations in the skin of neonatal mice. Arch Dermatol 1995;11:590–595.

51 Andersen SR, Jensen OA, Kristensen EB, Norn MS: Benign mucous membrane pemphigoid. III. Biopsy. Acta Ophthalmol 1974;52:455–563.

52 Sacks EH, Jakobiec FA, Wieczorek R, Donnenfeld E, Perry H, Knowles DM: Immunophenotypic analysis of the inflammatory infiltrate in ocular cicatricial pemphigoid. Further evidence for a T cell-mediated disease. Ophthalmology 1989;96:236–243.

53 Rice BA, Foster CS: Immunopathology of cicatricial pemphigoid affecting the conjunctiva. Ophthal-mology 1990;97:1476–1483.

54 Roat MI, Sossi G, Lo CY, Thoft RA: Hyperproliferation of conjunctival fibroblasts from patients with cicatricial pemphigoid. Arch Ophthalmol 1989;107:1064–1067.

55 Fine JD, Neises GR, Katz SI: Immunofluorescence and immunoelectron microscopic studies in cicatricial pemphigoid. J Invest Dermatol 1984;82:39–43.

56 Bernard P, Prost C, Lercerf V, Intrator L, Combemale P, Bedane C, Roujeau JC, Revuz J, Bonnetblanc JM, Dubertret L: Studies of cicatricial pemphigoid auto-antibodies using direct immunoelectron microscopy and immunoblot analysis. J Invest Dermatol 1990;94:630–635.

57 Chan LS, Hammerberg C, Cooper KD: Cicatricial pemphigoid. Identification of two distinct sets of epidermal antigens by IgA and IgG class circulating autoantibodies. Arch Dermatol 1990;126: 1466–1468.

58 Domloge-Hultsch N, Anhalt GJ, Gammon WR, Lazarova Z, Briggaman R, Welch M, Jabs DA, Huff C, Yancey KB: Antiepiligrin cicatricial pemphigoid. A subepithelial bullous disorder. Arch Dermatol 1994;130:1521–1529.

59 Kirtschig G, Marinkovich PM, Burgeson RE, Yancey KB: Anti-basement membrane autoantibodies in patients with anti-epiligrin cicatricial pemphigoid bind the alpha subunit of laminin 5. J Invest Dermatol 1995;105:543–548.

60 Bernard P, Prost C, Durepaire N, Basset-Seguin N, Didierjean L, Saurat JH: The major cicatricial pemphigoid is a 180-kD protein that shows immunologic cross-reactivities with the bullous pemphigoid antigen. J Invest Dermatol 1992;99:174–179.

61 Balding SD, Prost C, Diaz LA, Bernard P, Bedane C, Aberdam D, Giudice GJ: Cicatricial pemphigoid autoantibodies react with multiple sites on the BP 180 extracellular domain. J Invest Dermatol 1996;106:141–146.

62 Ghohestani RF, Rousell P, Nicolas JF, Sassolas B, Faure M, Claudy AI: Heterogeneity of the target antigens in the cicatricial pemphigoid. J Invest Dermatol 1996;106:278.

63 Tyagi S, Bhol K, Natarajan K, Livir-Ralatos C, Foster CS, Ahmed AR: Ocular cicatricial pemphigoid antigen: Partial sequence and biochemical characterization. Proc Natl Acad Sci USA, 1996;93: 14714–14719.

64 Woodley DT, Briggaman RA, O'Keffe EJ, Inman AO, Queen LL, Gammon WR: Identification of the skin basement membrane autoantigen in epidermolysis bullosa acquisita. N Engl J Med 1984; 310:1007–1113.

65 Zone JJ, Taylor TB, Kadunce DP, Meyer LJ: Identification of the cutaneous basement membrane zone antigen and isolation of antibody in linear immunoglobulin A bullous dermatosis. J Clin Invest 1990;85:812–820.

66 Wojnarowska F, Whitehead P, Leigh IM, Bhogal BS, Black MM: Identification of the target antigen in chronic bullous disease of childhood and linear IgA disease of adults. Br J Dermatol 1991;124: 157–162.

67 Marinkovich MP, Taylor TB, Keene DR, Burgeson RE, Zone JJ: LAD-1, the linear IgA bullous dermatosis autoantigen, is a novel 120-kDa anchoring filament protein synthesized by epidermal cells. J Invest Dermatol 1996;106:734–738.

68 Zambruno G, Manca V, Kanitakis J, Cozzani E, Nicolas JF, Gianetti A: Linear IgA bullous dermatosis with autoantibodies to a 290 kD antigen of anchoring fibrils. J Am Acad Dermatol 1994;31:884–888.

69 Pas HH, Kloosterhuis GJ, Heeres K, van der Mer JB, Jonkman MF: Bullous pemphigoid and linear IgA dermatosis sera recognize a similar 120-kDa keratinocyte collagenous glycoprotein with antigenic cross-reactivity to BP180. J Invest Dermatol 1997;108:423–429.

70 Marren P, Wojnarowska F: The diagnosis of immuno-bullous diseases. J Eur Acad Dermatol Venereol 1992;1:255–264.

71 Helm KF, Peters MS: Immunodermatology update: The immunologically mediated vesiculobullous diseases. Mayo Clin Proc 1991;66:187–202.

72 Leonard JN, Haffenden GP, Ring NP, McMinn RMH, Sidgwick A, Mowbray JF, Unsworth DJ, Holborow EJ, Blenkinsopp WK, Swain AF, Fry L: Linear IgA disease in adults. Br J Dermatol 1982;107:301–316.

73 Pouliquen Y, Patey A, Foster CS, Goichot L, Savodelli M: Drug-induced cicatricial pemphigoid affecting the conjunctiva. Light and electron microscopic features. Ophthalmology 1986;93:775–783.

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Linear IgA Disease

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Definition

Linear IgA disease is a chronic acquired subepidermal disease of children and adults characterised by IgA auto-antibodies to the basement membrane zone. The disease manifests cutaneous and mucosal involvement, is usually dapsone or sulphonamide responsive, and has a tendency to remit.

Two main clinical syndromes are distinguished: chronic bullous disease of childhood commencing in childhood and adult linear IgA disease commencing after puberty. Although they differ in age of presentation and clinical signs, there is much overlap, and the immunopathology and immunogenetics are common to both diseases.

Epidemiology

Linear IgA disease affects all ages, from babies of a few months to the elderly. The majority of children present as toddlers or pre-school children. Teenagers and young adults also present with the disease and there is a second peak over 60 years [1]. Linear IgA disease is one of the rarer subepidermal blistering diseases in Western Europe with an incidence of less than 0.5 per million in Western Europe, about half that of cicatricial pemphigoid [2, 3]. The sex incidence is about equal.

Clinical Features

The cutaneous clinical features of the children and adults will be described separately as they are distinctive.

Chronic Bullous Disease of Childhood

The mean age of onset is before 5 years. The onset is abrupt with symptoms ranging from absent or mild pruritus to severe burning. The face and perineum are frequently involved in younger children. The peri-oral area, the eyelids (fig. 1), ears, and scalp may be affected. The involvement of the perineum and vulva may give rise to suspicion of sexual abuse in some patients. The eruption may spread to the trunk, thighs, limbs and hands and feet.

Linear IgA Disease of Adults

This commences at any age from teenagers to the very old, most commonly after 60. The onset may be insidious or more usually abrupt. Symptoms vary from mild to severe pruritus and burning. The trunk is almost always involved, and the limbs, the face and scalp, hands and feet, are commonly affected. The lesions comprise urticated plaques, papules and vesicles, and blisters.

Mucosal Involvement

Mucosal involvement occurs in the majority of children and adults. The mouth may be involved with ulcers and erosions, which can be persistent and scar [1, 4]. Hoarseness is a manifestation of pharyngeal involvement and there is often nasal stuffiness, crusting and bleeding [1, 5]. Involvement of the genitals including the vagina can occur [1, 6]. Some patients are difficult to differentiate from cicatricial pemphigoid.

Ocular Lesions

Some patients develop recurrent acute or persistent conjunctivitis indistinguishable from that found in patients with cicatricial pemphigoid, though only a minority of patients with linear IgA disease progress to scarring [5, 7–9]. Loss of vision is very rare, though blindness is reported as young as 12 years [5]. As in cicatricial pemphigoid, conjunctival involvement typically occurs in both eyes, it may, however, be highly asymmetrical. Linear IgA disease should be suspected if conjunctival involvement is found in younger patients, though true cases of childhood cicatricial pemphigoid have been described [7, 10].

Symptoms vary from patient to patient. The conjunctivae may be itchy, sore, red and oedematous and there may be discharge. The eye may feel watery or dry. Not infrequently, patients report frank blisters on the lid margins or, rarely, on the tarsal conjunctiva. Few patients develop ocular scarring without ever complaining of symptoms.

Early tarsal scarring is sometimes linear or appears as a 'feltwork'. Scarring may be found also at the canthi with loss of the normal folded pattern of the plica. Shrinkage of the conjunctival fornices may develop, first as a blunting of the usual angle of reflection from globe to tarsus and later as

Fig. 1. A child with cutaneous involvement of the eyelids; she also has entropion. Reproduced with permission from Wojnarowska et al. [5].
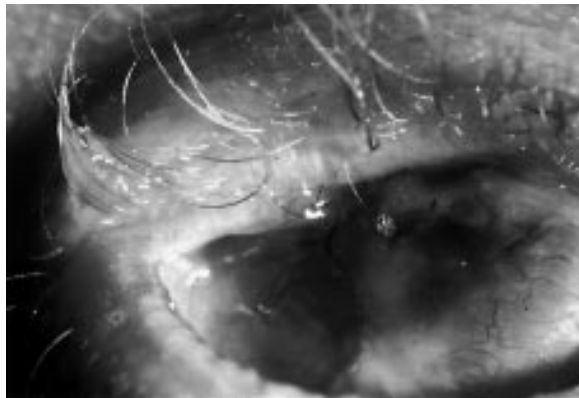

Fig. 2. End stage disease in linear IgA disease. Reproduced with permission from Wojnarowska et al. [5].

frank adhesions or symblepharon between the tarsal and bulbar conjunctivae. These appear especially in the inferior fornices if the lower lid is pulled away from the globe, forming a tenting of the tethered conjunctiva. Rarely, in advanced disease, scarring of the lid margins may cause trichiasis and the final stages of progressive corneal vascularisation and opacification (fig. 2) [5, 11]. Loss of the goblet cell function and dryness may contribute to symptoms. Conjunctival changes may be staged according to type or degree in a similar manner to cicatricial pemphigoid [12, 13].

Findings on direct and indirect immunofluorescence have been variably reported, as either linear IgA and/or IgG may be found in conjunctivae [9, 14, 15]. This investigation alone therefore cannot be relied upon to establish a diagnosis.

Ocular symptoms and signs may respond to systemic therapy, similar to cicatricial pemphigoid, as discussed below. Topical corticosteroids may be helpful in controlling the symptoms associated with acute episodes of conjunctivitis.

Clinical Associations

A number of precipitating factors are observed, in about a quarter of adult and a higher proportion of children: namely infection, antibiotics (often penicillins) [1, 16] and the presence of building work [17]. Drug-induced linear IgA disease does exist, vancomycin being the drug most frequently implicated and diclofenac less commonly [18–20].

Patients with linear IgA disease were originally grouped with dermatitis herpetiformis, but it is now clear that these are distinct diseases and there is no association of linear IgA disease with gluten-sensitive enteropathy [1, 21]. There is no overall association with clinical auto-immune disease, although auto-antibodies are common [1].

There is an increased incidence of lymphoproliferative disorders in adults with linear IgA disease which may occur after remission of the skin disease [16, 22, 23]. There are a few case reports of various malignancies in association with linear IgA disease [16, 24].

Methods of Diagnosis

Immunofluorescence
Direct Immunofluorescence. This can be from clinically uninvolved skin, and the back is often convenient (and out of sight) in a child. The forearm is the least satisfactory site [25]. Mucosal biopsies from the mouth and the conjunctiva may be also positive but may not be justified [14].

In all cases there is linear deposition of IgA along the basement membrane zone. There may also be other immunoreactants, IgG, IgM or C3, on direct immunofluorescence [1, 21].

Indirect Immunofluorescence. Indirect immunofluorescence for IgA basement membrane zone antibodies is more often positive in the serum from children (about 80%) than adults (about 30%) [1]. Blister fluid is an alternative
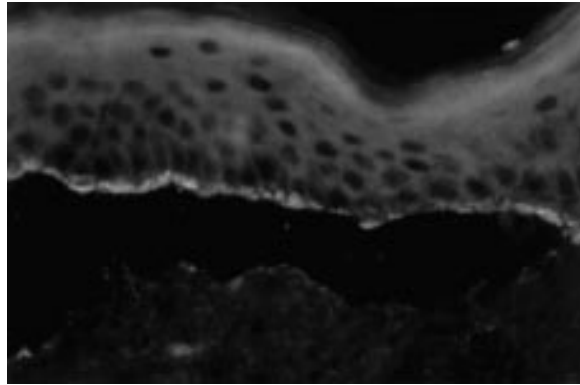
Fig. 3. Indirect immunofluorescence: epidermal binding of IgA auto-antibodies to salt split skin.
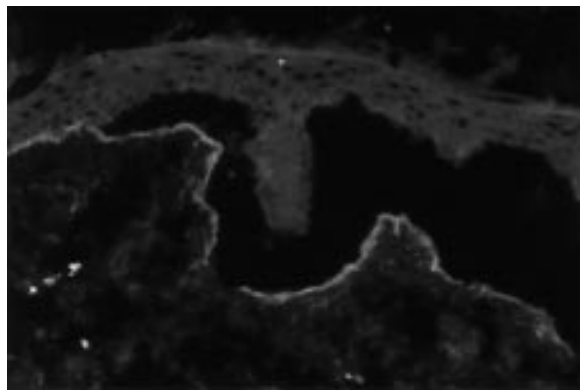

Fig. 4. Indirect immunofluorescence: dermal binding of IgA auto-antibodies to salt split skin.

to serum for indirect immunofluorescence and may be easier to obtain in a child. The titres are usually low, 1:5 or 1:10 but may be higher. The use of normal human skin split through the lamina lucida with 1 *M* salt increases the sensitivity and gives additional information about the site of the target antigen. The majority of sera demonstrate a binding to the epidermis (fig. 3), implying an antigen associated with hemidesmosomes or the upper lamina lucida, a few have a combined pattern, and a larger minority bind to the dermal aspect of the artificial blister (fig. 4), suggesting a lower lamina lucida or dermal antigen [26–28]. The sensitivity of indirect immunofluorescence can be increased by the use of normal conjunctiva as substrate as well as normal skin [14].

The presence of circulating antibodies of other isotypes, such as IgG, makes the diagnosis problematic, as such patients seem to represent an overlap between linear IgA disease and bullous or cicatricial pemphigoid.

Immuno-Electron Microscopy

Immuno-electron microscopic studies have shown the immunoreactants and target antigens in multiple locations within the basement membrane zone, namely with the hemidesmosomes, within the lamina lucida, in the sub-basal lamina zone, or in a mirror image pattern on each side of the lamina densa [29–33].

Immunoblotting

Immunoblotting studies have demonstrated a variety of target antigens, in keeping with the multiple localisations found with split-skin and immuno-electron microscopy studies. The antigens are present in both epidermal and dermal extracts. Two antigens with molecular weights of 285 and 97/120 kD may be unique to linear IgA disease [34–36]. The BP230 and BP180 antigens are shared with bullous and cicatricial pemphigoid. Other target antigens include collagen VII, the anchoring fibril component, and some still uncharacterised antigens [20, 34–40].

Aetiology

There is a strong association between linear IgA disease and the auto-immune haplotype HLA B8, CW7, DR3, and possession of this haplotype is associated with early disease onset [41, 42]. This haplotype predicts that the unusual allele of TNF will be present in these patients which also seems to confer a worse prognosis [43].

There is indirect evidence that the basement membrane zone auto-antibodies are pathogenic, as they cause splitting of skin and binding of neutrophils to the basement membrane zone in culture [44, 45].

Treatment

A few patients have mild disease and can be controlled with topical steroids alone. The treatment of children can be difficult, because side effects limit the dosage of drugs used, but the drugs used are identical in children and adults [46]. Dapsone in doses starting at <0.5 mg/kg, may be slowly increased to a dose of >1 mg/kg usually 100–150 mg in an adult to keep the

patient comfortable and without significant side effects. Side effects include haemolytic anaemia, which does not reach its maximum for a month, and patients at risk of glucose-6-phosphate dehydrogenase deficiency should be screened prior to treatment. Methaemoglobinaemia is common, plateauing at about 2 weeks, and may cause cyanosis, breathlessness, and angina. Early and potentially fatal side-effects include hepatitis, the dapsone syndrome (lymphadenopathy and hepatitis) and agranulocytosis. Motor neuropathy may occur. Most complications occur in the first 3 months.

Sulphapyridine is an alternative, although it is often poorly tolerated and may cause allergic reactions, hepatitis or agranulocytosis. Sulphapyridine, 250 mg to 3 g daily, usually controls the eruption rapidly, but the dose may need frequent adjustment. Sulphamethoxypyridazine (adult dose 250 mg to 1.5 g daily) is an alternative, which is often better tolerated. Dapsone and sulphonamides can be combined. Some patients do not respond to either of these and corticosteroids may need to be added. A few patients are very difficult to control and may need azathioprine or cyclosporin. Success has been reported with tetracyclines and nicotinamide [47]. The cutaneous lesions are always much more responsive than the mucosal lesions which can be treated with topical steroids.

In view of the ultimate spontaneous recovery in the majority of patients and of the rarity of blindness, attempts should be made to avoid over-treatment and in particular in children the production of side-effects with steroids.

Prognosis

Spontaneous remission occurs in the majority of patients after an average of 3–6 years [1, 48]. Initially there were no reports of childhood disease extending beyond puberty, but cases have now been documented [1, 49]. The prognosis in the presence of mucosal scarring may be much poorer [5].

References

1   Wojnarowska F, Marsden R, Bhogal B, Black M: Chronic bullous disease of childhood, childhood cicatricial pemphigoid and linear IgA disease of adults, a comparative study demonstrating clinical and immunopathological overlap. J Am Acad Dermatol 1988;19:792–805.
2   Bernard P, Vaillant L, Labeille B, et al.: Incidence and distribution of subepidermal autoimmune bullous skin diseases in three French regions. Arch Dermatol 1995;131:48–52.
3   Zillikens D, Wever S, Roth A, Weidenthaler-Barth B, Hashimoto T, Bröcker EB: Incidence of autoimmune subepidermal blistering dermatoses in a region of Central Germany. Arch Dermatol 1995;131:957–958.
4   Leonard J, Wright P, Williams D, et al.: The relationship between linear IgA disease and benign mucous membrane pemphigoid. Br J Dermatol 1984;110:307–314.

5 Wojnarowska F, Marsden R, Bhogal B, Black M: Childhood cicatricial pemphigoid with linear IgA deposits. Clin Exp Dermatol 1984;9:407–415.

6 Jacobson M, Krumholz B, Franks A: Desquamative inflammatory vaginitis. A case report. J Reprod Med 1989;34:647–650.

7 Langeland T: Childhood cicatricial pemphigoid with linear IgA deposits: A case report. Acta Derm Venereol (Stockh) 1985;65:354–355.

8 Kumar V, Rogozinski T, Yarborough M: A case of cicatricial pemphigoid or cicatricial linear IgA bullous dermatosis. J Am Acad Dermatol 1980;2:327–331.

9 Webster G, Raber I, Penne R, Jacoby R, Beutner E: Cicatrizing conjunctivitis as a predominant manifestation of linear IgA bullous dermatosis. J Am Acad Dermatol 1994;30(pt 2):355–357.

10 Kirtschig G, Wojnarowska F, Marsden R, Edwards S, Bhogal B, Black M: Acquired bullous diseases of childhood: Re-evaluation of diagnosis by indirect immunofluorescence examination of 1 M NaCl split skin and immunoblotting. Br J Dermatol 1994;130:610–616.

11 Marsden R, Greaves M: Atypical bullous dermatosis of childhood with entropion. J R Soc Med 1982;75:39–41.

12 Foster C, Wilson L, Ekins M: Immunosuppressive therapy for progressive ocular cicatricial pemphigoid. Ophthamology 1982;89:340–353.

13 Mondino B: Cicatricial pemphigoid and erythema multiforme. Ophthamology 1990;939:952.

14 Kelly S, Frith P, Millard P, Wojnarowska F, Black M: A clinico-pathological study of mucosal involvement in linear IgA disease. Br J Dermatol 1988;119:161–170.

15 Leonard J, Hobday C, Haffenden G: Immunofluorescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.

16 Godfrey K, Wojnarowska F, Leonard J: Linear IgA disease of adults: Association with lymphoproliferative malignancy and possible role of other triggering factors. Br J Dermatol 1990;123:447–452.

17 Collier P, Wojnarowska F: Linear IgA disease and chronic bullous disease of childhood. Eur J Dermatol 1993;3:623–634.

18 Collier P, Wojnarowska F: Drug-induced linear immunoglobulin A disease. Clin Dermatol 1993;11:529–533.

19 Kuechle M, Stegemeir E, Maynard B, Gibson L, Lieferman K, Peters M: Drug-induced Linear IgA bullous dermatosis: Report of six cases and review of the literature. J Am Acad Dermatol 1994;30:187–192.

20 Paul C, Wolkenstein P, Prost C: Drug-induced linear IgA disease: Target antigens are heterogeneous. Br J Dermatol 1997;136:406–411.

21 Leonard JN, Haffenden GP, Ring NP: Linear IgA disease in adults. Br J Dermatol 1982;107:301–316.

22 Mobacken H, Kastrup W, Ljunghall K: Linear IgA dermatosis: A study of ten adult patients. Acta Derm Venereol (Stockh) 1983;63:230–239.

23 McEvoy M, Connolly S: Linear IgA dermatosis: Association with malignancy. J Am Acad Dermatol 1990;22:59–63.

24 Rodenas J, Herranz M, Tercedor J, Concha A: Linear IgA disease in a patient with bladder carcinoma. Br J Dermatol 1997;257–259.

25 Collier P, Wojnarowska F: Variation in the deposition of the antibodies at different anatomical sites in linear IgA disease of adults and chronic bullous disease of childhood. Br J Dermatol 1992;127:482–484.

26 Aboobaker J, Wojnarowska F, Bhogal B, Black M, McKee P: The localisation of the binding site of circulating IgA antibodies in linear IgA disease of adults, chronic bullous disease of childhood, and cicatricial pemphigoid. Br J Dermatol 1987;116:293–302.

27 Willsteed E, Bhogal B, Black M, McKee P, Wojnarowska F: Use of 1 M NaCl split skin in the indirect immunoflourescence of the linear IgA bullous dermatoses. J Cutan Pathol 1990;17:144–148.

28 Wojnarowska F, Collier P, Allen J, Millard P: The localisation of the target antigens and antibodies in linear IgA disease is heterogeneous and dependent on the methods used. Br J Dermatol 1995;132:750–757.

29 Bhogal B, Wojnarowska F, Marsden R, Das A, Black M, McKee P: Linear IgA bullous dermatosis of adults and children: An immunoelectronic microscopic study. Br J Dermatol 1987;117:289–296.

3
A

30 Prost C, De Leca A, Combermale P, et al.: Diagnosis of adult linear IgA disease by immunoelectron microscopy in 16 patients with linear IgA deposits. J Invest Dermatol 1989;92:39–45.

31 Karpati S, Stolz W, Meurer M, Kreig T, Braun-Falco O: Ultrastructural immunogold studies in two cases of linear IgA dermatosis. Are there two distinct types of this disease? Br J Dermatol 1992;127:112–118.

32 Haftek M, Zone J, Taylor T, Kowalewski C, Chorzelski T, Schmitt D: Immunogold localization of the 97-kD antigen of linear IgA bullous dermatosis (LABD) detected with patient serum. J Invest Dermatol 1994;103:656–659.

33 Ishiko A, Shimizu H, Masunaga T: 97-kDa Linear IgA bullous dermatosis (LAD) antigen localizes to the lamina lucida of the epidermal basement membrane. J Invest Dermatol 1996;106:739–743.

34 Zone J, Taylor T, Kadunce D, Meyer L: Identification of the cutaneous basement membrane zone antigen and isolation of antibody in linear immunoglobulin A bullous dermatosis. J Clin Invest 1990;85:812–820.

35 Wojnarowska F, Whitehead P, Leigh I, Bhogal B, Black M: Identification of the target antigen in chronic bullous disease of childhood and linear IgA disease of adults. Br J Dermatol 1991;124: 157–162.

36 Marinkovich M, Taylor T, Keene D, Burgeson R, Zone J: LAD-1, the linear IgA bullous dermatosis autoantigen, is a novel 120-kDa anchoring filament protein synthesized by epidermal cells. J Invest Dermatol 1996;106:734–738.

37 Collier P, Wojnarowska F, Allen J, Kirtschig G: Molecular overlap of the IgA target antigens in the subepidermal blistering diseases. Dermatology 1994;189(suppl 1):105–107.

38 Kanitakis J, Mauduit G, Cozzani E, Badinand P, Faure M, Claudy A: Linear IgA bullous dermatosis of childhood with auto-antibodies to a 230-kDa epidermal antigen. Pediatr Dermatol 1994;11: 139–144.

39 Zambruno G, Manca V, Kanitakis J, Cozzani E, Nicholas J-F, Giannetti A: Linear IgA bullous dermatosis with autoantibodies to a 290 kD antigen of anchoring fibrils. J Am Acad Dermatol 1994;31:884–888.

40 Hashimoto T, Ishiko A, Shimizu H: A case of linear IgA bullous dermatosis with IgA anti-type VII collagen autoantibodies. Br J Dermatol 1996;134:336–339.

41 Sachs J, Leonard J, Awad J: A comparative serological and molecular study of linear IgA disease and dermatitis herpetiformis. Br J Dermatol 1988;118:759–764.

42 Collier P, Wojnarowska F: MHC class I and II antigens in linear IgA dermatosis (abstract). J Invest Dermatol 1992;98:526.

43 Collier P, Wojnarowska F, McGuire W, Welsh K: Polymorphism of tumour necrosis factor α promotor region of the MHC is strongly associated with linear IgA disease and affects prognosis (abstract). Br J Dermatol 1994;131(suppl 44):22.

44 Niwa Y, Sakane T, Shingu M, Yanagida I, Komura J, Miyachi Y: Neutrophil-generated active oxygens in linear IgA bullous dermatosis. Arch Dermatol 1985;121:73–78.

45 Hendrix J, Mangum K, Zone J, Gammon W: Cutaneous IgA deposits in bullous diseases function as ligands to mediate adherence of activated neutrophils. J Invest Dermatol 1990;94:667–672.

46 Marsden R: The treatment of benign chronic bullous dermatosis of childhood and bullous pemphigoid beginning in childhood. Clin Exp Dermatol 1982;7:653–663.

47 Peoples D, Fivenson D: Linear IgA bullous dermatosis: Successful treatment with tetracycline and nicotinamide. J Am Acad Dermatol 1992;26:498–499.

48 Marsden R, McKee P, Bhogal B, Black M: A study of benign chronic bullous dermatosis of childhood, a comparison with dermatitis herpetiformis and bullous pemphigoid occurring in childhood, Clin Exp Dermatol 1980;5:159–172.

49 Davies M, Marks R, Nuki G: Dermatitis herpetiformis, a skin manifestation of a generalised disturbance in immunity. Q J Med 1978;186:221–248.

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Mucocutaneous Paraneoplastic Disorders

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The skin often mirrors internal diseases, and specific skin findings have been linked to internal malignancy [1]. Skin manifestations causally related to cancer, but not induced by direct tumour invasion or metastasis have been designated as paraneoplastic dermatoses. A potential relationship between cutaneous changes and a malignant neoplasm may exist if the disorders appear concurrently, and have a parallel clinical course. In addition there may be a genetic association between the two processes. A specific tumour site or cell type may be the origin of the malignancy and target the skin for paraneoplasia [2]. The most important criteria for the diagnosis of para-neoplasia include appearance of the dermatosis following the development of the malignant tumour and a parallel course of clinical activity between the skin signs and the internal malignancy. These correlates also indicate that removal of the tumour typically results in clearing of the dermatosis and relapse of the cancer usually causes recurrence of the paraneoplastic dermatosis (table 1).

The eye is derived in part from the embryonic ectoderm and therefore many congenital and acquired syndromes may affect both the skin and the eyes. Paraneoplastic disorders of the skin can present with conjunctival and lid margin lesions as observed in malignancy-associated acanthosis nigricans [3, 4]. Skin signs occurring in conjunction with malignant internal disorders may characteristically feature periorbital lesions. Dermatomyositis is typically identified by violaceous erythema with or without oedema involving the perior-bital skin and called a heliotrope rash. The upper lids are mainly involved. Lid involvement may be discrete and appear as a mild discolouration along the eyelid margin [5]. Necrobiotic xanthogranuloma is often associated with

Table 1. Criteria for paraneoplasia that associate the skin signs and internal malignancy

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- Both conditions develop at the same time
- Both entities follow a parallel clinical course
- Concurrence of a specific malignant tumour and a certain dermatosis
- Rare or unique paraneoplastic dermatoses known to develop only in connection with malignancy
- Statistical association identified between the two conditions
- The two conditions may be part of a genetic syndrome such that the onset and clinical course are not completely co-ordinated and concurrent

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monoclonal gammopathy and frequently involves the periorbital region [6]. Ocular and periocular involvement by multicentric reticulohistiocytosis associated with paraproteinaemia demonstrates typical skin pathology [7]. Febrile neutrophilic dermatosis (Sweet's syndrome) develops as a result of myeloproliferative disorders in about 20% of the cases and may initially present as conjunctivitis or may be associated with ocular complications such as glaucoma, iritis or development of posterior synechia [8]. Malignant melanoma rarely may produce paraneoplastic retinopathy, sometimes with an angiographic finding of retinal phlebitis that mimics retinal vasculitis [9–11]. Recently, Morioka et al. [12] showed that the association ratio of internal malignancies with pemphigus and bullous pemphigoid was significantly higher than that of the controls aged over 70 years. Bystrn et al. [13] described a unique paraneoplastic mixed bullous skin disease associated with anti-skin antibodies and a B cell lymphoma with IgM paraproteinaemia. The mucocutaneous lesions lead to conjuctivitis and ocular scarring. This patient featured clinically, histologically and in immunofluorescence investigations a mixture of cicatricial pemphigoid and pemphigus vulgaris. However, only a single mucocutaneous paraneoplastic syndrome has been identified more recently which exhibits a high percentage of ocular involvement. The condition is called paraneoplastic pemphigus. This chapter will mainly focus on paraneoplastic pemphigus as an unique immunobullous disorder involving the skin, eye and mucous membranes.

Anhalt et al. [14] defined a distinct bullous disorder in patients with underlying malignancy and named it paraneoplastic pemphigus. Five criteria which characterise the syndrome were identified: (1) painful mucosal lesions and cutaneous eruption; (2) histological acantholysis and spongiosis at multiple levels of the epidermis; (3) vacuolisation of the basal layer and necrosis of keratinocytes with intercellular IgG and complement deposition and C3

Fig. 1. Patient with paraneoplastic pemphigus showing blistering lesions on mucocutaneous surfaces.

localised to the basement membrane zone by direct immunofluorescence examination of epidermis; (4) auto-antibodies detected by indirect immunofluorescence that bind intercellular substance expressed on different epidermal and mucosal cells, and (5) detection of specific antigens of 250 kD molecular mass (desmoplakin I), 230 kD (bullous pemphigoid antigen), 210 kD (desmoplakin II), and 190 kD (unidentified). Auto-antibodies in sera of the first reported patients were found by immunoprecipitation techniques which identified a unique complex of epidermal protein antigens. The complex of auto-antibodies were different from those documented previously in the sera of patients with pemphigus vulgaris, pemphigus foliaceus and pemphigoid [14].

Fig. 2. Close-up view of periocular changes in paraneoplastic pemphigus.

Clinical Findings

Cutaneous lesions are typically polymorphous and pruritic. Confluent erythema of the upper trunk with development of vesicles, bullae, erosions and crusting is often observed [15]. Localised erythematous and papulosquamous lesions with central vesicles resembling target lesions of erythema multiforme may be an initial finding [16]. Involvement of palms and soles often occurs. More recently, lichen planus pemphigoides-like eruptions [17], erosive lichen planus [18], annular bullous [19], and diffuse morbilliform reactions [16] have been reported as clinical variations of paraneoplastic pemphigus.

Involvement of mucous membranes is an important feature of paraneoplastic pemphigus (fig. 1, 2). Persistent painful blisters and erosions occur on labial, gingival, buccal, pharyngeal and lingual mucosa [20] (fig. 3). Involvement of the lungs has been documented [16, 21]. Paraneoplastic pemphigus with manifestations in the gastro-intestinal tract occurred in a patient reported by Lam et al. [21]. Genital erosions have been observed in several studies [14, 16, 22]. Ocular manifestations in patients with paraneoplastic pemphigus are common (table 2). Erosive conjunctivitis may result in symblepharon formation and the clinical findings share similarities with cicatricial pemphigoid [21, 23].
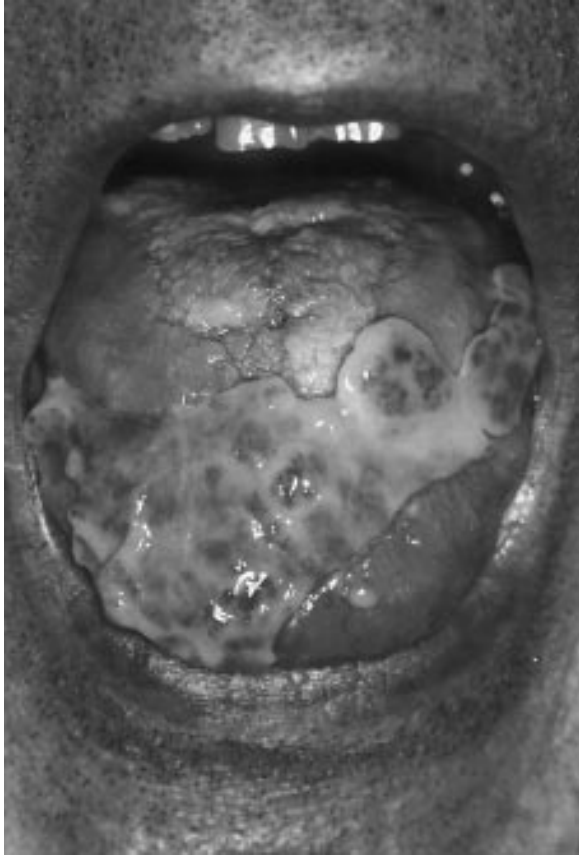
Fig. 3. Erosive lesions of the tongue.

Associated Neoplasm

Zillikens and Bröcker [31] reviewed 23 cases of paraneoplastic pemphigus and emphasised that most of the underlying malignancies were lymphomas or chronic lymphatic leukaemia. In addition, sarcoma, Waldenström's disease, and bronchogenic carcinoma were documented. Recently, a patient with Waldenström's macroglobulinaemia who subsequently developed paraneoplastic pemphigus after treatment with systemic interferon alpha 2A has been documented [32]. Castelman tumour, a benign angiofollicular lymph node hyperplasia and benign thymoma also have been associated with the clinical features of paraneoplastic pemphigus. Ostezan et al. [29] have recently described a patient with the clinical, histopathologic and immunofluorescence findings of paraneoplastic pemphigus, but no underlying disease process could be found.

Table 2. Review of the literature on patients with paraneoplastic pemphigus and ocular involvement

Refer- ence	Clinics	Histology from the conjunctiva	Underlying neoplasm	Treatment
23	case 1 bilateral bulbar conjunctival hyperaemia and sloughing of conjunctival epithelium	suprabasal acantholysis, IgG and C3 deposits	non-Hodgkin lymphoma	topical and systemic immuno- suppression
	case 2 bilateral conjunctivitis	idem	lymphoma	idem
14	case 1 vesicles and bleeding erosions of the conjunctiva	–	lymphoma	immuno- suppression
	case 3 conjunctival erosions	–	lymphoma	immuno- suppression
	case 5 blisters and erosions over conjunctiva	–	sarcoma	immunosuppres- sion and surgery
22	erosions of the cornea and conjunctiva resulting in symblepharon of the left conjunctiva	–	chronic lymphocytic leukaemia	chemotherapy
16	bilateral conjunctival effusions, erosions on right cornea	–	lymphoma	chemotherapy
24	case 1 + 2 identical with those of [23]			
	case 3 involvement of right bulbar conjunctiva	–	lymphoma	chemotherapy
25	lid erythema and congested oedematous conjunctivae	–	lymphoma	immuno- suppression
19	conjunctival erythema	–	lymphoma	immuno- suppression
26	erosions of conjunctival mucosa	–	lymphoma	chemotherapy and radiation
27	periorbital erosions, conjunctival injection, eyelid ulcer	–	Waldenström macro- globulinaemia	immuno- suppression
20	prominent inflammation of the lower palpebral conjunctival surfaces	–	non-Hodgkin lymphoma	immuno- suppression
21	scarring conjunctivitis with symblepharon	consistent with pemphigus	bronchogenic squamous cell carcinoma	immunosuppres- sion, external beam radiation
28	bilateral conjunctivitis	–	non-Hodgkin lymphoma	immunosuppres- sion, chemotherapy, radiation
29	purulent bilateral conjunctivitis with erythema and oedema	–	–	cyclosporin
30	Bilateral conjunctivitis	–	chronic lymphatic leukaemia	cyclosporin, cyclophosphamide, prednisone
30	redness and irritation of the eyes, bilateral conjunctivitis, severe bacterial keratitis	–	chronic lymphatic leukaemia	cyclosporin, prednisone, cyclophosphamide
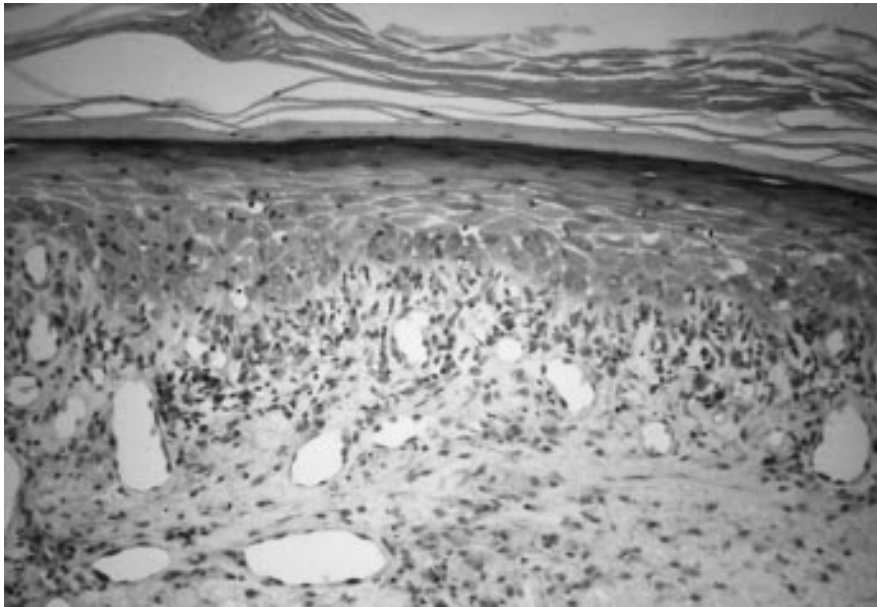
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Fig. 4. Light-microscopic findings with interface changes and epidermal exocytosis of inflammatory cells.

Course and Outcome

In general, patients with paraneoplastic pemphigus have a progressive course and immunosuppressive treatment modestly influences the mucocutaneous lesions [14]. However, single cases with clearance of the skin lesions following treatment with immunosuppression have been reported [16, 21, 30]. Interestingly, the mucosal erosions are least responsive to treatment. In patients with associated benign tumours, resection leads to clearing of the mucocutaneous lesions [31]. Perniciaro et al. [22] documented a case of paraneoplastic pemphigus and chronic lymphocytic leukaemia with prolonged survival. The patient lived almost 8 years after onset of paraneoplastic pemphigus.

Histologic Features, Immunofluorescence and Results of Molecular Biology

Light-microscopic findings of specimens from patients with paraneoplastic pemphigus reflect the various clinical spectrum of the mucocutaneous
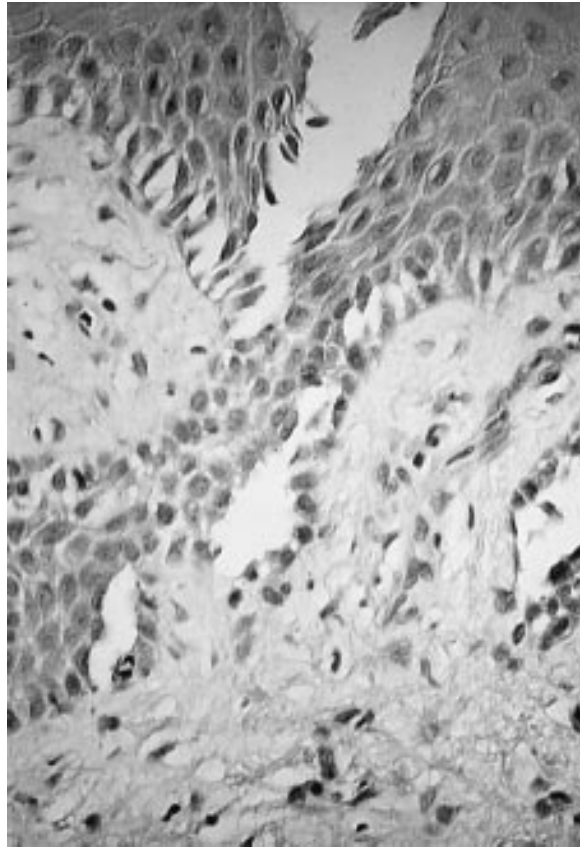
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Fig. 5. Acantholysis with suprabasal cleft formation.

lesions. The most important features are acantholysis, suprabasal cleft formation, necrotic keratinocytes, interface changes and epidermal exocytosis of inflammatory cells [33, 34] (fig. 4, 5). Horn and Anhalt [33] defined major and minor findings of histologic features of paraneoplastic pemphigus. Major findings include epidermal acantholysis, suprabasal clefts or blister formation, dyskeratotic keratinocytes, basal vacuolisation and epidermal exocytosis of inflammatory cells. Minor findings consisted of acantholysis and dyskeratosis of follicular and eccrine epithelia, lymphocytic satellitosis, polymorphous superficial perivascular infiltrate of lymphocytes and neutrophils, melanin incontinence, melanophages, necrotic blister roof, absence of apoptotic keratinocytes in the dermis and epidermal regeneration from adnexal epithelium. Direct immunofluorescence examination shows IgG and complement deposition along the basement membrane zone and/or in the
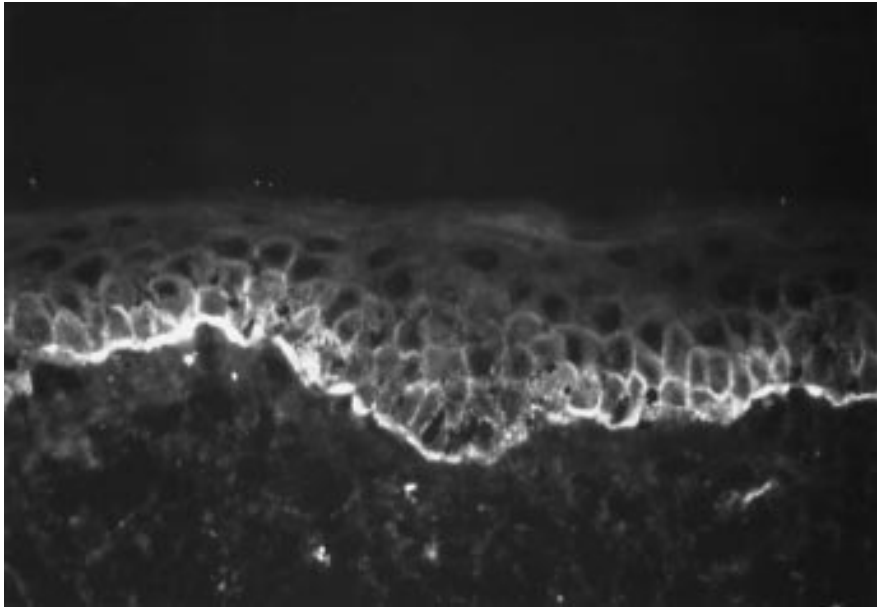
Fig. 6. Direct immunofluorescence staining with IgG deposition along the basement membrane zone and in the epidermal intercellular spaces.

epidermal intercellular spaces [33, 34] (fig. 6). Indirect immunofluorescence of monkey oesophagus, human skin and mouse skin shows cell surface staining on all substrates. Paraneoplastic pemphigus differs from the other types of pemphigus in that the auto-antibodies also bind by indirect immunofluorescence to simple columnar and transitional epithelia such as urinary bladder, respiratory epithelium [16], gastro-intestinal epithelium [14] and myocardium specimens [14, 15, 33]. Indirect immunofluorescence on rat bladder transitional epithelium has been found to be a simple and inexpensive test [35]. The test has been found to be highly specific and sensitive for paraneoplastic pemphigus [35]. Subsequently, Helou et al. [36] have suggested that indirect immunofluorescence on murine bladder epithelium is an adequate screening test for paraneoplastic pemphigus, but as great as one fourth of cases may show negative or indeterminate results. In a series of patients with pemphigus, Kanitakis et al. [37] found one patient who also reacted with epithelium of mouse bladder. However, Western blotting using serum was negative. In this study, the authors did not apply strict histological and indirect immunofluorescence criteria for
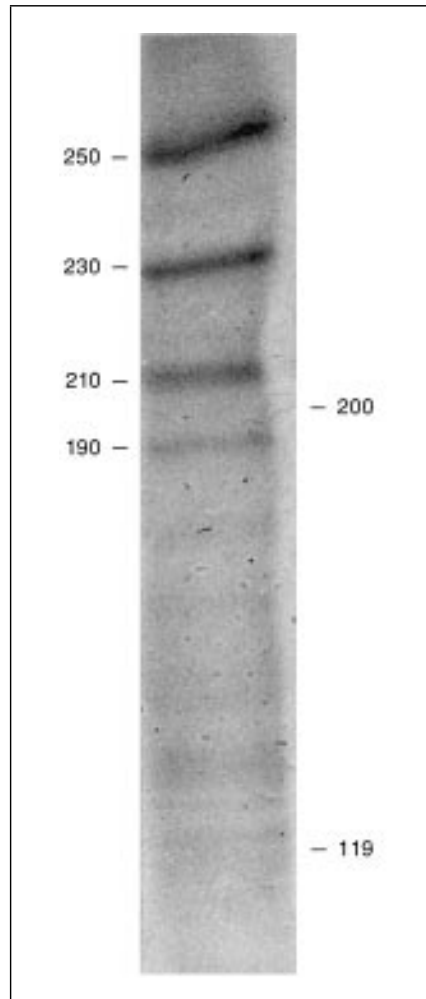
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Fig. 7. Immunoprecipitation analysis detects specific antigens with defined molecular mass (kD).

diagnosis of paraneoplastic pemphigus. They concluded that Western blotting and immunoprecipitation are more sensitive techniques for the diagnosis of paraneoplastic pemphigus.

The auto-immune response in paraneoplastic pemphigus involves intracellular and extracellular desmosomal antigens, and the antigens are shared partially with other auto-immune bullous skin disorders. Immunoprecipitation analysis using human keratinocyte extracts have demonstrated 250-, 230-, 210- and 190-kD proteins. In addtion, a 170-kD antigen has often been found in

paraneoplastic pemphigus [38, 39] (fig. 7). The 250-kD polypeptide has been identified as desmoplakin I, the 210-kD polypeptide as desmoplakin II, and the 230-kD protein is the bullous pemphigoid antigen associated with hemides-mosomes [40]. The 190-kD protein has not yet been identified or associated with any function. The apparent transmembranous protein of 170 kD may be a critical antigen in the pathophysiology of tissue injury in paraneoplastic pemphigus [33]. In some patients, 135- and 130-kD proteins are also detected [39, 41]. The 130-kD protein represents desmoglein. A recent immunoblot study showed that there was a slight but constant difference of mobility on the gel between the 210-kD protein detected in sera of patients with paraneoplastic pemphigus and desmoplakin II [39]. These findings shed some doubt on the hypothesis that the 210-kD protein reacting with auto-antibodies in patients with paraneoplastic pemphigus is, in fact, desmoplakin II.

The exact pathogenesis of paraneoplastic pemphigus is still unknown but the evidence suggests that the auto-antibodies are pathogenic. Several authors propose that non-neoplastic B cells are involved in the production of auto-antibodies [14, 20, 24]. Further theories include immune dysregulation and induction of auto-antibodies by the tumour [17].

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References

1   Poole S, Fenske NA: Cutaneous markers of internal malignancy. II. Paraneoplasic dermatoses and environmental carcinogens. J Am Acad Dermatol 1993;28:147–164.
2   McLean DI: Toward a definition of cutaneous paraneoplastic syndrome. Clin Dermatol 1993;11: 11–13.
3   Tabandeh H, Gopal S, Teimory M, Wolfensberger T, Luke IK, Mackie I, Dilly N: Conjunctival involvement in malignancy-associated acanthosis nigricans. Eye 1993;7:648–651.
4   Kuckelkorn R, Wilgenbus K, Lentner A, Reim M: Augenveränderungen bei Acanthosis nigricans maligna – möglicher Einfluss von Wachstumsfaktoren in der Ätiopathogenese dieses paraneoplas-tischen Krankheitsbildes. Klin Mbl Augenheilk 1992;201:169–173.
5   Stonecipher MR, Callen JP, Jorizzo JL: The red face: Dermatomyositis. Clin Dermatol 1993;11: 261–273.
6   Mehregan DA, Winkelmann RK: Necrobiotic xanthogranuloma. Arch Dermatol 1992;128:94–100.
7   Buckley CA, Bron AJ: Ocular and periocular features of multicentric reticulohistiocytosis with paraproteinaemia. A report of two cases. Trans Ophthalmol Soc N Z 1981;33:143–146.
8   Cohen PR: Sweet's syndrome presenting as conjunctivitis. Arch Ophthalmol 1993;111:587–588.
9   Kellner U, Bornfeld N, Foerster MH: Severe course of cutaneous melanoma associated paraneoplas-tic retinopathy. Br J Opththalmol 1995;79:746–752.
10 Rush JA: Paraneoplastic retinopathy in malignant melanoma. Am J Ophthalmol 1993;115:390–391.
11 Remulla JFC, Pineda R, Gaudio AR, Milam AH: Cutaneous melanoma-associated retinopathy with retinal periphlebitis. Arch Ophthalmol 1995;113:854–855.
12 Morioka S, Sakuma M, Ogawa H: The incidence of internal malignancies in autoimmune blistering diseases: Pemphigus and bullous pemphigoid in Japan. Dermatology 1994;189(suppl 1):82–84.
13 Bystryn JC, Hodak E, Gao SQ, Chuba JV, Amorosi EL: A paraneoplastic mixed bullous skin disease associated with anti-skin antibodies and a B-cell lymphoma. Arch Dermatol 1993;129: 870–875.

14 Anhalt GJ, Kim SC, Stanley JR, Korman NJ, Jabs DA, Kory M, Izumi H, Patrie HI, Mutasim D, Ariss-Abdo L, Labib RS: Paraneoplastic pemphigus. An autoimmune mucocutaneous disease associated with neoplasia. N Engl J Med 1990;323:1729–1735.

15 Mutasim DF, Pelc NJ, Anhalt GJ: Paraneoplastic pemphigus. Dermatol Clinics 1993;11:473–481.

16 Fullerton SH, Woodley DT, Smoller BR, Anhalt GJ: Paraneoplastic pemphigus with autoantibody deposition in bronchial epithelium after autologous bone marrow transplantation. JAMA 1992; 267:1500–1502.

17 Stevens SR, Griffiths CEM, Anhalt GJ, Cooper KD: Paraneoplastic pemphigus presenting as a lichen planus pemphigoides-like eruption. Arch Dermatol 1993;129:866–869.

18 Jansen T, Plewig G, Anhalt GJ: Paraneoplastic pemphigus with clinical features of erosive lichen planus associated with Castleman's tumor. Dermatology 1995;190:245–250.

19 Tankel M, Tannenbaum S, Parekh S: Paraneoplastic pemphigus presenting as an unusual bullous eruption. J Am Acad Dermatol 1993;29:825–828.

20 Helm TN, Camisa C, Valenzuela R, Allen CM: Paraneoplastic pemphigus. A distinct autoimmune vesiculobullous disorder associated with neoplasia. Oral Surg Oral Med Oral Pathol 1993;75: 209–213.

21 Lam S, Stone MS, Goeken JA, Massīcotte SJ, Smith AC, Folberg R, Krachmer JH: Paraneoplastic pemphigus, cicatricial conjunctivitis, and acanthosis nigricans with pachydermatoglyphy in a patient with bronchogenic squamous cell carcinoma. Ophthalmology 1992;99:108–113.

22 Perniciaro C, Kuechle MK, Colon-Otero G, Raymond MG, Spear KL, Pittelkow MR: Paraneoplastic pemphigus: A case of prolonged survival. Mayo Clin Proc 1994;69:851–855.

23 Meyers SJ, Varley GA, Meisler DM, Camisa C, Wander AH: Conjunctival involvement in paraneoplastic pemphigus. Am J Ophthalmol 1992;114:621–624.

24 Camisa C, Helm TN, Liu YC, Valenzuela R, Allen C, Bona S, Larrimer N, Korman NJ: Paraneoplastic pemphigus: A report of three cases including one long-term survivor. J Am Acad Dermatol 1992;27:547–553.

25 Rybojad M, Leblanc T, Flageul B, Bernard Ph, Morel P, Schaison G, D'Agay MF, Borradori L: Paraneoplastic pemphigus in a child with a T-cell lymphoblastic lymphoma. Br J Dermatol 1993; 128:418–422.

26 Fried R, Lynfield Y, Vitale P, Anhalt G: Paraneoplastic pemphigus appearing as bullous pemphigoid-like eruption after palliative radiation therapy. J Am Acad Dermatol 1993;29:815–817.

27 Scully RE, Mark EJ, McNeely WF, McNeely BU: Paraneoplastic pemphigus, with involvement of skin, mucosa (buccal cavity), larynx and trachea. N Engl J Med 1992;326:1276–1284.

28 Nishibori Y, Hashimoto T, Ishiko A, Shimizu H, Nishikawa T: Paraneoplastic pemphigus: The first case from Japan. Dermatology 1995;191:39–42.

29 Ostezan LB, Fabré VC, Caughman W, Swerlick RA, Korman NJ, Callen JP: Paraneoplastic pemphigus in the absence of a known neoplasm. J Am Acad Dermatol 1995;33:312–315.

30 Stahle-Bäckdahl M, Hedblad MA, Skoglund C, Fagerholm P, Anhalt GJ: Paraneoplastic pemphigus: A report of two patients responding to cyclosporin. Eur J Dermatol 1995;5:671–675.

31 Zillikens D, Bröcker EB: Paraneoplastischer Pemphigus. Induktion von Autoantikörpern gegen Strukturproteine der Haut. Hautarzt 1994;45:827–833.

32 Kirsner RS, Anhalt GJ, Kerdel FA: Treatment with alpha interferon associated with the development of paraneoplastic pemphigus. Br J Dermatol 1995;132:474–478.

33 Horn TD, Anhalt GJ: Histologic features of paraneoplastic pemphigus. Arch Dermatol 1992;128: 1091–1095.

34 Mehregan DR, Oursler JR, Leiferman KM, Muller SA, Anhalt GJ, Peters MS: Paraneoplastic pemphigus: A subset of patients with pemphigus and neoplasia. J Cutan Pathol 1993;20:203–210.

35 Liu AY, Valenzuela R, Helm TN, Camisa C, Melton AL, Bergfeld WF: Indirect immunofluorescence on rat bladder transitional epithelium: A test with high specifity for paraneoplastic pemphigus. J Am Acad Dermatol 1993;28:696–699.

36 Helou J, Allbritton J, Anhalt GJ: Accuracy of indirect immunofluorescence testing in the diagnosis of paraneoplastic pemphigus. J Am Acad Dermatol 1995;32:441–447.

37   Kanitakis J, Wang YZ, Roche P, Cozzani E, Nicolas JF, Sarrety Y, Thivolet J: Immunohistopathological study of autoimmune pemphigus. Lack of strictly histological and indirect immunofluorescence criteria for paraneoplastic pemphigus. Dermatology 1994;188:282–285.

38   Chorzelski TP, Hashimoto T, Korman NJ, Jablonska S, Kozlowska A, Ismail M, Rywik H: Atypical pemphigus associated with malignant thymoma and autoimmune response restricted to 170 kD antigen: Is it a variant of paraneoplastic pemphigus? Eur J Dermatol 1995;5:31–35.

39   Hashimoto T, Amagai M, Watanabe K, Chorzelski TP, Bhogal BS, Black MM, Stevens HP, Boorsuna DM, Korman NJ, Gamou S, Shimizu N, Nishikawa T: Characterization of paraneoplastic pemphigus autoantigens by immunoblot analysis. J Invest Dermatol 1995;104:829–834.

40   Oursler JR, Labib RS, Ariss-Abdo L, Burke T, O'Keefe EJ, Anhalt GJ: Human autoantibodies against desmoplakins in paraneoplastic pemphigus. J Clin Invest 1992;89:1775–1782.

41   Joly P, Thomine E, Gilbert D, Verdier S, Delpech A, Prost C, Lebbe C, Lauret P, Trou F: Overlapping distribution of antibody specificities in paraneoplastic pemphigus and pemphigus vulgaris. J Invest Dermatol 1994;103:65–72.

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Drug-Induced Conjunctival Cicatrisation

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Clinically apparent, chronically progressive conjunctival cicatrisation may arise as a rare adverse effect of certain drugs. Drug-induced conjunctival cicatrisation (DICC) has been referred to as pseudopemphigoid, although in certain cases the condition is indistinguishable from ocular cicatricial pemphigoid (OCP), both clinically and pathologically. Thus it is preferable to use the term *drug-induced conjunctival cicatrisation* rather than *pseudopemphigoid.* Various topical and systemic medications have been implicated in the aetiology of DICC, but the underlying mechanism remains unknown. Other effects of topical medication, some subclinical, may provide clues as to the aetiology of DICC. In the present review, the subject of adverse effects of medication on the conjunctiva is discussed.

Systemically Administered Drugs Causing Conjunctival Cicatrisation

Conjunctival cicatrisation occurring as a side-effect of systemically administered drugs is exceptionally rare (table 1). Patients treated with practolol (a β-receptor-blocking agent) have been reported to suffer both retroperitoneal and conjunctival fibrotic reactions [1, 2] and for this reason, the drug is rarely used today. In addition, conjunctival cicatrisation has been reported in a group of patients who had taken orally administered iodide or bromide [3]. However, these patients had been concurrently administered topical mercurous chloride and it was not possible, therefore, to know which drug to implicate.

Table 1. Drugs that may cause conjunctival cicatrisation

Systemic medication:	practolol
	? iodide or bromide
Topical medication:	idoxuridine
	adrenaline
	guanethidine
	dipivefrine
	pilocarpine
	β-blockers
	ecothiopate iodide
	demecarium bromide
	various combinations

Table 2. The spectrum of effect of topical medications on the conjunctiva [4, 5]

1. Total tolerance	no clinical signs or symptoms
2. Subclinical effect (cellular and ultrastructural)	no clinical signs or symptoms
3. Non-specific conjunctival irritation	symptoms, no clinical signs
4. Metabolite deposition/dyschromia	clinical signs, no symptoms
5. Anaphylactoid (allergic) acute or chronic conjunctivitis (type I hypersensitivity)	clinical signs and symptoms
6. Allergic contact (dermato-) conjunctivitis (type IV hypersensitivity)	clinical signs and symptoms
7. Papillary conjunctivitis (non-immunological irritation to factors such as pH, tonicity, contamination)	clinical signs and symptoms
8. Follicular conjunctivitis (immunological and characterised by lymphoid follicles)	clinical signs and symptoms
9. Cicatrising conjunctivitis (DICC or pseudopemphigoid)	severe clinical signs and symptoms

Topically Administered Drugs Causing Conjunctival Cicatrisation

Conjunctival reaction to topical drugs is dependent on multiple factors and can occur to varying degrees. Subclinical effects are almost certainly universal, but clinical effects form a wide spectrum ranging from total tolerance and absence of clinical signs to severe symptomatic cicatrisation, indistinguishable from OCP (table 2).

Allergic or non-specific toxic reactions, non-specific papillary conjunctivitis or specific follicular conjunctivitis are much more common than DICC

and in many patients the adverse effect is due to the medication's preservative. In these cases, administration of preservative-free drops should be tried. This is often curative, although less so with follicular conjunctivitis. Cvetkovic et al. [6] reported the development of conjunctival follicles after long-term (>1 year) exposure to pilocarpine and believed that this was unrelated to the presence or absence of preservatives. Furthermore, there is no evidence that preserved topical β-blockers induce a follicular response, suggesting that preservatives are not directly involved in the aetiology of follicular conjunctivitis.

Furthermore, the cicatrising conjunctival reaction itself is considered to represent a spectrum of disease ranging from a self-limiting form to a progressive type.

Fibrosis secondary to the instillation of topical medication was first reported in 1951 in a case of dacryostenosis following administration of furmethide iodide [7]. Since then there have been several reports of conjunctival cicatrisation attributed to topical medications. Various drugs have been implicated in the aetiology of DICC (table 1) including idoxuridine [8], adrenaline [9–11], guanethidine with or without adrenaline [11–13], dipivefrine [11], pilocarpine [11, 14, 15], β-blockers [11], ecothiopate [11, 13], demecarium bromide [14] and various combinations of therapy [11, 15–17].

Kristensen and Norn [9] reported on 29 eyes with 'benign mucous membrane pemphigoid' and found that 14 of the eyes had been treated with long-term adrenaline. They were the first to suggest an association between the use of topical therapy and ocular cicatrisation. A few years later, Patten et al. [13] described the first 2 cases of DICC (introducing the term 'pseudopemphigoid') induced by topical ecothiopate iodide applied for 6 and 9 years. Both patients had received topical ecothiopate iodide to the affected eye and the conjunctiva was compared with that of the unaffected eye. Both affected eyes demonstrated features similar to OCP. The clinical features included symblepharon, conjunctival cicatrisation, inferior punctal occlusion, epidermalisation, trichiasis and corneal opacification with vascularisation. Hoffer [10] also reported a case of DICC, secondary to 4 years use of adrenaline (1:1,000) and 5 further cases were reported by Leonard et al. [16] occurring after long-term (4–12 years) administration of unspecified anti-glaucomatous medication. Two further cases were reported by Hirst et al. [14] – 1 occurred following application of demecarium bromide for 3–4 years and the other following long-term use of pilocarpine (6 years) with minimal exposure to adrenaline. In both patients, the drugs were administered unilaterally and non-progressive cicatricial conjunctivitis occurred in the treated eye with no changes evident in the untreated fellow eye. Tseng et al. [15] reported another 2 cases occurring after long-term (12 years) exposure to anti-glaucoma medications (case 1: pilocarpine, case 2: various, including ecothiopate iodide). Case 1 differed from previously

reported cases in that the condition was progressive, despite discontinuation of pilocarpine and aggressive treatment with cyclophosphamide. A further 5 cases were reported by Fiore et al. [17] in patients who had been treated with various anti-glaucoma medications. Although 1 patient had been treated with various medications, conjunctival shrinkage occurred after 4 months therapy with timolol alone and the authors considered the β-blocker to be the causative agent in this case.

Although most cases of DICC arise in eyes exposed to anti-glaucoma medications, other topical agents have been implicated. Ostler et al. [18] reported the occurrence of conjunctival scarring and keratinisation in 17 eyes subjected to topical medication. Most of the cases were associated with combination therapy with either antibiotics and antivirals, antibiotics and artificial tears or various anti-glaucoma medications. In another report idoxuridine-induced DICC was reported in 4 patients [8].

The Clinical Features of Drug-Induced Conjunctival Cicatrisation

In its late stages, DICC is usually clinically indistinguishable from OCP. Even in its early stages, or in mild forms, DICC can be difficult to differentiate from OCP, although when induced by topical drugs the fibrotic reaction predominates in the lower fornix. Wright [12], in an analysis of patients receiving combined guanethidine and adrenaline, showed that inferior forniceal shallowing and subconjunctival fibrosis may occur. An analysis of this subtle reaction to topical anti-glaucoma medications has been reported in a more recent study [19]. The depth of the inferior fornix of 179 glaucoma patients receiving topical medications was compared with that of 420 control subjects who had no history of ocular disease and had not been exposed to topical medications. The glaucoma patients were divided into three groups. The first group comprised 68 patients, 50 years and older, using miotics (pilocarpine, carbachol or ecothiopate) for 3 years or longer. The second group comprised 88 patients, 40 years and older, using non-miotic therapy (adrenaline, dipivefrine and/or β-blockers) for 3 years or more, regardless of miotic therapy. The third group comprised 23 patients, 50 years and older, taking non-miotics for at least 3 years and excluded those exposed to miotics for more than 6 months. A significant foreshortening of the fornix was found with increasing age and, after taking this into account, also after at least 3 years exposure to topical anti-glaucoma medications, irrespective of type. The fact that different classes of topically administered medications were associated with conjunctival shrinkage indicated that there may be a common pathway resulting in inflammation or toxicity.

Table 3. Clinical features of DICC

1. Absence of systemic features of mucous membrane pemphigoid (by definition)
2. Unilateral (in some cases)
3. Non-specific chronic papillary conjunctivitis (early)
4. Inferior fornix foreshortening (early)
5. Shallowing of canthal recesses
6. Canthal keratinisation
7. Obliteration of conjunctival folds
8. Flattening of the plica and caruncle
9. Conjunctival thickening
10. Increased conjunctival vascularisation
11. Subepithelial fibrosis (white-lines)
12. Punctal occlusion (especially inferior)
13. Symblephara
14. Conjunctival ulceration
15. Complete cicatrisation and/or epidermalisation
16. Secondary features/complications
17. Lack of progression following cessation of topical medication administration (in some cases only)

Suspicion of DICC should arise if the clinical features are unilateral and may be confirmed if the history reveals that topical medication has been administered to the affected eye alone (see also table 3). Figures 1–3 show the eyes of a gentleman who had a combination of topical drugs applied to his right eye alone, over a period of many years. Further evidence for DICC may be provided following withdrawal of the topical drug which may prevent progression. However, this does not occur in all cases and progression may occur unabated following cessation of medication.

Subtle Clinical Effects of Topical Medication on the Conjunctiva

Most patients who have administered topical medications over long periods of time are essentially symptom free, although minor complaints are not uncommon on direct questioning. Sympathomimetics, in particular, are often associated with local effects including subjective irritation, epithelial adrenochrome deposition, reactive hyperaemia, blepharoconjunctivitis and corneal oedema [20–22], but there is growing evidence that other medications also affect the conjunctiva. Despite the many ways in which the conjunctiva can react to topical medications, the majority of patients are able to tolerate such therapy and clinically obvious conjunctival changes remain the exception rather than the rule. Clinical signs are not always striking and subtle changes are easily missed without a rigorous examination. Wright [23], for example,

Fig. 1. The face of a male patient with a long-standing history of right herpes simplex viral keratitis who developed DICC following many years of topical therapy (various combinations). The conjunctiva of the right eye is abnormal, being inflamed and showing evidence of cicatrisation. The left eye which had not been exposed to any topical medications is normal.
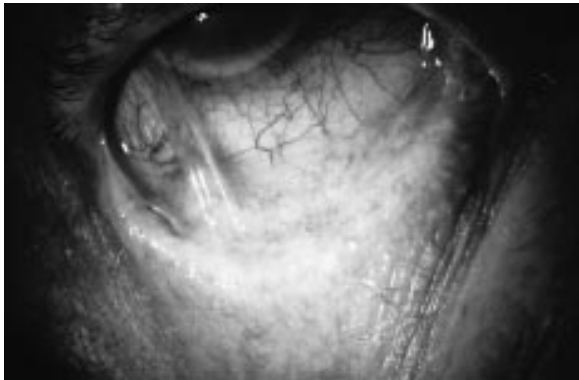

Fig. 2. The abnormal right eye showing clear evidence of cicatrisation (injection, symblepharon, subepithelial scarring, loss of the inferior fornix and an abnormal lower eyelid margin).

reported the development of relatively subtle squamous metaplasia or epidermalisation as an adverse reaction to topical medication in 6 patients treated with a variety of drugs, mainly for glaucoma. The first detectable change was a loss of epithelial wetting by the tear film with disturbance of the light reflex from the conjunctival surface. Increasing keratinisation created a whitish, slightly foamy appearance and if this remained unnoticed eventually a thick
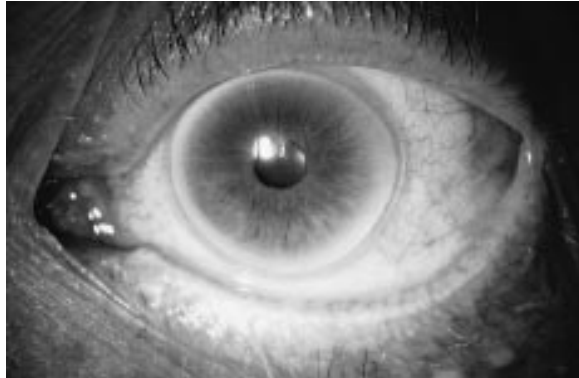
Fig. 3. The normal left eye.

white mass of keratin formed. A similar reaction has been reported after use of topical dipivefrine and metipranolol [24]. The main clinical sign was of leucoplakia and histological features included epithelial thickening and keratinisation, loss of goblet cells and subepithelial inflammation. In view of the pharmacological similarities between metipranolol and practolol, known to cause ocular cicatrisation [1, 2], the authors considered the β-blocker to be the cause of the conjunctival reaction. A further 2 cases of chronic conjunctivitis (without leucoplakia) related to the use of metipranolol were mentioned in support of the hypothesis.

Subclinical Effects of Topical Medication on the Conjunctiva

A number of studies have made an attempt to determine the effect of topical anti-glaucoma medications on the cellular content of the conjunctiva [25–30]. The findings have been variable, but it would appear that both the type of medication and the duration of therapy are important factors in alteration of the cell profile of the conjunctiva. Certainly, long-term therapy (perhaps more than 4 years) [29] with multiple topical agents results in a significant decrease in the number of goblet cells and increase in the number of fibroblasts, macrophages, lymphocytes and mast cells in the subepithelial tissues [28].

A few studies have looked at the effect of either single or multiple topical medications on specific features of the conjunctival surface. Surface changes themselves may have little effect on the development of cicatrisation, but they may be indicative of subepithelial changes of more importance. Therapy with pilocarpine has been reported to have a greater effect in the development of conjunctival follicles compared with timolol [6]. However, it has been reported

that relatively short-term treatment with topical timolol alone [6, 31–33] or in combination with dipivefrine [33] can induce a dry-eye state with primary and/or potential secondary effects on the conjunctiva. Such findings have not been confirmed by other workers [34–36], but this may reflect short duration of exposure to topical therapy or small numbers of patients/animals examined. Steuhl et al. [37] investigated the effect of topical anti-glaucoma medications on conjunctival cell differentiation. Minimal differences were found after pilocarpine application, there being a minor increase in desquamation of small electron-dense epithelial cells and a decrease in the number of microvilli. Patients treated with β-blockers, however, had a significant reduction in number of secretory epithelial cells and a pronounced increase in cells, with rough endoplasmic reticulum often showing vacuolisation and dilation. In addition to a loss of desmosomes and tight junctions (zonulae occludens), a reduction in the number and degenerative changes associated with the microvilli and microplicae was noted after at least a year of therapy with any of the medications. Brandt et al. [38] used conjunctival impression cytology to assess the effect of topical anti-glaucoma medication on the degree of epithelial metaplasia. A control group had the lowest cumulative metaplasia score, consistent with healthy conjunctiva. The metaplasia grade in 3 of the treatment groups (β-blockers, β-blockers with pilocarpine and β-blockers together with pilocarpine and sympathomimetics) was significantly increased in comparison with the control group. The largest difference was seen in the comparison between control group and triple therapy group suggesting that the metaplastic change was associated with the number of anti-glaucoma medications used, the presence of sympathomimetic, longer duration of therapy and/or medication interactions.

In vitro Evidence for an Adverse Effect of Topical Medications on the Conjunctiva

In vitro studies are not truly representative of the in vivo state. The instillation of eye drops, for example, exposes the conjunctiva to a drug for a short period of time, whereas in tissue culture there is continuous exposure. Furthermore, in vitro experimentation does not allow for recuperative processes or natural replacement of damaged tissue during intervals between drug installation. Nevertheless, to determine whether cellular changes in the conjunctiva are a direct effect of topical medications, tissue culture studies enable carefully controlled experimentation.

Takahashi [39–43] studied the effect of anti-glaucoma medications and their preservatives on cultured human conjunctival epithelial cells. Preservatives (benzalkonium chloride at concentrations above 0.005%) were found to be cytotoxic. Neither pilocarpine (1%) alone or timolol (0.5%) were cytotoxic.

Adrenaline (1.25%) alone showed some cytotoxicity after exposure of more than an hour. Befnolol (0.5%) showed some cytotoxicity after exposure of more than an hour and bupranolol (0.25%) alone was the most cytotoxic, revealing a differential effect of the β-blockers tested. However, since the experiments utilised epithelial cells, the results are of only limited value in adding to our understanding of the effect that topical medications have on subepithelial fibroblast proliferation, which is of more importance with respect to cicatrisation. However, Williams et al. [44] have investigated the effects of the preservative benzalkonium chloride and commerically available β-blockers (with or without preservative) on tissue cultures of human Tenon's capsule fibroblasts. None of the tested drugs stimulated fibroblast proliferation but, instead, were found to be toxic to fibroblasts at concentrations used clinically. It was found that pure preparations of the β-blockers prevented attachment at a higher concentration than did the commercially available, preservative-containing versions, and benzalkonium chloride was therefore implicated as a toxic component in terms of cellular attachment. With respect to acute effects on cell proliferation, the preservative-free timolol preparation was significantly less toxic than the preservative-containing timolol. This finding again implicated benzalkonium chloride, which was identified as the most toxic agent tested. However, findings similar to those with timolol were not found with the other β-blockers (levobunolol or betaxolol). From the study of delayed effects on cell proliferation, using the commercially available preparations, levobunolol was found to be the only drug that became less toxic after the cells were washed free of drug and recultured. Since the benzalkonium chloride concentration in the preservative-containing levobunolol preparation was 2.5-fold less than in the other β-blockers, it was proposed that the cells exposed to levobunolol may have been more able to recuperate from the initial injury in comparison to those exposed to the other drugs with more preservative.

These results do not support the hypothesis that anti-glaucoma medications directly stimulate fibroblast growth. Thus they favour the concept of topical therapy causing tissue irritation, associated low-grade chronic inflammation and indirect fibroblast stimulation (see also table 4).

The Role of Preservatives

Although a number of workers have implied that preservatives may play a role in the adverse effects exerted by topical medications [4, 19, 23, 37–39, 41, 42, 45], surprisingly little work has been done to clarify the effect of preservatives on the conjunctiva. Of course, ethical issues prevent simple in vivo studies using preservative alone in patients. However, a recently published animal study compared the effect of various topical medications, with and

Table 4. Potential factors of importance in the aetiology of the adverse effect of topical medication on the conjunctiva

1. Type of medication
2. Type of preservative
3. Number of medications
4. Combination therapy interaction effects
5. Duration of therapy
6. Cumulative duration of therapy
7. Frequency of application
8. Physical properties of preparation
   (pH, tonicity, concentration, temperature)

without preservatives, on the histopathology of the rabbit conjunctiva [46]. Using special stains for collagen, a slight increase in the thickness of subepithelial collagen was evident with the medications containing a preservative (metipranolol 0.3% with benzalkonium chloride 0.01% and pilocarpine 2% with cetrimonium chloride 0.004%). In addition, therapy with the preserved preparation of pilocarpine increased the amount of collagen type IV and α-smooth muscle actin identified by immunohistochemistry. It was proposed that preservatives may have an additional effect on the conjunctiva in comparison with the medications alone. In certain individuals it is probable that preservatives can induce DICC but their exact role remains unknown.

The Pathology of Drug-Induced Conjunctival Cicatrisation

Pathological features mirror the spectrum of clinical disease. Thus, subclinical disease is characterised by relatively minor histopathological change, whereas severe progressive cicatrisation is associated with extensive histological, cellular and subcellular change.

Patten et al. [13] described histopathological and immunohistological features including subepithelial fibrosis, an increase in the number of subepithelial lymphocytes and plasma cells, a reduction in the number of goblet cells and in one case basement membrane staining for IgG. An immune or toxic reaction was proposed, but not substantiated. Conjunctival biopsies were obtained from the patients with subtle squamous metaplasia reported by Wright [23] and the histological findings included loss of goblet cells, epithelial keratinisation, keratohyaline granule formation, subepithelial infiltration with lymphocytes and plasma cells and in some cases an increase in the number of fibroblasts and degree of fibrosis. Again an immune aetiology was favoured

but not substantiated. The clinical signs resolved after cessation of the therapy, but since the conjunctiva was not re-biopsied it is not known whether the histological changes were completely reversed. Of the 5 cases of DICC reported by Leonard et al. [16], basement membrane immunofluorescent testing was positive in 3 and negative in 2 of the cases. The authors favoured an immunological aetiology and proposed that in certain patients certain drugs could trigger a reaction indistinguishable from OCP. In the 2 cases of Hirst et al. [14], the histopathological changes were as previously described and ultrastructural findings included bullous separation of the epithelium and basement membrane thickening. Direct and indirect immunofluorescence testing, however, was negative in both cases, except that in the first case subepithelial plasma cells stained for IgA. Pouliquen et al. [11] described the histopathological and ultrastructural features of conjunctival biopsies taken from 10 patients with DICC. Three patients had used pilocarpine, 2 adrenaline, 2 ecothiopate iodide, 1 timolol, 1 timolol and dipivefrin and 1 a combination of timolol, pilocarpine and adrenaline. In the majority of cases there were changes identical to those of OCP, including a reduction in the number of goblet cells, epithelial keratinisation, squamous metaplasia, increased numbers of desmosomes, basement membrane changes, subepithelial inflammatory cell infiltration and reduced intravascular space. Immunohistochemistry was not performed, but the patients responded to immunosuppressive therapy, this being suggestive of an immune aetiology. The authors proposed that their patients were perhaps destined to develop OCP and that use of topical medications induced a more rapid emergence of the pathology. In support of their theory they referred to the later appearance of pemphigoid in the untreated eyes of 4 patients with unilateral DICC. Four of the 5 patients with DICC reported by Fiore et al. [17] had a conjunctival biopsy analysed by immunofluorescence and there was no evidence of basement membrane immunoglobulin deposition in any of them. The authors proposed that DICC represented a spectrum of diseases ranging from a self-limiting, toxic form to a progressive, immunological form. In their report on DICC secondary to various topical medications Ostler et al. [18] undertook skin patch testing and immunofluorescent staining of conjunctival biopsies. Patch testing of antibiotics or preservatives (benzalkonium, thimerosal and parabens), present in the topical medications used, was positive in 7 patients. Immunofluorescent staining was either negative or non-specific in most cases (9 of 13 patients investigated). The authors suggested that either a delayed hypersensitivity or toxic reaction, to either the medications or their preservative, was responsible. In a further report immunofluorescent staining of conjunctival biopsies from patients with idoxuridine-induced DICC was again either negative or non-specific and the authors favoured a non-immunological aetiology [8].

Table 5. Cellular and histopathological features of DICC

1. Reduction in the number of goblet cells
2. Epithelial keratinisation
3. Squamous metaplasia
4. Loss of microvilli
4. Increased numbers of desmosomes
5. Bullous separation of the epithelium
6. Increase in the number of subepithelial fibroblasts and degree of fibrosis
7. Reduced intravascular space
8. Increase in the number of subepithelial lymphocytes and plasma cells
9. Basement membrane thickening
10. Basement membrane staining for immunoglobulin (in some cases)

At a cellular level, the process of conjunctival cicatrisation involves a cascade of events initiated by subepithelial inflammation which subsequently induces fibroblast proliferation and eventual collagen deposition in a manner similar to that seen in wound healing. The localised inflammatory cell population, and in particular macrophages produce a cocktail of fibrogenic cytokines which incites a fibroblast reaction. The main fibrogenic cytokines thought to play a role include transforming growth factor beta, platelet-derived growth factor, basic fibroblast growth and tumour necrosis factor alpha [47–49]. However, the process is undoubtedly highly complex involving many cellular interactions. The cell profile associated with most chronic conjunctival diseases is mixed suggesting that the differences in natural history between the conditions is dependent on the activity of the cells rather than their actual number or type. Studies investigating the roles of cytokines and lymphokines are thus crucial to our further understanding of the pathogenesis of DICC and all other cicatrising conditions (table 5).

Pathogenesis
Essentially, the pathogenesis of DICC is unknown and the immunological features reported to date have been inconsistent. Self-limiting cicatrisation is thought to represent a 'toxic reaction' but this is poorly understood. Progressive DICC, which continues to advance despite drug cessation is thought to represent an 'immunological reaction' similar to that which is associated with OCP. Hence, direct immunofluorescence of the conjunctival basement membrane has shown the deposition of immunoglobulin in certain cases of DICC. This has been indistinguishable from the immunostaining associated with OCP and thus conjunctival biopsy is of no value in differentiating DICC from OCP. In view of the similarities between certain cases of DICC and OCP, it is possible

Table 6. Potential aetiologies for DICC [11, 17, 50, 51]

1. Drug use and the development of conjunctival cicatrisation may be independent events and occur coincidentally
2. The drug may cause conjunctival scarring with mimics OCP, but is not OCP because the scarring is usually non-progressive
3. The drug may trigger the development of OCP, to which the patient may be predisposed
4. The drug may be the cause of OCP, DICC being identical to OCP
5. DICC represents a spectrum of diseases ranging from a self-limiting, toxic form to a progressive, immunological form
6. Pre-existing but undiagnosed OCP causes scarring which may obstruct collecting vessels of the outflow pathway and result in glaucoma requiring topical therapy which induces DICC

that factors associated with the administration of topical medications somehow induce a pathological process identical to OCP which itself is induced by other unknown factors. Chronic low-grade conjunctival inflammation and/or direct tissue damage following chronic administration of topical agents may alter the antigenicity of the basement membrane inducing an auto-immune reaction identical to that of OCP. Such a reaction may only occur in certain patients, perhaps those predisposed to pemphigoid. Against this hypothesis is the fact that reported cases of DICC have not been associated with extraocular mucous membrane cicatrisation, although to counteract this, it is unknown whether extraocular mucous membrane basement membranes have the same antigenicity as the conjunctival basement membrane (table 6).

The Sequelae of Drug-Induced Conjunctival Cicatrisation

Secondary changes associated with DICC are as for OCP and are discussed in a separate section (pp. 176–191).

Management of Drug-Induced Conjunctival Cicatrisation

DICC is not always progressive after withdrawal of the causative drug. Thus it is essential that suspected medications are withdrawn as early as possible to maximise the chance of aborting the fibrotic response before the onset of the irreversible sequelae. It should not, however, be assumed that cessation of the suspected medication will result in suspension of the disease process, and the patient should be carefully monitored for the progressive type

of cicatrisation. In situations where a topical preparation is suspected, but discontinuation of the drug is not considered a suitable option, the medication should be represcribed as an unpreserved preparation. Again, the patient should be carefully monitored and other treatment options for the primary disorder considered if the change in preparation makes no difference. Management of the progressive forms of DICC is as for OCP and is discussed later in this volume (pp. 192–241). An important point to make is that conjunctival inflammation secondary to the sequelae of the cicatricial process, such as superadded bacterial conjunctivitis or trichiasis, may be attributed to the cicatricial disease process incorrectly. Treatment of these complications may be all that is required. Furthermore, such complications will almost always exert an adverse effect on the cicatricial process itself and should thus be dealt with promptly or, better still, prevented. The management of sequelae and concurrent disease is discussed elsewhere (pp. 192–241).

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References

1 Wright P: Untoward effects associated with practolol administration: Oculomucocutaneous syndrome. Br Med J 1975;i:595–598.
2 Rahi AHS, Chapman CM, Garner A, Wright P: Pathology of practolol-induced ocular toxicity. Br J Ophthalmol 1976;60:312–323.
3 Grant WM: Toxicology of the Eye. Springfield, Thomas, 1974, p 653.
4 Wilson FM: Adverse external ocular effects of topical ophthalmic medications. Surv Ophthalmol 1979;24:57–88.
5 Theodore FH: Drug sensitivities and irritations of the conjunctiva. JAMA 1953;151:25–30.
6 Cvetkovic D, Parunovic A, Kontic DJ: Konjunktivale Veränderungen bei der lokalen langjährigen medikamentösen Therapie des Glaukoms. Fortschr Ophthalmol 1986;83:407–409.
7 Schaffer RN, Ridgway WL: Furmethide iodide in the production of dacryostenosis. Am J Ophthalmol 1951;34:718–720.
8 Lass JH, Thoft RA, Dohlman CH: Idoxuridine-induced conjunctival cicatrization. Arch Ophthalmol 1983;101:747–750.
9 Kristensen EB, Norn MS: Benign mucous membrane pemphigoid. 1. Secretion of mucus and tears. Acta Ophthalmol 1974;52:266–281.
10 Hoffer KJ: Pemphigoid related to epinephrine treatment (letter). Am J Ophthalmol 1977;83:601.
11 Pouliquen Y, Patey A, Foster CS, Goichot L, Savoldelli M: Drug-induced cicatricial pemphigoid affecting the conjunctiva: Light and electron microscopic features. Ophthalmology 1986;93:775–783.
12 Wright P: Cicatrizing conjunctivitis. Trans Ophthalmol Soc UK 1986;105:1–17.
13 Patten JT, Cavanagh HD, Allansmith MR: Induced ocular pseudopemphigoid. Am J Opthalmol 1976;82:272–276.
14 Hirst LW, Werblin T, Novak M, Green WR, Pollack I: Drug-induced cicatrizing conjunctivitis simulating ocular pemphigoid. Cornea 1982;1:121–128.
15 Tseng SCG, Maumenee AE, Stark WJ, Maumenee IH, Jensen AD, Green WR, Kenyon KR: Topical retinoid treatment for various dry-eye disorders. Ophthalmology 1985;92:717–727.
16 Leonard JN, Hobday CM, Haffenden GP, Griffiths CEM, Powles AV, Wright P, Fry L: Immunofluorescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.
17 Fiore PM, Jacobs IH, Goldberg DB: Drug-induced pemphigoid: A spectrum of diseases. Arch Ophthalmol 1987;105:1660–1763.

18   Ostler HB, Okumoto M, Daniels T, Constant M: Drug-induced cicatrization of the conjunctiva; in O'Connor GR (ed): Immunologic Diseases of the Mucous Membranes: Pathology, Diagnosis and Treatment. New York, Masson, 1980, pp 149–158.

19   Schwab IR, Linberg JV, Gioia VM, Benson WH, Chao GM: Foreshortening of the inferior conjunctival fornix associated with chronic glaucoma medications. Ophthalmology 1992;99:197–202.

20   Liesegang TJ: Bulbar conjunctival follicles associated with dipivefrin therapy. Ophthalmology 1985; 92:228–233.

21   Corwin ME, Spencer WH: Conjunctival melanin deposits. Arch Ophthalmol 1963;69:317–321.

22   Podos SM: Pharmacology of ocular drugs. 2. Epinephrine. Ophthalmology 1980;87:721–723.

23   Wright P: Squamous metaplasia or epidermalization of the conjunctiva as an adverse reaction to topical medication. Trans Ophthalmol Soc UK 1979;99:244–246.

24   Derous D, de Keizer RJW, de Wolff-Rouendaal D, Soudijn W: Conjunctival keratinisation, an abnormal reaction to an ocular beta-blocker. Acta Ophthalmol 1989;67:333–338.

25   Sherwood MB, Grierson I, Millar L, Hitchings RA: Long-term morphologic effects of antiglaucoma drugs on the conjunctiva and Tenon's capsule in glaucomatous patients. Ophthalmology 1989;96: 327–335.

26   Smith DL, Skuta GL, Kincaid MC, Rabbani R, Cruess DF, Kao SF: The effects of glaucoma medications on Tenon's capsule and conjunctiva in the rabbit. Ophthalmic Surg 1991;22:336–340.

27   Gerstenberger A, Marquardt R: Die Becherzelldichte unter Pilocarpineinfluss. Fortschr Ophthalmol 1986;83:46–50.

28   Broadway DC, Grierson I, O'Brien C, Hitchings RA: Adverse effects of topical antiglaucoma medication. I. The conjunctival cell profile. Arch Ophthalmol 1994;112:1437–1445.

29   Baun O, Heegaard S, Kessing SV, Prause JU: The morphology of conjunctiva after long-term topical anti-glaucoma treatment: A quantitative analysis. Acta Ophthalmol Scand 1995;73: 242–245.

30   Baudouin C, Fillacier KM, Bechetoille A, Ettaiche M, Gastaud P: Immunohistological evidence of inflammatory cells and fibroblasts in conjunctival and trabecular specimens in glaucoma (abstract). Ophthalmology 1995;102:135.

31   Herreras JM, Pastor JC, Calonge M, Asensio VM: Ocular surface alteration after long-term treatment with an antiglaucomatous drug. Ophthalmology 1992;99:1082–1088.

32   Varga M, Follmann P: Feinstrukturelle Untersuchungen der Bindehautoberfläche nach Langzeitbehandlung mit Timolol. Fortschr Ophthalmol 1986;83:155–157.

33   Sagdic Yalvac I, Gedikoglu G, Karagoz Y, Akgun U, Nurozler A, Koc F, Kasim R, Duman S: Effects of antiglaucoma drugs on ocular surface. Acta Ophthalmol Scand 1995;73:246–248.

34   Meyer E, Scharf Y, Zonis S: Effect of topical timolol on the rabbit and human conjunctiva: Ultrastructural study. Glaucoma 1989;11:55–60.

35   Quaranta CA, Russo L, Rossi Brunori P, Vigasio F, Ballini S, Grigolato PG: Osservazioni ultrastrutturali sugli ipotetici danni congiuntivali da farmaci beta-bloccanti. Boll Oculist 1988; 67(suppl 3):345–351.

36   Hoffman JP: Timoptic product information summary: Toxicological properties – Animals. West Point, Merck, Sharp & Dohme, 1978, p 7.

37   Steuhl KP, Knorr M, Frohn A, Thiel H-J: Über den Einfluss antiglaukomatöser Augentropfen auf die Zelldifferenzierung der Konjunktiva. Fortschr Ophthalmol 1991;88:865–869.

38   Brandt JD, Wittpenn JR, Katz LJ, Steinmann WN, Spaeth GL: Conjunctival impression cytology in patients with glaucoma using long-term topical medication. Am J Ophthalmol 1991;112:297–301.

39   Takahashi N: Cytotoxicity of preservatives on cultured human conjunctival cells. Acta Soc Ophthalmol Jpn 1980;84:1171–1176.

40   Takahashi N: Cytotoxic effects of antiglaucoma agents on cultured human conjunctival cells. Acta Soc Ophthalmol Jpn 1981;85:1046–1052.

41   Takahashi N: Cytotoxicity of mercurial preservatives in cell culture. Ophthalmic Res 1982;14:63–69.

42   Takahashi N: Quantitative cytotoxicity of preservatives evaluated in cell culture with Chang's human conjunctival cells – Effect of temperature on cytotoxicity. Jpn J Ophthalmol 1982;26:234–238.

43 Takahashi N: A new method evaluating quantitative time-dependent cytotoxicity of ophthalmic solutions in cell culture. Beta-adrenergic blocking agents. Graefe's Arch Clin Exp Ophthalmol 1983; 220:264–267.

44 Williams DE, Nguyen KD, Shapourifar-Tehrani S, Kitada S, Lee DA: Effects of timolol, betaxolol, and levobunolol on human Tenon's fibroblasts in tissue culture. Invest Ophthalmol Vis Sci 1992; 33:2233–2241.

45 Pande M, Ghanchi F: The role of preservatives in conjunctival toxicity of subconjunctival gentamicin injection. Br J Ophthalmol 1992;76:235–237.

46 Mietz H, Niessen U, Krieglstein GK: The effect of presevatives and antiglaucomatous medication on the histopathology of the conjunctiva. Graefe's Arch Clin Exp Ophthalmol 1994;232:561–565.

47 Saks EH, Jakobiec FA, Wieczorek R, Donnenfeld E, Perry H, Knowles DM: Immunophenotypic analysis of the inflammatory infiltrate in ocular cicatricial pemphigoid: Further evidence for a T-cell mediated disease. Ophthalmology 1989;96:236–243.

48 Rice BA, Foster CS: Immunopathology of cicatricial pemphigoid affecting the conjunctiva. Ophthalmology 1990;97:1476–1483.

49 Bernauer W, Wright P, Dart JK, Leonard J, Lightman S: The conjunctiva in acute and chronic mucous membrane pemphigoid: An immunohistochemical analysis. Ophthalmology 1993;100:339–346.

50 Tauber J, Melamed S, Foster CS: Glaucoma in patients with ocular cicatricial pemphigoid. Ophthalmology 1989;96:33–37.

51 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.

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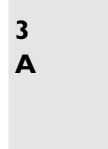
Immunological Investigations in the Evaluation of Patients with Cicatrising Conjunctivitis

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Introduction

The principal aims in the evaluation of patients with cicatrising conjunctivitis are: (1) the distinction between temporally limited and chronic progressive conditions; (2) the classification of progressive disease; (3) the assessment of disease activity, and (4) the investigation of disease mechanisms. While immunological tests are important in this assessment, they do not always provide diagnostic information and therefore cannot replace clinical considerations, as described in the Introduction and in the chapter on 'Monitoring of Activity and Progression'. The only *diagnostic* technique currently available is the analysis of biopsy specimens by immunopathological methods. Additional laboratory investigations provide exciting insights into disease mechanisms, but do not help in the clinical management of these patients [1, 2].

Diagnostic Techniques

Detection of Immunoreactants at the Basement Membrane Zone
Ocular cicatricial pemphigoid (OCP) is postulated to be initiated by the binding of circulating antibodies to the basement membrane of the conjunctiva with subsequent complement activation. This results in the biopsy finding of *linear* deposits of immunoglobulin and/or complement at the conjunctival basement membrane and is generally regarded as diagnostic of OCP [3–6]. This finding is characteristic but non-specific. Identical biopsy findings are
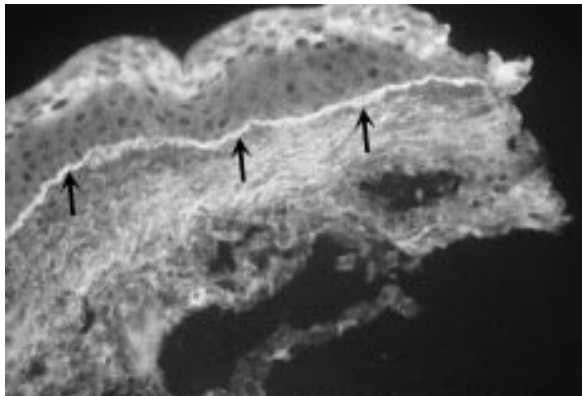
Fig. 1. OCP: immunofluorescence photograph of a conjunctival biopsy specimen. The snap-frozen tissue has been cryostat-sectioned and stained with fluorescein-labelled anti-human IgA (direct immunofluorescence). Note the line of homogeneous fluorescence of the epithelial BMZ revealing the presence of deposits of IgA in this region. Original magnification × 100.

seen in conjunctivae of patients with bullous pemphigoid, linear IgA disease, certain paraneoplastic syndromes and some progressive forms of drug-induced conjunctival cicatrisation (see previous chapters pp. 64–101) [7–13].

In ocular disease, biopsies are typically taken from the bulbar conjunctiva, in the presence of extra-ocular manifestations also from other mucous membranes and lesional skin. Direct immunofluorescence is the classical investigation to demonstrate the diagnostic linear deposits of immunoreactants at the basement membrane zone (BMZ). This technique is routinely used in most dermatology units, and the ophthalmologist should seek collaboration with a dermatologist for the evaluation of such patients.

Direct Immunofluorescence. Principles: immunofluorescence techniques are usually performed on tissue that had been deep frozen. This ensures that labile antigens are not damaged by fixatives. For direct immunofluorescence, the test solution of fluoresceinated antibody is applied to the section in a drop, incubated and washed off. Any bound antibody is then revealed under the microscope; UV light is directed onto the section through the objective, thus the field is dark and areas with bound fluorescent antibody fluoresce green (fig. 1–4) [1, 14].

Technique for the detection of immunoglobulin and complement deposition as used by the authors: the biopsy specimens are obtained under local anaesthesia, immediately embedded in OCT medium (Lab-Tek Inc.), frozen in liquid nitrogen and stored at –70 °C. Cryostat sections (4 μm) of the tissues
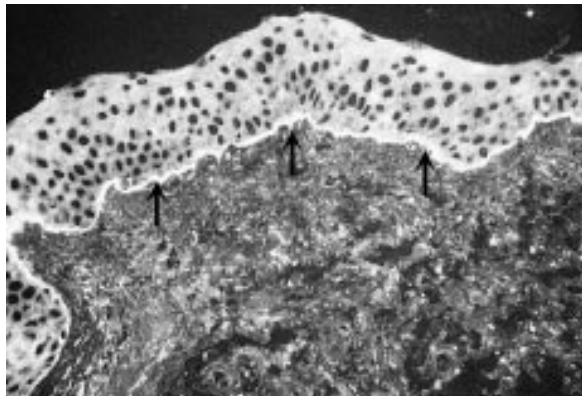
Fig. 2. Cicatricial pemphigoid with involvement of skin and mucous membranes. Skin biopsy specimen. Direct immunofluorescence shows homogeneous linear deposition of IgG. Frozen section. Original magnification × 100.
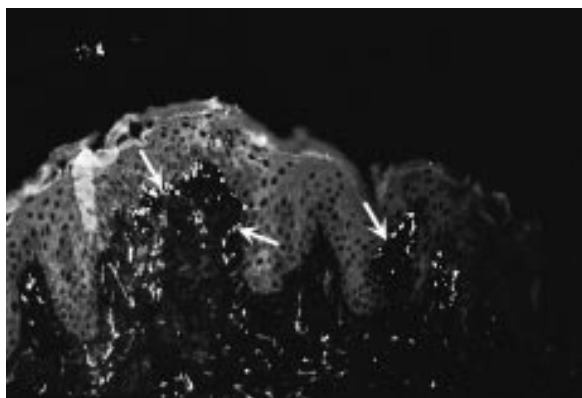

Fig. 3. In contrast to cicatricial pemphigoid, perilesional skin in dermatitis herpetiformis shows *granular* deposits of IgA in direct immunofluorescence. Frozen section. Original magnification × 80.

are cut and air dried on gelatin-coated slides. Fluoresceinated sheep antihuman antibodies (Dako Laboratories), appropriately diluted in phosphate-buffered saline, are then applied to each tissue. Antibodies that are routinely used include those directed against human IgA, IgG, IgM, C3, and fibrinogen. After 30 min of incubation at room temperature the tissues are thoroughly washed, mounted and viewed in a Zeiss fluorescence microscope.
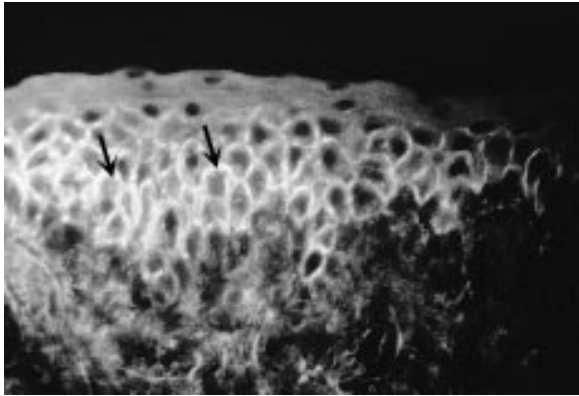
Fig. 4. Pemphigus vulgaris. Direct immunofluorescence of perilesional skin demonstrating IgG in the intercellular space. Frozen section. Original magnification × 280.

Comment: results of immunofluorescence analysis unfortunately are frequently negative or inconclusive. Positive results can be expected in about 50% of the patients with OCP [15, 16]. A negative biopsy, therefore, does not rule out this diagnosis. Furthermore, patients with cicatricial pemphigoid who have positive conjunctival findings at a first assessment can become negative after clinical remission following treatment, indicating that these deposits vary with disease activity [15]. It is our experience that negative biopsy findings are often associated with severe conjunctival inflammation and we found that the number of deposits is highest in *mild* ocular inflammation [15]. The acute inflammatory process may destroy the linear deposits.

The use of split tissue by chemical separation through the lamina lucida is an established method to increase the sensitivity of immunofluorescence techniques in the analysis of BMZ pathology [17]. Split tissue with good morphology can be easily obtained with skin or large mucous membrane specimens. Conjunctival biopsies from patients with cicatrising conjunctivitis, however, typically are tiny and delicate to handle. This technique is therefore hardly applicable for routine evaluation [17].

The use of the avidin-biotin immunoperoxidase technique in addition to immunofluorescence analysis was suggested to increase the diagnostic yield in patients with clinical suspicion of OCP [16]. In a large series, Power et al. [16] found that processing of immunofluorescent-negative or inconclusive conjunctival biopsies by the immunoperoxidase technique increased the sensitivity from 52% with immunofluorescence alone to 83% [16]. We used very thin sections of glycol-methacrylate-embedded tissues with excellent morphology for the peroxidase technique and found that a differentiation between

diffuse tissue staining due to the normal presence of immunoreactants in inflamed conjunctiva and the actual linear deposits was not possible in most cases [unpubl. data]. Visualization of the diagnostic linear deposits from the 'background noise' of signals is still best achieved by the immunofluorescence technique.

Investigative Procedures

The presence and titre assessment of circulating antibodies against dermal or epidermal components are helpful for the evaluation and monitoring of several bullous skin disorders [18]. In contrast, sera from patients with OCP have a low prevalence of circulating antibodies against BMZ components [3, 19–21]. There is growing evidence, however, that anti-BMZ auto-antibodies play an essential role in its pathogenesis [21–24]. Anti-BMZ auto-antibodies are occasionally found in drug-induced conjunctival cicatrisation [6] and it is likely that sensitive methods will detect them in cicatrising *ocular disease* associated with linear IgA disease and some paraneoplastic disorders. Currently, there is only little information on the target-antigen(s) of auto-antibodies within the conjunctival BMZ and the dermo-epidermal junction, and this is a central subject of ongoing research (see also the chapter on 'The Immunologic Target: Antigenic Aspects of Basement Membranes').

The immunological techniques that are used most frequently for the investigation of auto-immune disorders of the BMZ include, in addition to direct immunofluorescence with its variations, indirect immunofluorescence for the assessment of circulating antibodies, and immunoblotting and immunoprecipitation for the characterisation of potential target antigens.

Detection of Circulating Anti-Basement Membrane Zone Antibodies
Indirect Immunofluorescence. Principles: the patient's serum is added to sections of *normal* tissue and then the pathological binding site of the antibodies is determined by immunofluorescence.

Technique for the detection of circulating anti-BMZ antibodies as used by the authors: the sera are diluted 1:10 in phosphate-buffered saline and incubated with the sections of substrate for 30 min at room temperature. 1 mol/l of sodium chloride salt-split-skin and mucosa, including normal human conjunctiva, serve as substrates. The slides are washed three times in phosphate-buffered saline, and then incubated with the antihuman antibodies. The further steps are as for direct immunofluorescence.

Comments: the presence of circulating anti-BMZ antibodies in the serum of patients with OCP is variable, their detection dependent on the technique

and the substrate that is used [6, 17]. With *fresh conjunctiva* as a substrate, it was shown that anti-BMZ antibodies are present in up to 40% of patients with cicatricial pemphigoid, but when the same sera were tested on non-split oral mucous membrane or skin, no antibodies were detected [6]. Chemical splitting of the substrate tissue may increase the sensitivity of indirect immuno-fluorescence up to 88% in cicatricial pemphigoid [17]. The detected anti-BMZ antibodies are most frequently of the IgG and IgA class [6, 17], but IgM antibodies have also been described [6, 17].

Indirect immunofluorescence data alone do not allow any conclusions on the clinical features or course of cicatricial pemphigoid.

Detection of Target Antigens

Immunoblot Analysis. Principles: immunoblotting is a technique to identify and characterise previously unknown antigens from a complex mixture. In immunoblotting for the evaluation of bullous disorders, cell lysates – typically of cultured keratinocytes – are resolved in analytical separation gels. By using different gels (sodium dodecyl sulphate, iso-electric focusing or peptide-mapping gels), it is possible to obtain data on the size, iso-electric point and molecular relationships of the antigens that are being investigated. After this initial separation, the resolved molecules are electrophoretically transferred to membranes (blots) and treated with the patient's serum. A radiolabelled conjugate is added to detect the antibodies that are bound to the blot. Finally, the blot is placed in contact with x-ray film to visualise the antigen bands which have bound the antibody.

Immunoprecipitation. An antigen may become so denatured by the gel separations and blotting procedures that some of its epitopes are destroyed and can no longer bind to particular antibodies. In these cases, immunoprecip-itation instead of immunoblotting is used to identify which antigen an antibody binds to. Immunoprecipitation is more sensitive than immunoblotting.

Principles: in the evaluation of bullous disorders, the antigens are derived from the surface of keratinocytes from cell cultures, which are solubilised with detergents. This antigen mixture being tested is biosynthetically radiolabelled and patient's serum added, which then binds to its specific antigens. These complexes are precipitated by the addition of co-precipitating agents, such as anti-immunoglobulin antibodies. The insoluble complexes are spun down and washed to remove any unbound labelled antigens. Then the precipitate is resolubilised and the components separated on analytical gels. After running, the fixed gels are autoradiographed, to show the position of the specific labelled antigen.

Comments: in cicatricial pemphigoid several potential target antigens have been identified on immunoblots. These include a 45-kD molecule in pure OCP

[23], epidermal components of 90-kD [22], 120-kD [25], 130-kD, 140-kD, 205-kD [22], and the 170- to 180-kD and 220- to 230-kD bullous pemphigoid antigens [20–23, 26]. Reactivity to the 120-kD keratinocyte antigen, however, occurs also in normal serum samples and therefore does not represent a specific cicatricial pemphigoid autoantigen [21]. Immunoprecipitation techniques identified a single large protein with a molecular weight greater or equal to 600 kD that bound to *IgG* antibodies of certain patients with cicatricial pemphigoid. Further analyses identified this multi-subunit protein as epiligrin/laminin 5 [24, 27, 28], a major component of the extracellular matrix of cultured human keratocytes (see also the chapter on 'The Immunologic Target: Antigenic Aspects of Basement Membranes').

This large number of potential cicatricial pemphigoid antigens may appear confusing and indicates heterogeneity among patients with similar clinical features [29]. There are, however, findings that may help to further classify and understand the group of disorders encompassed by the term 'cicatricial pemphigoid': two studies found that sera of patients with pure OCP (ocular lesions only) did not react with the bullous pemphigoid antigens [21, 23]. Their *IgA* antibodies reacted with a 45-kD BMZ antigen [21] and it was suggested to classify these patients as a separate clinical and immunopathological entity, called 'pure ocular cicatricial pemphigoid' [21]. It was further suggested that antibodies of the *IgA class* that are directed against the bullous pemphigoid antigens (170–180 and 220–230 kD) may be related to the occurrence of mucosal lesions [21, 22]. Patients with anti-epiligrin antibodies typically have lesions of their mucous membranes *and* skin [24, 28]. These lesions are indistinguishable from those in other forms of cicatricial pemphigoid. Based on the immunological findings, anti-epiligrin cicatricial pemphigoid is now recognised as a pathologically distinct subgroup [24, 27, 28] and an animal model that uses injections of laminin 5 into newborn mice is being investigated [30].

Prospects

Patients with acquired auto-immune bullous diseases have antibodies that target specific antigens in normal human skin and mucous membranes, including the conjunctiva. These auto-antibodies serve as diagnostic markers. In chronic progressive conjunctival cicatrisation, the investigation of auto-antibodies has only started recently. It may be expected, however, that the assessment of antilaminin 5 and further auto-antibodies will help to evaluate patients with cicatrising conjunctivitis in the future.

References

1   Helm KF, Peters MS: Immunodermatology update: The immunologically mediated vesiculobullous diseases. Mayo Clin Proc 1991;66:187–202.

2   Marren P, Wojnarowska F: The diagnosis of immuno-bullous diseases. J Eur Acad Dermatol Venerol 1992;1:255–264.

3   Mondino BJ, Ross AN, Rabin BS, Brown SI: Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 1977;83:443–450.

4   Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.

5   Bean SF, Waisman M, Michel B, Thomas SI, Knox JM, Levine M: Cicatricial pemphigoid. Arch Dermatol 1972;106:195–199.

6   Leonard JN, Hobday CM, Haffenden GP, Griffiths CEM, Powles AV, Wright P, Fry L: Immunofluorescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.

7   Leonard JN, Haffenden GP, Ring NP, McMinn RMH, Sidgwick A, Mowbray JF, Unsworth DJ, Holborow EJ, Blenkinsopp WK, Swain AF, Fry L: Linear IgA disease in adults. Br J Dermatol 1982;107:301–316.

8   Frith PA, Venning VA, Wojnarowska F, Millard PR, Bron AJ: Conjunctival involvement in cicatricial and bullous pemphigoid: A clinical and immunopathological study. Br J Ophthalmol 1989;73:52–56.

9   Anhalt GJ, Kim S, Stanley JR, Korman NJ, Jabs DA, Kory M, Izumi H, Ratrie III H, Mutasim D, Ariss-Abdo L, Iabib RS: Paraneoplastic pemphigus: An autoimmune mucocutaneous disease associated with neoplasia. N Engl J Med 1990;323:1729–1735.

10 Helm TN, Camisa C, Valenzuela R, Allen CM: Paraneoplastic pemphigus. A distinct autoimmune vesiculobullous disorder associated with neoplasia. Oral Surg Oral Med Oral Pathol 1993;75:209–213.

11 Bystrin JC, Hodak E, Gao S-Q, Chuba VJ, Amorosi EL: A para-neoplastic mixed bullous skin disease associated with anti-skin antibodies and a B-cell lymphoma. Arch Dermatol 1993;129:870–875.

12 Pouliquen Y, Patey A, Foster CS, Goichot L, Savodelli M: Drug-induced cicatricial pemphigoid affecting the conjunctiva. Light and electron microscopic features. Ophthalmology 1986;93:775–783.

13 Fiore PM, Jacobs IH, Goldberg DB: Drug-induced pemphigoid. A spectrum of diseases. Arch Ophthalmol 1987;105:1660–1663.

14 Beutner EH, Nisengard RJ, Kumar VJ: Defined immunofluorescence: Basic concepts and their application to clinical immunodermatology; in Beutner EH, Chorzelski TP, Bean SF (eds): The Immunopathology of the Skin. New York, Wiley, 1979, pp 29–72.

15 Bernauer W, Elder MJ, Leonard J, Wright P, Dart JKG: The value of biopsies in the evaluation of chronic progressive conjunctival cicatrisation. Graefe's Arch Clin Exp Ophthalmol 1994;232:533–537.

16 Power WJ, Neves RA, Rodriguez A, Dutt JE, Foster CS: Increasing the diagnostic yield of conjunctival biopsy in patients with suspected ocular cicatricial pemphigoid. Ophthalmology 1995;102:1158–1163.

17 Kelly SE, Wojnarowska F: The use of chemically split tissue in the detection of circulating anti-basement membrane zone antibodies in bullous pemphigoid and cicatricial pemphigoid. Br J Dermatol 1988;118:31–40.

18 Pye RJ: Bullous eruptions; in Rook A, Wilkinson DS, Ebling FJG (eds): Textbook of Dermatology edited by Champion RH, Burton JL, Eblin FJG. Oxford, Blackwell, vol 3, 1992, pp 1623–1673.

19 Fine JD, Neises GR, Katz SI: Immunofluorescence and immunoelectron microscopic studies in cicatricial pemphigoid. J Invest Dermatol 1984;82:39–43.

20 Bernard P, Prost C, Lercerf V, Intrator L, Combemale P, Bedane C, Roujeau JC, Revuz J, Bonnetblanc JM, Duberet L: Studies of cicatricial pemphigoid autoantibodies using direct immunoelectron microscopy and immunoblot analysis. J Invest Dermatol 1990;94:630–635.

21 Chan LS, Yancey KB, Hammerberg C, Soong HK, Regezi JA, Johnson K, Cooper KD: Immune-mediated subepithelial blistering diseases of mucous membranes. Arch Dermatol 1993;129:448–455.

22 Chan LS, Hammerberg C, Cooper KD: Cicatricial pemphigoid. Identification of two distinct sets of epidermal antigens by IgA and IgG class circulating autoantibodies. Arch Dermatol 1990;126:1466–1468.

23 Smith EP, Taylor TB, Meyer LJ, Zone JJ: Identification of a basement membrane zone antigen reactive with circulating IgA antibody in ocular cicatricial pemphigoid. J Invest Dermatol 1993; 101:619–623.

24 Domloge-Hultsch N, Anhalt GJ, Gammon WR, Lazarova Z, Briggaman R, Welch M, Jabs DA, Huff C, Yancey KB: Antiepiligrin cicatricial pemphigoid. A subepithelial bullous disorder. Arch Dermatol 1994;130:1521–1529.

25 Sarret Y, Reano A, Nicolas JF, Su H, Thivolet J: Bullous pemphigoid and cicatricial pemphigoid: Immunoblotting detection of involved autoantigens. Autoimmunity 1989;2:145–153.

26 Balding SD, Prost C, Diaz LA, Bernard P, Bedane C, Aberdam D, Giudice GJ: Cicatricial pemphigoid autoantibodies react with multiple sites on the BP 180 extracellular domain. J Invest Dermatol 1996;106:141–146.

27 Kirtschig G, Marinkovich PM, Burgeson RE, Yancey KB: Anti-basement membrane autoantibodies in patients with anti-epiligrin cicatricial pemphigoid bind the alpha subunit of laminin 5. J Invest Dermatol 1995;105:543–548.

28 Yancey KB, Kirtschig G, Yee C, Lazarova Z: Studies of patients with anti-epiligrin cicatricial pemphigoid. J Dermatol 1995;22:829–835.

29 Ghohestani RF, Rousell P, Nicolas JF, Sassolas B, Faure M, Claudy AI: Heterogeneity of the target antigens in the cicatricial pemphigoid. J Invest Dermatol 1996;106:278.

30 Lazarova Z, Yee C, Darling T, Briggaman RA, Yancey KB: Passive transfer of anti-laminin 5 antibodies induces subepidermal blisters in neonatal mice. J Clin Invest 1996;98:1509–1518.

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Monitoring of Activity and Progression in Cicatrising Conjunctivitis

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The objectives of management of patients with chronic progressive cicatrising conjunctivitis are to resolve any conjunctival inflammation, to treat other ocular and – if there are any – systemic features of disease and to prevent progression of cicatrisation. For the assessment of disease activity and progression, and to determine the effect of therapeutic interventions, reproducible systems are required to allow monitoring of conjunctival changes. This section discusses the various means of documenting cicatrising conjunctivitis and examines how the various grading systems may be clinically useful.

Important indices to assess at each clinical visit are the amount of conjunctival cicatrisation, the inflammatory disease activity and the corneal changes. For cicatrising conjunctivitis, there are two established staging systems in use that have been developed for ocular cicatricial pemphigoid (OCP): that of Mondino [1–3] (table 1) and the system of Foster [4] (table 2A). More recently, there has been a modification of Foster's classification to try to more accurately define the subtle signs of progression (table 2B) [5].

At Moorfields Eye Hospital we use a standardised protocol for the monitoring of these patients (table 3). Drawings are used in conjunction with standard position photographs to allow documentation of subtle signs and to compare the clinical data of our patients with those defined and ranged by Mondino and Foster [6].

Classification of the Cicatrisation

The clinical grading of conjunctival cicatrisation (fig. 1) is of outermost importance in the management of cicatrising conditions and has several uses.

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Table 1. Mondino's staging system [1–3]

Mondino Stages	Characteristics
I	0–25% loss of inferior fornix depth
II	25–50% loss of inferior fornix depth
III	50–75% loss of inferior fornix depth
IV	75–100% loss of inferior fornix depth

Table 2A. Foster's staging system [4]

Foster stages	Characteristics
I	subconjunctival scarring and fibrosis
II	fornix foreshortening of any degree
III	presence of symblepharon, any degree
IV	ankyloblepharon, frozen globe

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First is to document the ongoing effects of disease. Second, it can be used as clinical guide to the adequacy of treatment. Third, it is required to assess the outcome of new therapeutic agents. Lastly, it may be diagnostic in that it ultimately distinguishes between progressive and non-progressive disease.

Mondino's system uses only the lower fornix depth and subdivides patients into four categories depending on the degree of lower fornix shortening. For example, if the fornix is reduced by 0–25% of normal, then it is stage I, if it is reduced by 25–50% of normal, it is stage II (table 1). The inferior fornix depth is normally 11.0 ± 0.12 mm for 60- to 69-year-olds and 10.1 ± 0.17 mm in patients 80 years and older (mean $\pm$ SE) [7]. Therefore, the stages of Mondino's system can be reproducibly quantified by measuring the depth, either using a 'dipstic' as described by Schwab et al. [7] or the vertical height of the light beam of the slit lamp. The lower fornix inferior to the 6 o'clock limbus should be used because it is a definable and repeatable location.

Foster's system relies on the presence of specific clinical signs. For example, stage I is cicatrisation of any clinically detectable degree, stage II is fornix foreshortening of any degree, stage III is the presence of symblepharon and stage IV is ankyloblepharon (table 2A). As the signs are very specific and easy to diagnose, the patient can be accurately assigned to the appropriate category.

Standardised drawings (table 3) allow a detailed documentation of various patterns of scarring. Such different patterns are described and illustrated in the Introduction of the volume; additional examples are shown in figures

Table 2B. Modification of Foster's staging system to describe degrees within stages [5]

Modified stages	Characteristics
I	subconjunctival scarring and fibrosis
II	fornix foreshortening
a–d	describes loss of inferior fornix depth
a	0–25%
b	25–50%
c	50–75%
d	75–100%
III (n)	presence and number of symblephara countable
a–d	describes horizontal involvement by symblephara
a	0–25%
b	25–50%
c	50–75%
d	75–100%
IV	ankyloblepharon, frozen globe

Example: IIbIIIb(2) = 50% fornix loss, 50% horizontal involvement of 2 discrete symblephara.
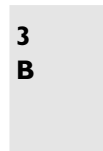
1–4. These drawings are best used in combination with clinical photographs. Photographs are independent of the observer and this helps to minimise inter-rater inconsistencies. Several pictures are required: the eye in the primary position, a view of the medial canthus with the eye in lateral gaze, a view of the lateral canthus with the eye in medial gaze, a view of the superior conjunctiva with the eye in downgaze, a view of the lower conjunctiva and fornix with the lower lid everted and a view of the upper lid tarsus with the lid everted (table 3B). The quality of the photographs very strongly depends on the photographer's skills, and good liaison is essential between them and the clinicians.

Documentation of the Conjunctival Inflammation

The amount of conjunctival inflammation present is an important variable in guiding daily management. Four stages of inflammation have been described, ranging from nil to severe and designated as +, + +, + + +, + + + + [3, 6]. We use a similar classification more accurately defined as: − = *absence* of conjunctival inflammation; + = *mild* inflammation: conjunctival hyperaemia

Table 3. Protocol for the monitoring of patients with progressive cicatrising conjunctivitis as it is used at Moorfields Eye Hospital for study purposes

Table 3A. Chronic progressive conjunctival cicatrisation: Cover Sheet

Surname	Name (label)	Cover-Sheet Date of examination Examined by

Personal medical history
- General health

- Autoimmune disorders
  (rheumatoid arthritis, lupus
  erythematosus,...)

- Disease onset
  – Onset of extra-ocular
    manifestations (date):
  – Onset of ocular involve-
    ment (date):

- Extra-ocular manifestations
  – Skin involvement (specify)   Y / N
  – Involvement of extra-ocular mucous   Y / N
    membranes
    (mouth, pharynx, nose, larynx, oesophagus,
    urethra, vagina, anus)

- Ocular disease
  – Visual handicap (unable to read, date)   RE
                                                 LE
  – Previous treatments (specify)

- Differential diagnosis (specify)
  ○ Physical
  ○ Chemical
  ○ Infections
  ○ Oculocutaneous/mucocutaneous
    disorders
  ○ Other systemic disorders
  ○ Drug induced
  – previous systemic medications
  – previous topical medications

Diagnosis:
Date of biopsy:
Biopsy results:

Table 3B. Chronic progressive conjunctival cicatrisation: follow-up

Surname	Name (label)	Follow-Up
		Date of examination
		Examined by

Current treatment

Present status
extra-ocular manifestations
  – Skin involvement (specify)   Y / N
  – Involvement of extraocular   Y / N
    mucous membranes
    (mouth, pharynx, nose, larynx,
    oesophagus, urethra, vagina, anus)

Complaints	RE	LE
Redness	○ no	○ no
	○ yes	○ yes
	○ no change	○ no change
	○ improved	○ improved
	○ worse	○ worse
Foreign body sensation	○ no	○ no
	○ yes	○ yes
	○ no change	○ no change
	○ improved	○ improved
	○ worse	○ worse

Photographs (seven photographs   Y / N
  each eye)
In primary gaze
Gaze right
Gaze left
Up-gaze (with lower lid gently pulled down,
  revealing the inferior fornix)
Down-gaze (with upper lid retracted)
Upper tarsus (flipped upper lid)
Lower tarsus (everted lower lid)

Findings	RE	LE
Visual acuity		

**3
B**

Table 3C. Chronic progressive conjunctival cicatrisation: follow-up

Surname	Name (label)	Follow-Up
		Date of examination
		Examined by

RE **Central lower fornix depth in mm (slit beam):**
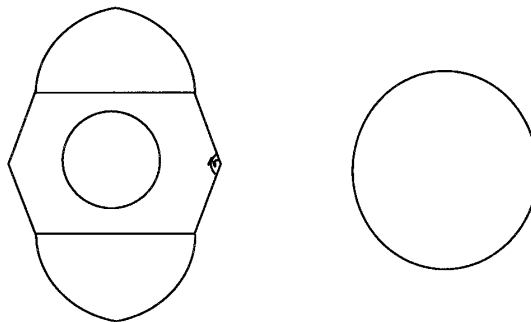
LE **Central lower fornix depth in mm (slit beam):**
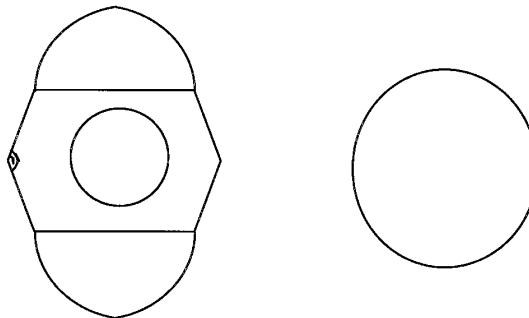
Shrinkage

Keratinisation

Superficial scarring/synechia

Punctate keratitis

Subepithelial fibrosis

Table 3D. Chronic progressive conjunctival cicatrisation: follow-up

Surname	Name (label)	Follow-Up
		Date of examination
		Examined by

Grading

RE   LE

Lids and lashes

○ normal	(0) ○ normal
○ canthal involvement	(2) ○ canthal involvement
○ trichiasis by aberrant lashes	(3) ○ trichiasis by aberrant lashes
○ entropion	(4) ○ entropion

Conjunctival inflammation

○ absent	(0) ○ absent
○ mild (mild hyperaemia and stromal swelling)	(1) ○ mild (mild hyperaemia and stromal swelling)
○ moderate (marked hyperaemia and thickening)	(2) ○ moderate (marked hyperaemia and thickening)
○ severe (all quadrants, limbitis, ulcers)	(4) ○ severe (all quadrants, limbitis, ulcers)

Symblepharon formation

○ absent	(0) ○ absent
○ one symblepharon	(2) ○ one symblepharon
○ two or more symblephara	(3) ○ two or more symblephara
○ ankyloblepharon	(4) ○ ankyloblepharon

Lower fornix shrinkage

○ absent, normal fornices	(0) ○ absent, normal fornices
○ 25% or less	(2) ○ 25% or less
○ 25–50%	(5) ○ 25–50%
○ 50–75%	(7) ○ 50–75%
○ obliteration of the fornices	(9) ○ obliteration of the fornices

Tear film

○ normal	(0) ○ normal
○ reduced break-up time	(1) ○ reduced break-up time
○ dry eye	(2) ○ dry eye

Ocular surface changes

○ no keratinisation	(0) ○ no keratinisation
○ keratinisation in one area	(2) ○ keratinisation in one area
○ keratinisation of the ocular surface	(4) ○ keratinisation of the ocular surface

Keratopathy

○ normal cornea	(0) ○ normal cornea
○ punctate keratitis	(1) ○ punctate keratitis
○ limbitis and/or peripheral scarring (not axis)	(3) ○ limbitis and/or peripheral scarring (not axis)
○ central opacities and/or vascularisation	(4) ○ central opacities and/or vascularisation

3
B

1a

1b

3
B

2a

2b

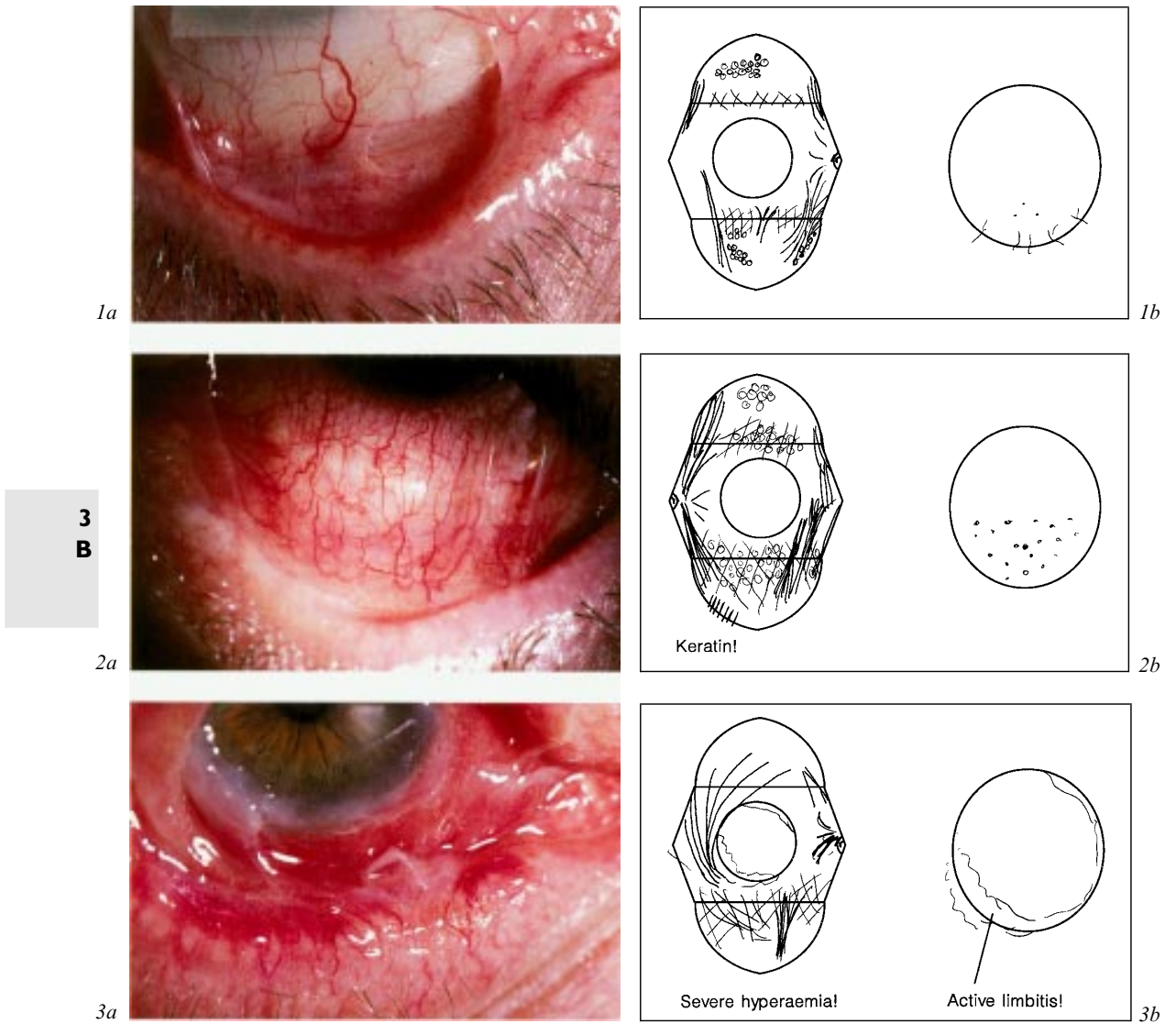Keratin!

3a

Severe hyperaemia!   Active limbitis!

3b

Fig. 1. *a* Mild inflammation in an eye with OCP showing a shortened lower fornix and two symblephara. *b* Changes as in schematic drawing.

Fig. 2. *a* Moderately inflamed conjunctiva in OCP. Note the conjunctival hyperaemia, oedema and tissue thickening. *b* Changes as in schematic drawing.

Fig. 3. *a,b* Severe inflammation and associated limbitis in OCP.
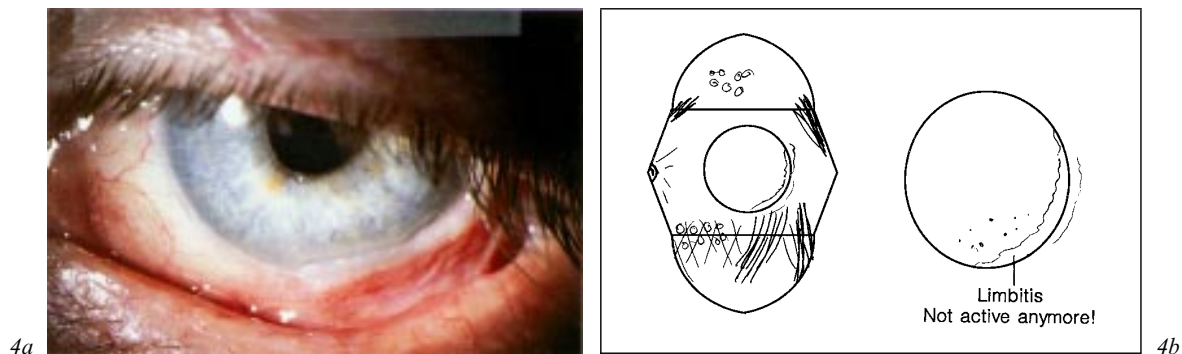
4a

4b

Limbitis
Not active anymore!

Fig. 4. a,b Limbitis with the characteristic white appearance that occurs as the inflammation resolves.

and mild stromal oedema; $+ + =$ *moderate* inflammation: extensive or marked conjunctival hyperaemia with stromal oedema and significant tissue thickening; there are no conjunctival ulceration or limbitis (fig. 2); $+ + + =$ *severe* inflammation: there is inflammation in all quadrants with severe ocular oedema; limbitis and conjunctival ulceration may be present (fig. 3).

3
B

Documentation of the Corneal Changes

The corneal disease that often accompanies conjunctival cicatrisation also needs careful documentation. This is first achieved by accurately measuring the visual acuity using standardised charts. Topical or systemic immunosuppression may cause refractive changes which must be accounted for, as must any lens opacities that may develop. Once these aspects have been considered, visual acuity is a quantifiable means of determining global corneal function. Early corneal disease mainly affects the epithelium either as punctate keratitis or as dysplasia. This requires particular clinical and pictorial documentation. Although keratoconjunctivitis sicca is not a typical feature of OCP cicatrisation until very late, tests such as Schirmer's and tear film break-up times are also useful means of documenting the ocular surface environment.

Clinical Assessment of the Staging Systems

In order to assess the best clinical staging system, one study compared both Mondino's and Foster's system [8]. The distribution of the various stages

Table 4. Distribution of patients according to the staging systems (n = 132 eyes) [8]

Stage	Foster	Mondino
I	3 (2)	5 (4)
II	30 (23)	26 (20)
III	80 (61)	45 (34)
IV	19 (14)	56 (42)
Total	132 (100)	132 (100)

Figures in parentheses are percentages.

among 132 eyes of 66 patients attending Moorfields Eye Hospital in London, UK, are given in table 4. Clearly, stage I is uncommon in clinical practice (2 and 4%) as is ankyloblepharon (14%). The majority of eyes are Foster's stage III (61%) while Mondino's classification has a more even distribution of eyes in stages II, III & IV (20, 34 and 42%, respectively).

Progression of disease can be defined as a shift from one stage to another although by definition, stage IV patients cannot progress. Using these definitions, Mondino [2] showed that when OCP patients do not receive systemic treatment or any anti-inflammatory topical treatment, stage I progressed in 50% of cases (9/18), stage II progressed in 75% (9/12) and stage III progressed in 78% (7/9). The mean time of follow-up was 22 months with a range of 10–53 months.

Another definition of progression can be 'any increase in conjunctival shrinkage including loss of fornix or new symblepharon formation' [2, 3]. In another trial where patients did not receive systemic or topical anti-inflammatory treatment, progression occurred in 40% of eyes in stage I (13/42), 62% of stage II (16/26) and 73% of eyes in stage III (8/11) [2]. There was a mean follow-up of 24 months. In this study, the definition of progression was much more sensitive than previously although the results are very similar, implying that progression can be assessed by stages alone. These two studies are the only adequate natural history data for untreated patients with OCP and are the gold standard for progression over a 2-year period using these definitions. Rates of progression in other cicatrising disease are unknown.

In OCP patients that have received medical treatment and were followed for more than 2 years, using Mondino's staging system, 70% of stage II eyes and 50% of stage III eyes progressed whereas only 2/46 of patients changed stages when classified using Foster's staging system [8].

Clinical Outcome and the Staging Systems

To assess whether the amount of cicatrisation could be correlated with clinical outcome, Elder et al. [8] examined the correlation between means of documenting conjunctival cicatrisation in OCP and various parameters of outcome. Among Foster's stages I–IV, the percentage of eyes with acuities of 6/18 or better were 67, 77, 66 and 26%, respectively. For those eyes displaying severe visual impairment or blindness ($>6/60$), the percentages were 33, 6, 18 and 74%, respectively. Therefore, only Foster's stage IV was associated with a poorer visual acuity and this was statistically significant ($p < 0.05$). This also implies that symblepharon was not associated with a poorer final visual outcome per se.

For Mondino's stages I–IV, the eyes with acuities of 6/18 or better were 100, 77, 60 and 55%, respectively. For those eyes displaying severe visual impairment or blindness ($<6/60$), the percentages were 0, 8, 20 and 36%, respectively. Therefore, there is a weak correlation between a shallower inferior fornix and impaired visual acuity. For example, an eye with a fornix less than 3 mm deep has an acuity of 6/18 or better in 55%. However, this trend was not statistically significant. This illustrates that the visual acuity may be maintained despite tremendous conjunctival cicatrisation and implies specific keratopathic mechanisms.

In this same study there were significant differences in the amounts of cicatrisation and the need for systemic immunosuppression during follow-up [8]. For example, 62% of the group who never required immunosuppression were Mondino's stage I or II, whereas only 10% on treatment belonged to these groups. Similarly, for Foster's staging, 87% of the treated group had symblepharon (stage III) compared to 41% of the untreated subgroup. These data are clinically useful. Patients who present with symblepharon are ultimately more likely to need systemic immunosuppression (87 vs. 41%, $2.1 \times$) as are patients who present with lower fornices less than 5.5 mm deep (90 vs. 38%, $2.3 \times$). These groups probably have more rapidly progressive disease and a worse prognosis which accounts for their relatively advanced conjunctival shrinkage at presentation. Patients without symblepharon may have such slowly progressive disease that there will be no significant morbidity in their lifetime and therefore the risks associated with current immunosuppressive regimens, both topical and systemic, are not justified in these patients.

Conclusions

Duke-Elder [9] once said of OCP: 'the ultimate prognosis is invariably bad, should the patient survive long enough to allow the ocular changes to

attain their inevitable termination'. This need not be the case for the majority of patients, but to achieve good clinical outcomes, it is essential that the signs of conjunctival inflammation and fibrosis are adequately recorded. We would recommend that standardised drawings and photographs are used for cicatrising conjunctivitis. On the basis of these documents, patients can be classified according to Mondino's and Foster's systems to allow comparison of data.

References

1   Mondino BJ, Ross AN, Rabin BS, Brown SI: Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 1977;83:443–450.
2   Mondino BJ, Brown SI: Ocular cicatricial pemphigoid. Ophthalmology 1981;88:95–100.
3   Mondino BJ, Brown SI: Immunosuppressive therapy in ocular cicatricial pemphigoid. Am J Ophthalmol 1983;96:453–459.
4   Foster CS, Wilson LA, Ekins MB: Immunosuppressive therapy for progressive ocular cicatricial pemphigoid. Ophthalmology 1982;89:340–353.
5   Tauber J, Jabbur N, Foster CS: Improved detection of disease progression in ocular cicatricial pemphigoid. Cornea 1992;11:446–451.
6   Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
7   Schwab IR, Linberg JV, Gioia VM, Benson WH, Chao GM: Foreshortening of the inferior conjunctival fornix associated with chronic glaucoma medications. Ophthalmology 1992;99:197–202.
8   Elder MJ, Bernauer W, Leonard J, Dart JK: Progression of disease in ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:292–296.
9   Duke-Elder S: System of Ophthalmology. London, Kimpton, vol 8, pp 509.

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What Initiates the Immune Response? Possible Mechanisms from the Ophthalmologist's View

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Cicatricial pemphigoid, linear IgA disease, certain paraneoplastic syndromes and some forms of drug-induced conjunctivitis may lead to progressive conjunctival scarring. These conditions do not only have clinical similarities, but they potentially share a common pathogenesis [1–8].

Circumstantial evidence and the investigation of animal models have established the auto-immune character of ocular cicatricial pemphigoid (OCP). The findings of tissue-fixed basement membrane zone antibodies, complement deposition at the epithelial basement membrane zone and circulating auto-antibodies to the conjunctival basement membrane zone suggest all the involvement of immune mechanisms. The most direct test whether auto-immunity is responsible for the conjunctival lesions, however, is to induce auto-immunity deliberately in an experimental animal. This was shown to be effective for the induction of skin lesions by injection of IgG anti-laminin 5 antibodies into neonatal mice [9] (laminin 5 is one of the target auto-antigens in OCP). In another model, conjunctivitis developed in neonatal rabbits after injection of a murine monoclonal antibody against basement membrane of stratified squamous epithelium [2]. No animal models have yet been developed to study chronic progressive conjunctival cicatrisation with paraneoplastic syndromes or drug-induced conjunctivitis.

On a theoretical basis, the finding of auto-antibodies in chronic progressive conjunctival cicatrisation allows three possible inferences [10]:
–   the auto-immunity is responsible for producing the ocular lesions;
–   there is a disease process which, through the production of tissue damage, leads to the development of auto-antibodies;
–   there is a factor which produces both the lesions and the auto-immunity.

The first possibility, that the auto-immune process produces the lesions, applies to most diseases associated with auto-immunity, and this pathogenesis is responsible for the vast majority of patients with OCP and linear IgG disease (see above). Auto-antibodies are rarely induced following the release of auto-antigens simply by trauma (the second possibility), they may develop for example after myocardial infarction [10]. This mechanism, however, is likely to be involved in those patients with Stevens-Johnson syndrome who develop OCP up to 30 years after the acute initial inflammatory episode [11]. The ocular mucosal injury during the initial inflammation may render components of the basement membrane zone immunogenic, resulting in auto-immunisation with antigens normally not presented to the immune system. This mechanism and the third of the above possibilities must be considered for drug-induced cicatrisation. Drug-induced conjunctival shrinkage represents a spectrum of disease ranging from a self-limiting toxic form to a progressive, 'immunological' form (=pseudopemphigoid) [12]. Auto-antibodies have been found with this immunological form [3] and this condition typically remains progressive after withdrawal of the drugs. Different classes of topically administered medications are associated with drug-induced pemphigoid and in vitro studies have failed to show a stimulation of fibroblasts by antiglaucomatous drugs or their preservatives [13]. This may indicate that a mechanical factor is involved in the mucosal injury. The chronic tissue damage and the chronic low-grade inflammation that is found after long-term application of drugs [14, 15] may give rise to an auto-immune reaction in analogy to the cases of OCP after Stevens-Johnson syndrome. Alternatively, chronic low-grade inflammation and tissue damage may trigger the onset of pemphigoid in disposed patients. The fact that none of the reported patients with pseudopemphigoid had extraocular mucous membrane involvement does not support this hypothesis, but it should be considered that conjunctival and extraocular basement membranes may have different antigenicity.

Familial incidence of auto-immunity is well known and this is rather due to genetic than environmental factors. Evidence for the operation of genetic factors in auto-immune diseases comes from their tendency to be associated with particular major histocompatibility complex (MHC) specificities. The MHC system of man, also known as human leucocyte antigen (HLA) system, is a complex genetic region which encodes for cell surface products important in mediating interaction between cells of the immune system. Studies in OCP showed an association with HLA-B12 [16] and with the MHC class II antigens HLA-DR4 and HLA-DQ7 [17–19]. While HLA-B12 and HLA-DR4 are found in less than half of the OCP patients, the frequency of HLA-DQ7 is 95% in comparison with the estimated 13.5% of the general US Caucasian population [18]. These findings suggest that immunogenetic factors are operative in OCP.

There is, however, no direct relationship between HLA antigens and disease susceptibility. This is supported by a family study on monozygotic twins that found exactly the same HLA alleles but only one of the twins suffered from OCP [20]. Preliminary results indicate that patients with drug-induced pemphigoid have distinctly different HLA susceptibility associations [21].

The immunogenetic susceptibility is one important aspect in OCP and possibly also in other conditions associated with chronic progressive conjunctival cicatrisation. Further to the genetic background, other factors clearly have an influence on the development of ocular disease. These additional factors are largely unknown – tissue trauma and chronic inflammation may be two of them.

References

1 Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
2 Roat MI, Alstadt S, Carpenter AB, SundarRaj N, Thoft A: Antibasement membrane antibody-mediated experimental conjunctivitis. Invest Ophthalmol Vis Sci 1990;31:168–175.
3 Leonard JN, Haffenden GP, Ring NP, McMinn RMH, Sidgwick A, Mowbray JF, Unsworth DJ, Holborow EJ, Blenkinsopp WK, Swain AF, Fry L: Linear IgA disease in adults. Br J Dermatol 1982;107:301–316.
4 Anhalt Gj, Kim S, Stanley JR, Korman NJ, Jabs DA, Kort M, Izumi H, Ratrie III H, Mutasim D, Ariss-Abdo L, Labib RS: Paraneoplastic pemphigus: An autoimmune mucocutaneous disease associated with neoplasia. N Engl J Med 1990;323:1729–1735.
5 Helm TN, Camisa C, Valenzuela R Allen CM: Paraneoplastic pemphigus. A distinct autoimmune vesiculobullous disorder with neoplasia. Oral Surg Oral Med Oral Pathol 1993;75:209–213.
6 Bystrin JC, Hodak E, Gao S-Q, Chuba VJ, Amorosi EL: A para-neoplastic mixed bullous skin disease associated with anti-skin antibodies and a B-cell lymphoma. Arch Dermatol 1993;129:870–875.
7 Leonard JN, Hobday CM, Haffenden GP, Griffiths CEM, Powles AV, Wright P, Fry L: Immunofluorescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.
8 Pouliquen Y, Patey A, Foster CS, Goichot L, Savodelli M: Drug-induced cicatricial pemphigoid affecting the conjunctiva. Light and electron microscopic features. Ophthalmology 1986;93:775–783.
9 Lazarova Z, Yee C, Darling T, Briggaman RA, Yancey KB: Passive transfer of anti-laminin 5 antibodies induces subepidermal blisters in neonatal mice. J Clin Invest 1996;98:1509–1518.
10 Roitt I: Autoimmunity and autoimmune disease: in Roitt I, Brostoff J, Male D (eds): Immunology. London, Mosby, 1996, pp 27.1–27.12.
11 Chan LS, Soong KH, Foster CS, Hammerberg C, Cooper KD: Ocular cicatricial pemphigoid occurring as a sequela of Stevens-Johnson syndrome. JAMA 1991;266: 1543–1546.
12 Fiore PM, Jacobs IH, Goldberg DB: Drug-induced pemphigoid. A spectrum of diseases. Arch Ophthalmol 1987;105:1660–1663.
13 Williams DE, Nguyen KD, Shapourifar-Tehrani S, Kitada S, Lee DA: Effects of timolol, betaxolol and levobunolol on human Tenon's fibroblasts in tissue culture. Invest Ophthalmol Vis Sci 1992; 33:2233–2241.
14 Sherwood MB, Grierson I, Millar L, Hitchings RA: Long-term morphologic effects of antiglaucomatous drugs on the conjunctiva and Tenon's capsule in glaucoma patients. Ophthalmology 1989; 96:327–335.
15 Broadway DC, Grierson I, O'Brien C, Hitchings RA: Adverse effect of topical antiglaucoma medication. I. The conjunctival cell profile. Arch Ophthalmol 1994;112:1437–1445.

16 Mondino BJ, Brown SI, Rubin BS: HLA antigens in ocular cicatricial pemphigoid. Arch Ophthalmol 1979;97:479.
17 Zaltas MM, Ahmed AR, Foster CS: Association of HLA-DR4 with ocular cicatricial pemphigoid. Curr Eye Res 1989;8:189–193.
18 Haider N, Neuman R, Foster CS, Ahmed AR: Report on the sequence of DQB1*0301 gene in ocular cicatricial pemphigoid patients. Curr Eye Res 1992;11:1233–1238.
19 Yunis JJ, Mobini N, Yunis EJ, Alper CA, Deulofeut R, Rodriguez A, Foster CS, Marcus-Baglet D, Good RA, Ahmed AR: Common major histocompatibility complex class II markers in clinical variants of cicatricial pemphigoid. Proc Nat Acad Sci USA 1994;91:7747–7751.
20 Bhol K, Udell I, Haider N, Yunis JJ, Mohimen A, Neuman R, Grasso C, Ahmed AR, Foster CS: Ocular cicatricial pemphigoid. A case report of monozygotic twins discordant for the disease. Arch Ophthalmol 1995;113:202–207.
21 Tauber J, Foster CS: Cicatricial pemphigoid; in Mannis MJ, Macsai MS, Huntley AC (eds): Eye and Skin Disease. Philadelphia, Lippincott-Raven, 1996, pp 261–271.

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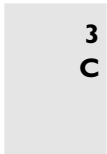
Antigen Processing and Presentation

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Lymphocytes generate immune responses following recognition and inter-action with two different forms of antigen. Antibodies on the surface of B lymphocytes, which generate 'humoral' responses, can recognise antigen in its native intact three-dimensional structural form. In contrast, the specific segments (epitopes) required for recognition by T lymphocyte receptors, which generate 'cell-mediated' responses, are short fragments of an antigenic peptide chain which are usually inaccessible for immune recognition in the intact unprocessed protein. The essential need for antigen *processing* is thus explained by the requirement of the T cell receptor to engage a peptide fragment bound with a major histocompatibility complex (MHC) molecule on the antigen-presenting cell (APC) or target cell surface. Antigen processing occurs within the target cell of that antigen or an APC: it involves degrading the antigenic protein into peptide fragments, prior to transport to the cell surface where they are presented to lymphocytes by MHC molecules (antigen *presentation*).

In this chapter, the constituents of the immune response to antigens will be briefly described and current understanding of both antigen processing and presentation will be reviewed.

Major Histocompatibility Complex Molecules

The MHC, referred to in man as the HLA (human leucocyte antigen) region, has been one of the most intensively studied regions of the genome in recent years. Among the most important gene products encoded are MHC class I and class II molecules, cell surface glycoproteins with complementary functions but similar structure. They are synthesised on the cytoplasmic face of the endoplasmic reticulum (ER), translocate to its lumen where they fold

into their three-dimensional protein structure and then migrate to the cell surface. Their main function is to present peptides to T cells: indeed the T cell receptor does not engage the peptide antigen unless it is bound with the MHC molecule. The structures of both classes of molecule have been determined by x-ray crystallography. MHC class I consists of two associated polypeptide chains, an α-chain comprising three segments or domains and a smaller β_2-microglobulin chain. The α_3-domain spans the cell membrane and the α_1- and α_2-domains together form a cleft on the MHC molecule surface within which peptide antigens bind. Class II molecules have a similar structure to class I, with α- and β-polypeptide chains each spanning the cell membrane and forming a cleft for peptide binding.

There is a striking difference in the distribution of the two classes of MHC molecule in normal tissues. Almost all nucleated cells express MHC class I molecules on the cell surface. Class II molecules are expressed on the surface of a much more restricted range of cells, principally B cells, dendritic cells and macrophages, known as 'professional APCs'. The resting levels of class I and class II MHC molecules can be greatly increased, both quantitatively and in the range of cells, in disease states, primarily as a consequence of exposure to cytokines released during inflammatory responses.

The presentation of processed antigenic peptides in the groove on the surface of MHC molecules to T cells is critical to the function of the immune response. Interaction of the T cell, peptide and MHC molecule (together known as the trimolecular complex) leads to activation of the T cell and generation of a clone of T cells which respond to the antigenic peptide. Because we know that peptides presented in association with class I molecules arise from intracellular proteins, the role of antigen presentation by MHC class I molecules is to enable the immune system to monitor the internal contents of intact cells. CD8 + T cells recognise peptide bound to class I molecules on the cell surface, thereby detecting virus infection or malignant transformation, and function primarily in direct cytotoxicity of diseased cells by release of injurious proteins such as perforin. MHC class II molecules present peptides generated from internalised exogenous antigens in B cells and macrophages. Class II molecules interact with CD4 + T cells, which are the major helper T cell phenotype and whose predominant function is to generate lymphokines that regulate essentially all other functions of the immune response.

Processing and Presentation of Endogenous Antigens

Antigens are categorised as *endogenous* or *exogenous* according to whether they are derived from within the cell or its exterior. Endogenous antigens are
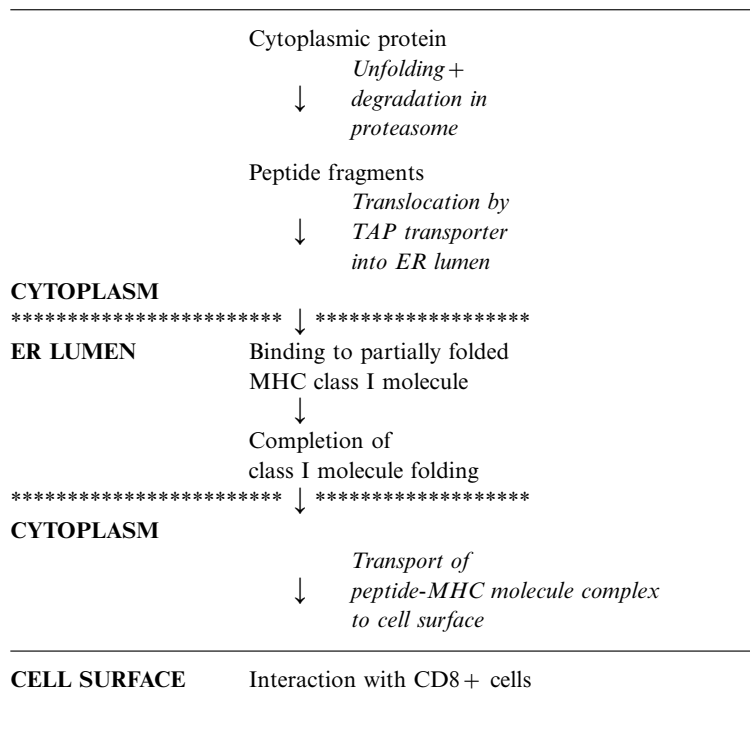
```
                    Cytoplasmic protein
                              Unfolding +
                    ↓         degradation in
                              proteasome

                    Peptide fragments
                              Translocation by
                    ↓         TAP transporter
                              into ER lumen
CYTOPLASM
*********************** ↓ *******************
ER LUMEN            Binding to partially folded
                    MHC class I molecule
                              ↓
                    Completion of
                    class I molecule folding
*********************** ↓ *******************
CYTOPLASM

                              Transport of
                    ↓         peptide-MHC molecule complex
                              to cell surface


CELL SURFACE       Interaction with CD8 + cells
```

Fig. 1. Processing of endogenous antigens.

synthesised in the cytoplasm or nucleus, for example viral proteins synthesised following virus infection of a cell. In contrast, bacterial proteins which are phagocytosed are exogenous.

In general, endogenous proteins are unfolded and degraded into peptide fragments in the cytoplasm by a large proteinase complex, called the proteasome. The transporters associated with antigen processing (TAP), which are integral membrane proteins in the ER, then transfer these peptides from the cytoplasm into the lumen of the ER where selected peptides complex with newly synthesised partially folded MHC class I molecules. Peptides binding to class I molecules are of defined length, usually nine amino acids. Moreover, only those peptides which fulfil the steric requirements for fitting into the binding groove of the class I molecule will bind. Peptide binding allows the class I molecule to fold completely and the MHC-peptide complexes are then transported via the Golgi apparatus to the cell surface for presentation to CD8 + T cells (fig. 1). This usually takes place in local lymph nodes. The T

cell receptor and the CD8 + molecule on the lymphocyte surface interact with the MHC-peptide complex on the APC surface, initiating signal transduction to the lymphocyte nucleus and interleukin-2 gene transcription. This cytokine induces expansion of a clone of antigen-specific activated CD8 + cells which migrate from the lymph node to the site of inflammation and lyse target cells expressing the appropriate class I-peptide complex.

Thus endogenous antigen processing can be conceived as a regulated series of protein-protein interactions that generates short peptide fragments in the cytoplasm, transports them to the ER, and directly loads some into class I molecules during folding and assembly. The combined effects of peptide transfer by TAP and the peptide-binding capabilities of the class I molecules themselves must allow the immune system to detect the presence of intracellular pathogens, and it must be possible to present a diverse array of peptides in order to optimise the chance of detecting, for example, cells infected by any one of a range of viruses. At the same time, TAP and class I molecules are necessary to select the repertoire of CD8 + T cells capable of recognising foreign peptide and eliminate those capable of responding to normal self cells.

Processing and Presentation of Exogenous Antigens

Exogenous proteins transported across the cell membrane are endocytosed in vesicles that are formed as the membrane in the region of the attached protein becomes invaginated. Once internalised, vesicles coalesce to form large vesicles termed endosomes. Within an hour of internalisation, endosomes fuse with lysosomes to form endolysosomes. In an acidic region of the cytoplasm, proteases within the endolysosome are activated and degrade the exogenous antigenic proteins. Antigen processing results in unfolding and cleavage of the internalised protein into peptide fragments. Specialised phagocytic cells such as macrophages or neutrophils can internalise large particles such as micro-organisms. These are internalised within large phagosomes, within which the endocytosed contents are later degraded by hydrolytic enzymes into amino acids, nucleotides and sugars.

Class II molecules are synthesised in the ER as α- and β-subunits, as-sembled with a third subunit called the invariant chain. After leaving the ER, the invariant chain remains associated with the class II α/β-dimer until it reaches the endosomal/lysosomal compartment, where it is proteolytically cleaved. By binding the class II α/β-dimer in the ER, the invariant chain blocks peptide binding in this compartment and directs the α/β-dimer to the endosomal compartment. In this way, the invariant chain ensures that class II molecules bind to peptides from a pool different from those which bind to
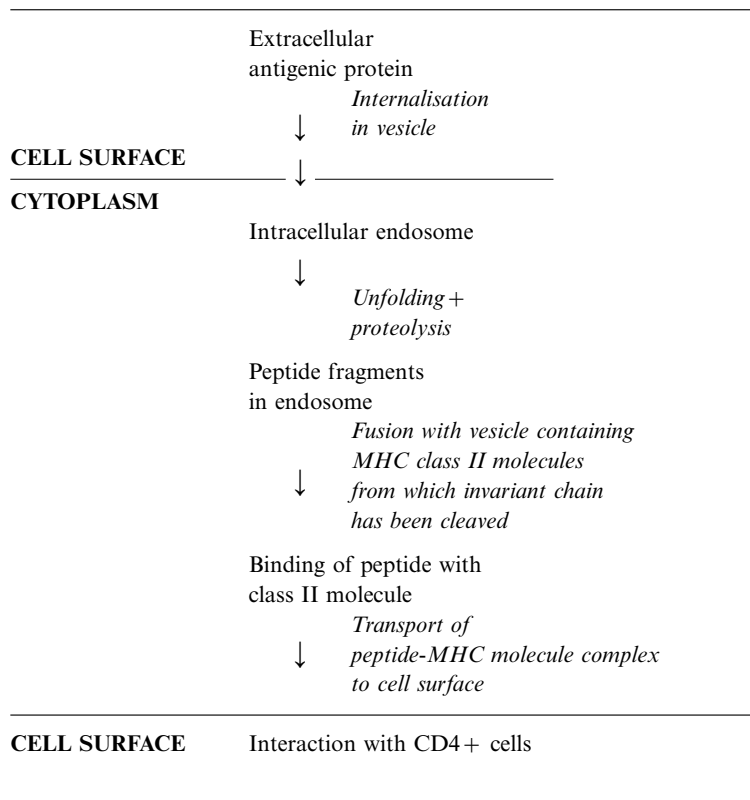
```
                          Extracellular
                          antigenic protein
                                    Internalisation
                          ↓         in vesicle
CELL SURFACE                ↓
CYTOPLASM

                          Intracellular endosome

                          ↓
                                    Unfolding +
                                    proteolysis

                          Peptide fragments
                          in endosome
                                    Fusion with vesicle containing
                                    MHC class II molecules
                          ↓         from which invariant chain
                                    has been cleaved

                          Binding of peptide with
                          class II molecule
                                    Transport of
                          ↓         peptide-MHC molecule complex
                                    to cell surface


CELL SURFACE              Interaction with CD4 + cells
```

Fig. 2. Processing of exogenous antigens.

class I molecules. Vesicles containing processed peptide fragments fuse with storage vesicles containing newly synthesised MHC class II molecules transported from the ER. The class II molecules engage the peptide in the binding cleft. In contrast to the peptide binding site in class I, the binding site in class II molecules is open at both ends, allowing for association of peptides of variable length (usually 10–17 amino acids). Bound antigenic peptides are then transported from endosomes to the cell surface where they can be surveyed by CD4+ T cells (fig. 2).

Occupancy of the T cell receptor by the class II MHC-peptide complex on the APC surface is accompanied by interaction between the CD4+ molecule on the T cell surface and the MHC molecule. When accompanied by ligation of additional accessary and co-stimulatory molecules, signals are transduced to the T cell nucleus which leads to transcription of the interleukin-2

gene. Production of this cytokine is crucial to proliferation of antigen-specific CD4+ T cells, which in turn activate B cells and macrophages.

Antigens and Auto-Immune Disease

Antigens are characterised by their capacity to provoke an immune response. Whether immune reactivity is induced and maintained or aborted depends on various properties of the antigen. These include its localisation (whether antigen reaches lymphoid tissue or remains outside), mode of presentation, distribution kinetics and the immune capacity of the responding individual. For auto-antigens, which are self-constituents, an additional constraint is the natural tolerance to them. The reason for tolerance to many auto-antigens may be that these are not picked up by migrating professional APCs such as dendritic cells: consequently they do not reach lymphoid tissues to induce mature T cells. A large number of different antibodies may be produced to an antigen because antigens are three-dimensional structures and present many different configurations to B cells. Different antibodies can bind to different epitopes of a particular antigenic protein.

For the purpose of this review, auto-immune disease is defined as one which results from the direct effects of a specific immune response against host tissues. Thus in auto-immune disease, the body's immune system turns against itself and generates lymphocytes capable of reacting with self-components. Auto-antigens induce auto-antibody production by B cells, but autoreactive T cells may also contribute to the immune response. It is primarily the interaction of auto-antibody and antigen which causes auto-immune disease, and the detection of circulating or tissue-bound auto-antibodies is one of the most consistent findings in auto-immune diseases. Auto-antigens can be based on various sites of origin, such as (1) cell membrane (e.g. erythrocyte membranes in haemolytic anaemia); (2) cytoplasm organelles (e.g. salivary duct cells in Sjögren's syndrome); (3) cell nuclei (e.g. DNA in systemic lupus), and (4) specialised tissue products (e.g. epithelial intercellular adhesion molecule in pemphigus vulgaris, epithelial basement membrane hemidesmosome adhesion structures in bullous pemphigoid).

Evidence that ocular cicatricial pemphigoid (OCP) is an auto-immune disease has accumulated in recent years. This evidence includes the finding of circulating auto-antibodies against the conjunctival basement membrane zone, the presence of auto-antibody at the conjunctival epithelial basement membrane [1], MHC class II expression in epithelial cell membranes, infiltration of affected conjunctiva by T lymphocytes, dendritic cells, macrophages and plasma cells [2, 3], and the response of clinical disease to immunosuppression.

Disease Association with MHC Haplotypes

The immune response is regulated in large part by genes of the MHC, encoded on the short arm of chromosome 6. These are the most polymorphic human genes. Structural analysis of MHC molecule peptide binding sites indicates that this marked genetic polymorphism leads to amino acid sequence variations. The repertoire of peptides that can be displayed at the cell surface is determined by the structure of the class I and II peptide binding sites, and so individuals with different MHC haplotypes will present different sets of peptides to T cells. This phenomenon may account for the finding that susceptibility to almost all auto-immune diseases is MHC-associated. Indeed, the much stronger association of more than 40 auto-immune diseases with the class II antigen HLA-DR than other antigens suggests MHC class II restriction in the case of auto-antigens. One may hypothesise that an auto-immune response follows the recognition by the T cell receptor of a self peptide selectively presented within the antigen-binding cleft of a disease-associated, self, class II molecule. The immunological attack may be primarily dictated by resident APCs in the target organ: in OCP, conjunctival dendritic cells or other APCs expressing disease-associated class II molecules may primarily induce a T cell response, with resulting auto-antibody production by B cell clones and tissue injury.

There is enhanced immunogenetic susceptibility to OCP in individuals with HLA-DR4 and -DQ7 haplotypes [1, 4]. This might not necessarily imply that the factor precipitating the initial episode is genetically controlled, but rather that disease progression from this initial process depends on a specific genetic predisposition. One published report of monozygotic twins discordant for OCP also argues against a single-gene theory for disease development and supports hypotheses of either multigenic aetiology or interaction with environmental factors [5].

References

1   Ahmed AR, Kurgis BS, Rogers RS: Cicatricial pemphigoid. J Am Acad Dermatol 1991;24:987–1001.
2   Rice BA, Foster CS: Immunopathology of cicatricial pemphigoid affecting the conjunctiva. Ophthalmology 1990;97:1476–1483.
3   Bernauer W, Wright P, Dart JK, Leonard JN, Lightman S: The conjunctiva in acute and chronic mucous membrane pemphigoid. Ophthalmology 1993;100:339–346.
4   Ahmed AR, Foster S, Zaltas M, Notani G, Awdeh Z, Alper CA, Yunis EJ: Association of DQw7 (DQB1*0301) with ocular cicatricial pemphigoid. Proc Natl Acad Sci USA 1991;88:11579–11582.
5   Bhol K, Udell I, Haider N, Yunis JJ, Mohimen A, Neuman R, Grasso C, Ahmed AR, Foster S: Ocular cicatricial pemphigoid. A case report of monozygotic twins discordant for the disease. Arch Ophthalmol 1995;113:202–207.

Recommended Reviews

Germain RN: MHC-dependent antigen processing and peptide presentation: Providing ligands for T lymphocyte activation. Cell 1994;76:287–299.

Parham P: Transporters of delight. Nature 1990;348:674–675.

Zinkernagel RM: Immunology taught by viruses. Science 1996;271:173–178.

Wucherpfennig KW, Strominger JL: Selective binding of peptides to disease-associated major histocompatibility complex (MHC) molecules: A mechanism for MHC-linked susceptibility to human autoimmune diseases. J Exp Med 1995;181:1597–1601.

Stanley JR: Autoantibodies against adhesion molecules and structures in blistering skin diseases. J Exp Med 1995;181:1–4.

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The Immunologic Target: Antigenic Aspects of Basement Membranes

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It is postulated that ocular cicatricial pemphigoid (OCP) is triggered by blood-borne antibodies that bind to naturally occurring antigens within the basement membrane zone (BMZ) of skin and mucous membranes. Similar mechanisms exist for bullous skin diseases and a whole host of systemic diseases. This section reviews the anatomy and physiology of normal basement membranes and then examines the pathophysiology of the BMZ diseases. Particular regard is given to OCP and the other mucocutaneous diseases.

In broad biological terms, organisms are composed of parenchyma and connective tissue; epithelia, endothelia, muscle and fat cells versus cells dispersed between connective tissue fibres and matrix. These tissue types are separated by an acellular sheet of specialised extracellular matrix – a basement membrane [1]. For epithelia, basement membrane connects the basal aspect of basal cells to the underlying stroma. For muscle, adipose and Schwann cells, it acts to envelop them [2]. The region of the basement membrane is intimately related to adjacent structures and this is referred to as the BMZ [1]. The various components of the BMZ are secreted by the adjacent cells and the membrane is assembled extracellularly [3].

The BMZ has three broad functions. First, it acts as a scaffold to aid appropriate cell replication, both physiologically and during tissue repair. Second, it acts to spatially orientate cells, particularly if they are polarized. Lastly, it acts as a boundary, a barrier and a channel between the epithelium and the underlying stroma [4].
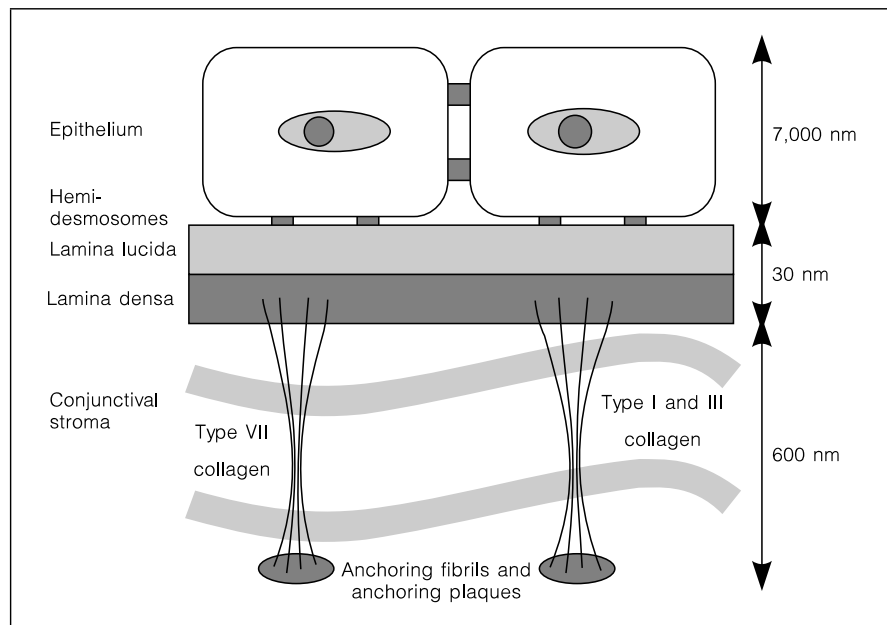
Fig. 1. A schematic outline of the components of the BMZ (not to scale).

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Anatomy of the Basement Membrane Zone

Overview

The BMZ is composed of a 15- to 65-nm-thick, superficial, electron-lucent lamina lucida, a 15- to 125-nm-thick lamina densa and a deep, 50- to 400-nm-thick lamina fibroreticularis [1, 4]. Associated with these layers are hemidesmosomes, anchoring filaments, sub-basal dense plates, anchoring fibrils and elastic microfibril bundles (figs. 1–3) [5]. Hemidesmosomes are specialised plaques on the plasma membrane of the basal cells that 'spot-weld' cells to the BMZ. Hemidesmosomes sit on the lamina lucida and attach directly to the lamina densa via transmembrane anchoring filaments. The lamina densa attaches to the stroma via anchoring fibrils and plaques within the stroma [1]. Compared to hemidesmosomes, desmosomes join adjacent cells together with each other. They contain a wide variety of proteins including plakoglobin, desmoplakin I and II, desmoglein, desmocollins I and II and desmocalmin. There is very little biochemical or immunological similarity between desmosomes and hemidesmosomes and none of the above proteins are found in hemidesmosomes [6].

Fig. 2. Electron microscopy of conjunctival epithelium, BMZ and stroma. The BMZ is seen at the junction of the middle and upper thirds of the illustration with the deep aspect of a basal cell located more superficially. Fibroblasts can be seen in the stroma of the lower third.

Fig. 3. Electron microscopy showing two desmosomes and associated structures between two basal cells of the conjunctival epithelium.

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Table 1. Components of the BMZ

Components	Location	Molecular weight, kD
Type I collagen	mainly lamina densa	550
Proteoglycans	mainly lamina lucida	130–185
Laminin	lamina densa and lucida	1,000
Epiligrin (laminin 5)	lamina densa and lucida	450, subunits of 140–170
Entactin	lamina densa and lucida	158
Nidogen	lamina densa and lucida	150
Fibronectin	all sites, esp. fibroreticularis	450 (2 × 220)
Antigen 19-DEJ-1	junction lamina lucida and densa	?
Fibrillin	microfibrils	350
Collagen type VI	fibroreticularis	?
Type VII collagen non-collagenous domain	hemidesmosomes	230

See text for references.

The Main Constituents of the Basement Membrane Zone

The main components of the BMZ are type IV collagen, proteoglycans and laminin although there are many others (table 1). The lamina lucida is mainly composed of heparin sulphate proteoglycans while the lamina densa is mainly type IV collagen and laminin [7]. The lamina fibroreticularis and the anchoring filaments are mainly type VII collagen [8]. Ultrastructurally, the basement membrane consists of a tridimensional network of type IV collagen filaments which is a non-fibrillar collagen unique to the BMZ. These filaments are sheathed by laminin and heparin sulphate to form 'cords' which are more tightly packed in the lamina densa than the lamina lucida. The other components of the BMZ are arranged between the cords [1]. The various BMZ constituents differ between tissues, for example type IV collagen is probably absent from the central cornea epithelial BMZ although it is present in Bowman's membrane [4, 9].

Anchoring Fibrils and Plaques

The BMZ needs to be firmly adherent to the underlying tissue and this is achieved by direct adhesion and by the anchoring fibril system. The fibrils extend from the BMZ to the anchoring plaques located within the extracellular matrix (fig. 1). The fibrils are composed of type VII collagen whereas the anchoring plaques are type IV collagen and laminin [10, 11]. The numbers of fibrils in a given tissue are a function of its mechanical stresses. For example, they are maximal in heel skin, and less in skin on the back of the hand compared to the palm [12].

Candidate Antigens for Basement Membrane Disease

Within the BMZ there are a complex series of proteins and sugars which are all candidates for involvement in immune-related disease and these are catalogued below. Their specific BMZ location and molecular weight are important in elucidating the probability of a protein being an antigen in a specific disease (table 1).

The proteoglycans are chains of repeating disaccharides (glycosaminoglycans) bound to a protein. For example, heparin sulphate, chondroitin sulphate and dermatan sulphate. The composition depends on the type of BMZ [13]. Proteoglycans are orientated within the BMZ such that the negative glycosaminoglycans are adjacent to the epithelium. This contributes to the 'charge barrier' and enhances the selective permeability of the BMZ.

Laminin is a family of glycoproteins that are important for cellular adhesion to the BMZ and for its structural integrity [14, 15]. The various isoforms play different roles and are antigenically different [16]. For example, the hereditary disease epidermolysis bullosa is due to mutations in the genes encoding laminin 5 (previously known as nicein, kalinin or BM600) [17, 18]. This isoform is associated with the anchoring filament-hemidesmosome complex and localises to the border between the lamina lucida and lamina densa, typically adjacent the hemidesmosomes [19–21].

Epiligrin is a set of three disulphide-linked polypeptides that are similar to, or identical to laminin 5 and localises to the interface of the lamina lucida and lamina densa [22–25]. It is secreted by keratocytes and functions as the ligand for integrin $\alpha 3\beta 1$. Epiligrin is a key candidate antigen for OCP (vide infra).

Collagen type VI is a filamentous collagen that may be present in the anchoring plaques in the fibroreticularis and hence has a role in epithelial adhesion [26]. It is also found loosely arranged around blood vessels, within Bowman's membrane and as part of the trabecular meshwork [2]. The collagen type VII molecule consists of three identical α-chains, each with a 145-kD non-collagenous domain (NC1) and a 145-kD carboxyl-terminal domain. These molecules aggregate in a lateral fashion to form the anchoring filaments [10, 11]. Its gene (COL7A1) is defective in hereditary epidermolysis bullosa [17].

Antigen 19-DEJ-1 is a primate-specific proteoglycan located in the mid-lamina lucida beneath the hemidesmosomes and is associated with the anchoring filaments [27, 28]. It is found in human skin, mucous membrane, oesophagus and corneal BMZ but not vascular BMZ and it may represent either an epitope of the anchoring filaments or of the sub-basal dense plate [27]. Entactin and nidogen are also found in the BMZ and are similar to laminin [1, 4]. Fibrillin is a 350-kD glycoprotein associated with the microfibrils [29].

Basement Membrane Pathophysiology

BMZ dysfunction is common. It can be due to over- or underproduction of one or more components of the BMZ or by proteolytic attack on the BMZ. Diseases of the former include diabetes and those of the latter include immune- and non-immune-related inflammation and infection [4]. In diabetes, the vascular BMZ is thickened due to increased amounts of type IV collagen and laminin. The amounts of proteoglycan are reduced and this reduces the charge barrier leading to increased permeability of the vasculature [4, 30].

A good example of immune-mediated anti-basement membrane disease is the family of glomerulonephritides. In Goodpasture's syndrome, the antigen is the NC1 globular domain of type IV collagen whereas in post-streptococcal glomerulonephritis, the auto-antigen is probably either heparin sulphate or laminin [4, 31]. In these diseases, complement activation triggers neutrophil and macrophage-mediated damage. In comparison, it is odd that there is not renal or pulmonary component to the bullous skin diseases which share similar antigens.

In classic auto-immune BMZ diseases like Goodpasture's syndrome, the antigen is a natural component of the basement membrane. However, antibody binding can also occur due to two other mechanisms. First, preformed antibody-antigen complexes can become embedded within the membrane. Second, antigen within the serum can become bound or lodged within the BMZ and antibody binding occurs as a secondary response. Either mechanism tends to produce a granular rather than linear deposition of IgG [32].

Systemic Diseases of the Skin and Mucous Membranes

All of the bullous skin diseases may be associated with cicatricial conjunctival changes although these ocular changes are uncommon, mild and frequently asymptomatic. These diseases include dermatitis herpetiformis, epidermolysis bullosa acquisita, pemphigus vulgaris and bullous pemphigoid and, as a family, they all have circulating antibodies to skin and mucous membrane basement membrane [33]. Antibodies and complement are bound to the BMZ of lesional and non-lesional BMZs. However, the diseases differ because the specific components of the BMZ that function as the antigens are unique (table 2).

Ocular Cicatricial Pemphigoid

OCP is postulated to be initiated by the binding of circulating antibodies to the basement membrane of mucous membranes including the conjunctiva.
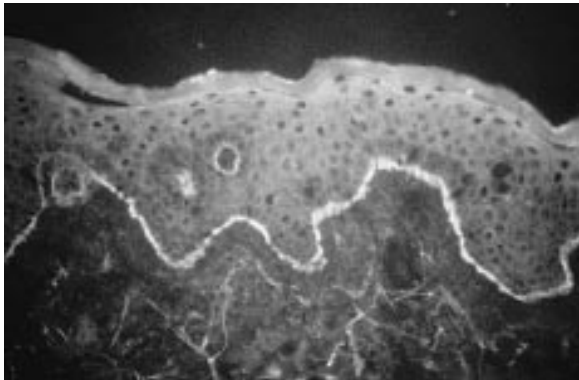
Fig. 4. Immunofluorescence for IgG which is bound to the basement membrane of the conjunctiva in OCP.

Table 2. The antibody-binding site in immune-related cicatrising conjunctivitides

Disease	Antibody binding site
OCP	BMZ, epiligrin and further BMZ components
Dermatitis herpetiformis	sublamina densa region of BMZ
Epidermolysis bullosa acquisita	type VII collagen in anchoring fibrils
Pemphigus vulgaris	intercellular cement substance
Bullous pemphigoid	
Antigen 1 (BPAG1)	220-kD component of hemidesmosome
Antigen 2 (BPAG2)	180-kD component of hemidesmosome
Stevens-Johnson syndrome	Blood vessel wall

This activates complement and initiates a type II cytotoxic hypersensitivity reaction that then damages the tissues at the site.

Circulating antibodies to conjunctival basement membrane are detected in about 50% of patients [34–40]. The antibody is directed against epidermal antigens of a molecular weight of 230 kD and/or 160–180 kD. There is some cross-reactivity to bullous pemphigoid antigen [41–43]. IgG, IgA and complement are bound to conjunctival basement membrane of OCP in up to 67% (fig. 4) [35, 44–46]. The binding is strictly linear and is always along the BMZ [41]. Various components of complement are also bound to the basement membrane, including C1q, C3, C4 and properdin [36–38, 45], and complement may be activated via both the classical and the alternative pathway. Lysis of

the desmosomal attachments of the basal layer of the epithelium causes the subepithelial bullae which may be clinically manifest. Ultrastructurally, the conjunctival BMZ in OCP becomes disorganised and discontinuous. This is accompanied by basal lamina disruption, disorganisation of the stromal collagen fibrils and increased desmosomes, tonofilaments and tonofibrils in the epithelium [47–49].

Additional evidence for the auto-immune basis of OCP comes from the development of an animal model in rabbits [50]. The injection of a monoclonal antibody to the basement membrane of conjunctival epithelium (MAb63) causes conjunctival inflammation, neutrophil infiltration and basement membrane deposition of IgG in a pattern similar to that seen in acute OCP.

Drug-Induced Cicatrising Conjunctivitis

The most similar ocular disease to OCP is drug-associated cicatrisation and this may occur due to the systemic or topical administration of drugs. Oral practolol causes cicatrising conjunctivitis, sclerosing peritonitis, a psoriasiform rash and secretory otitis media and is associated with circulating anti-nuclear antibodies and pemphigus-like antibodies [51–55]. Topical administration of ocular medication, particularly hypotensive glaucoma drugs, may cause cicatrising conjunctivitis (pseudopemphigoid), either by a local toxic effect or an immune-related effect [56, 57]. Clinically and histologically, it is identical to OCP and the most common aetiology is local toxicity, although some patients may have immunoglobulins bound to the conjunctival BMZ and/ or circulating conjunctival antibodies. Cicatrisation due to toxicity does not progress after the drug is withdrawn, whereas the immune-related cicatrisation may [35, 38]. The mechanisms are unknown. It is possible that OCP would have occurred whether the drug acted as an immunological catalyst in an OCP-predisposed patient, or whether the drug made the basement membrane immunogenic in an analogous manner to drug-induced pemphigus [35, 58].

Means of Determining the Antigen in BMZ Disease

At present, the antigens in OCP are poorly characterised. The determination of the antigen will come from the combination of data from three different sources. First, the patient's serum can be reacted with a variety of epidermal products to determine the size of antigen that the serum pathologically recognises. Second, the patient's serum can be added to sections of normal tissue. This determines the pathological binding site of the antibodies in the blood.

Third, patient tissue can be assessed to determine where abnormal antibodies have bound. This identifies the site of the antigen in the pathological tissues. Theoretically, the BMZ binding site of the patient's serum and that of the patient's tissue should be the same. By knowing the candidate antigen's approximate molecular size and its location within the BMZ, and knowing the BMZ components, the antigen can be deduced. This can then be confirmed by showing specific binding between the proposed antigen and the patient's serum.

Light microscopy is too insensitive for BMZ localisation, therefore electron microscopy combined with monoclonal antibodies (immuno-electron microscopy) is required. The antibodies may be added to the tissue before or after it is embedded in resin. The post-embedding technique utilizes normally fixed, embedded tissue. Ultrathin sections are incubated with the primary antibody and then visualised using a gold-conjugated secondary antibody. BMZ investigations optimally use 1-nm gold particles and these require 'enlarging' to 10–30 nm by 'silver enhancing ' [59–64]. The disadvantage of post-embedding is that BMZ antigens such as laminin 5 and 19-DEJ-1 may not withstand some processing protocols [21, 27, 60].

The pre-embedding technique involves incubating fresh, unfixed tissue in a buffered solution containing the primary antibody. The tissue is then washed, incubated with a gold-or peroxide-conjugated secondary antibody, embedded and visualised. There are several problems with this method. The peroxidase reaction products diffuse away from their original location [65]. The reaction may obscure the underlying tissue [27]. Immunogold of 5 nm or larger is too large to diffuse into the lamina lucida [59, 62, 63]. The silver-enhanced immunogold gives an uneven and variable staining pattern [28].Only one antibody can be visualised per tissue block and this is a major problem for tissues such as the human conjunctiva where there is a limited amount that can be harvested. Lastly, any technical problem with the pre-embedding renders the whole block unusable. Despite these shortcomings, excellent results have been obtained visualising human skin BMZ antigens with 1 nm immunogold [28]. Further, certain epitopes such as the BMZ components GB3 (laminin 5) and 19-DEJ-1 are only visualised with this method compared with post-embedding [21, 27].

The Ocular Circatricial Pemphigoid Antigen

Auto-Antibodies in the Serum
Using sera from patients with OCP, Bedane et al. [66] found that immune deposits localise to the lamina densa and the lower lamina lucida in regularly spaced clumps and that there was no staining of the cytoplasmic membrane

of the epithelium. They also used sera from epidermolysis bullosa acquisita and bullous pemphigoid for comparison. Epidermolysis bullosa acquisita antigen is known to be a component of the anchoring filaments which was found to be localised to the lamina densa and the anchoring fibrils [67]. The bullous pemphigoid antigen is a component of the hemidesmosomes and the sera localised to the cytoplasmic attachment plaque of the hemidesmosome [68]. Therefore, OCP antigen is different from epidermolysis bullosa acquisita and bullous pemphigoid and is possibly part of the anchoring filament system. Further, OCP serum seems restricted to the IgG subclasses of IgG1 (4/23 specimens) and IgG4 (10/23) and reacted only with 230-kD and/or 180-kD epidermal bands. Only IgG4 was able to fix complement [69].

Recently, Bernard et al. [42] used Western blotting and immunoprecipitation to show that serum from 10 patients with OCP had affinity for a 180-kD antigen (n = 8 and 10) and/or a 230-kD antigen (n = 4 and 2 for each assay, respectively). It is noted that the assays required much higher concentrations of serum compared to those with serum from patients with bullous pemphigoid [80–320 ×]. Further, they suggested that there are cross-reactivities between the OCP antigen and the 180-kD bullous pemphigoid antigen (BP180). BP180 is known to be an intracellular component of the epithelial desmosomes [64, 70]. Therefore, the OCP antigen could be part of the hemidesmosome or a component of the anchoring filaments.

Immuno-Electron Microscopy

Overall, the exact location of the OCP antigen is not well defined. Fine et al. [41] assessed buccal mucosa using immuno-electron microscopy (n = 5) and showed that IgG, IgA and C3 were bound only to the lamina lucida of the BMZ. The finding is similar to that of Nieboer et al. [71] (n = 1) and Honigsmann et al. [72] (n = 1). Prost et al. [73] used immuno-electron microscopy to study one lesional skin biopsy of OCP. They demonstrated complement (C3) deposition on the cytoplasmic membrane of the basal keratocytes and in a 'clump' pattern on the intracytoplasmic side of the basal cytoplasmic membrane. This was explained by an 'alteration in the basal cytoplasmic membrane' allowing C3 to penetrate into the cell. In the same study, bullous pemphigoid biopsies showed C3 adherent to the hemidesmosomes, consistent with recent knowledge [42, 64]. Direct immuno-electron microscopy on OCP skin/gingiva showed thick and discontinuous immunoglobulin and complement deposits localised mainly to the lamina densa but with some deposits in the lamina lucida [74]. Direct immuno-electron microscopy on the conjunctiva recently suggested that, in purely ocular OCP, there is immunolocalisation to just the lamina lucida whereas OCP with systemic features localises to both the lamina lucida and the lamina densa [75].

Anti-Epiligrin Auto-Antibodies in Ocular Cicatricial Pemphigoid

There is emerging evidence that there may be several pathophysiological causes for the clinical phenotype of OCP; 'anti-epiligrin cicatricial pemphigoid' and 'non-anti-epiligrin cicatricial pemphigoid'. Domloge-Hultsch et al. [23] described a subgroup of patients with cicatricial pemphigoid that have IgG auto-antibodies to epiligrin as demonstrated by immunoprecipitation, immunofluorescence and immuno-electron microscopy. These antibodies bind specifically to subunit α3 of laminin [24]. A clinically identical group of patients with cicatricial pemphigoid and patients with other bullous diseases had no auto-antibodies to epiligrin or laminin 5 using the same techniques [23, 24]. Shimuzu et al. [76] used indirect immuno-electron microscopy to show that serum from patients with anti-epiligrin cicatricial pemphigoid localises to the lower aspect of the lamina lucida at the interface with the lamina densa, approximately beneath the hemidesmosomes. This correlates with its binding to the dermal side of 1 M NaCl split skin. In contrast, non anti-epiligrin cicatricial pemphigoid serum binds to the hemidesmosomes and the junction between the hemidesmosomes and the plasma membrane in basal keratocytes and this correlates with its binding to the epidermal side of 1 M NaCl split skin [76]. Further, injection of an antibody to subunit α3 laminin causes detachment of basal keratocytes from the basement membrane, consistent with epiligrin playing an important role in the pathogenesis of bullous disease [25].

References

1   Inoue S: Ultrastructure of basement membranes. Int Rev Cytol 1989;117:57–98.
2   Marshall GE, Konstas AG, Lee WR: Collagens in ocular tissues. Br J Ophthalmol 1993;77:515–524.
3   Cooper AR, McQueen HA: Subunits of laminin are differentially synthesized in mouse eggs and early embryos. Devel Biol 1983;96:467–471.
4   Abrahamson DR: Recent studies on the structure and function of basement membranes. J Pathol 1986;149:257–278.
5   Fine JD: Structure and antigenicity of the skin basement membrane zone. J Cutan Pathol 1991; 18:401–408.
6   Schwarz MA, Owaribe K, Kartenbeck J, Franke WW: Desmosomes and hemidesmosomes: Constitutive molecular components. Ann Rev Cell Biol 1990;6:461–491.
7   Stanley JR, Woodley DT, Katz SI, Martin GR: Structure and function of basement membrane. J Invest Dermatol 1982;79(suppl):69–72.
8   Morris NP, Sakai LY, Keene DR, Glanville RW, Burgeson RE: The structure of type VII collagen and its relationship to anchoring filaments. J Cell Biol 1985;101:101.
9   Cleutjens JP, Havenith MG, Kasper M, Vallinga M, Bosman FT: Absence of type IV collagen in the centre of the corneal basement membrane. Histochem J 1990;22:688–694.
10 Prockop DJ, Kivirikko KI: Collagens: Molecular biology, diseases, and potentials for therapy. Annu Rev Biochem 1995;64:403–434.
11 Jones DA, Hunt SW, Prisayanh PS, Briggaman RA, Gammon WR: Immunodominant autoepitopes of type VII collagen are short, paired peptide sequences within the fibronectin type III homology region of the noncollagenous (NC1) domain. J Invest Dermatol 1995;104:231–235.

12   Keene DR, Sakai LY, Lunstrum GP, Morris NP: Type VII collagen forms an extended network of anchoring fibrils. J Cell Biol 1987;104:611–621.

13   Kuettner KE, Kimura JH: Proteoglycans: An overview. J Cell Biochem 1985;27:327–336.

14   Hogan B: Laminin and epithelial attachment. Nature 1981;290:737–738.

15   Timpl R, Engel J, Martin GR: Laminin – a multi-functional protein of basement membranes. Trends Biochem Sci 1983;8:207–209.

16   Yurchenco PD, O'Rear JJ: Basal lamina assembly. Curr Opin Cell Biol 1994;6:674–681.

17   Eady RA, Dunnill MG: Epidermolysis bullosa: Hereditary skin fagility diseases as paradigms in cell biology. Arch Dermatol Res 1994;287:2–9.

18   Marinkovich MP, Verrando P, Keene DR, Meneguzzi G, Lunstrum GP, Ortonne JP, Burgeson RE: Basement membrane proteins kalinin and nicein are structurally and immunologically identical. Lab Invest 1993;89:295–299.

19   Marchisio PC, Cremona O, Savoia P, Pellegrini G, Ortonne JP, Verrando P, Burgeson RE, Cancedda R, De Luca M: The basement membrane protein BM-600/nicein codistributes with kalinin and the integrin alpha 6 beta 4 in human cultured keratinocytes. Exp Cell Res 1993;205:205–212.

20   Vailly J, Verrando P, Champliaud MF, Gerecke D, Wagman DW, Baudoin C, Aberdam D, Burgeson R, Bauer E, Ortonne JP: The 100-kDa chain of nicein/kalinin is a laminin B2 chain variant. Eur J Biochem 1994;219:209–218.

21   Verrando P, Schofield O, Ishida-Yamamoto A, Aberdam D, Partouche O, Eady RA: Nicein (BM6OO) in junctional epidermolysis bullosa: Polyclonal antibodies provide new clues for a pathogenic role. J Invest Dermatol 1993;101:738–743.

22   Carter WG, Ryan MC, Gahr PJ: Epiligrin, a new cell adhesion ligand for integrin alpha 3 beta 1 in epithelial basement membranes. Cell 1991;65:599–610.

23   Domloge-Hultsch N, Anhalt GJ, Gammon WR, Lazarova Z, Briggaman R, Welch M, Jabs DA, Huff C, Yancey KB: Antiepiligrin cicatricial pemphigoid. A subepithelial bullous disorder. Arch Dermatol 1994;130:1521–1529.

24   Kirtschig G, Marinkovich MP, Burgerson RE, Yancey KB: Anti-basement membrane autoantibodies in patients with anti-epiligrin cicatricial pemphigoid bind the alpha subunit of laminin 5. J Invest Dermatol 1995;105:543–548.

25   Yancey KB, Kirtschig G, Yee C, Lazarova Z: Studies of patients with anti-epiligrin cicatricial pemphigoid. J Dermatol 1995;22:829–835.

26   Marshall GE, Konstas AG, Lee WR: Immunogold fine structural localisation of extracellular matrix components in aged human cornea. II. Types V and VI. Graefe's Arch Clin Exp Ophthalmol 1991; 229:164–171.

27   Fine JD, Horiguchi Y, Jester J, Couchman JR: Detection and partial characterization of a midlamina lucida hemidesmosome associated antigen (19-DEJ-1) present within human skin. J Invest Dermatol 1989;92:825–830.

28   McGrath JA, Ishida-Yamamoto A, Shimizu H, Fine JD, Eady RA: Immunoelectron microscopy of skin basement membrane: A pre-embedding method using 1-nm immunogold with silver enhancement. Acta Derm Venereol (Stockh) 1994;74:197–200.

29   Sakai LK, Keene DR, Engvall E: Fibrillin, a new 350 kD glycoprotein, is a new component of extracellular matrix. J Cell Biol 1986;103:2499–2509.

30   Sternberg M, Cohen-Forterre L, Peyroux J: Connective tissue in diabetes mellitus: Biochemical alterations of the extracellular matrix with special reference to proteoglycans, collagens and basement membranes. Diabete Metab 1985;11:27–50.

31   Weislander J, Barr JF, Butkowski RJ, Edwards SJ, Bygren P, Heingard D, Hudson BJ: Goodpasture antigen of the basement membrane: Localization to noncollagenous regions of type IV collagen. Proc Natl Acad Sci USA 1984;81:3838–3842.

32   Martinez-Hemandez A, Amenta PS: The basement membrane in pathology. Lab Invest 1983;48: 656–677.

33   Yancey KB: From bedside to bench and back. The diagnosis and biology of bullous diseases. Arch Dermatol 1994;130:983–987.

34   Dabelsteen E, Ullman S, Thomsen K: Demonstration of basement membrane autoantibodies in patients with benign mucous membrane pemphigoid. Acta Derm Venereol 1974;54:189–192.

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C

35 Leonard JN, Hobday CM, Haffenden GP: Immunofluorescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.

36 Mondino BJ, Ross AN, Rabin BS, Brown SI: Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 1977;83:443–450.

37 Mondino BJ, Brown SI, Rabin BS: Autoimmune phenomenon in the external eye. Trans Am Acad Ophthalmol 1978;85:801–817.

38 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.

39 Person JR, Rogers RS III: Bullous and cicatricial pemphigoid: Clinical, histopathologic, and immunopathologic correlations. Mayo Clin Proc 1977;52:54–66.

40 Waltman SR, Yarian D: Circulating autoantibodies in ocular pemphigoid. Am J Ophthalmol 1974; 77:891–894.

41 Fine JD, Neises GR, Katz SI: Immunofluoresence and immunoelectron microscope studies in cicatricial pemphigoid. J Invest Dermatol 1984;82:39–43.

42 Bernard P, Prost C, Durepaire N, Basset-Seguin N, Didierjean L, Saurat J: The major cicatricial pemphigoid antigen is a 180-kD protein that shows immunologic cross-reactivities with the bullous pemphigoid antigen. Invest Dermatol 1992;99:174–179.

43 Niimi Y, Zhu XJ, Bystryn JC: Identification of cicatricial pemphigoid antigens. Arch Dermatol 1992;128:54–57.

44 Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.

45 Rice BA, Foster CS: Immunopathology of cicatricial pemphigoid affecting the conjunctiva. Ophthalmology 1990;97:1476–1483.

46 Bernauer W, Wright P, Dart JKG, Leonard JN, Lightman S: The conjunctiva in acute and chronic mucous membrane pemphigoid: An immunohistochemical analysis. Ophthalmology 1993;100:339–346.

47 Carroll JM, Kuwabara T: Ocular pemphigus: An electron microscopic study of the conjunctival and corneal epithelium. Arch Ophthalmol 1968;80:683–695.

48 Gallbavy EJ, Foster CS: Ultrastructural characteristics of conjunctiva in ocular cicatricial pemphigoid Cornea 1985;4:127–136.

49 Chuah KL, Earley OT, Mahon G, Gardiner T, Archer DB: Progressive changes in ocular cicatricial pemphigoid. Invest Ophthalmol Vis Sci 1996;37:4700.

50 Roat MI, Alstradt SP, Carpenter AB, SundarRaj N, Thoft RA: Anti-basement membrane antibody-mediated experimental conjunctivitis. Invest Ophthalmol Vis Sci 1990;31:168–175.

51 Wright P: Cicatrizing conjunctivits. Trans Ophthalmol Soc UK 1986;105:1–17.

52 Wright P: Untoward effects associated with practolol administration: Oculocutaneous syndrome. Br Med J 1975;i:595–598.

53 Wright P: Skin reactions to practolol. Br Med J 1974;ii:560.

54 Amos HE, Brigden WD, McKerron RA: Untoward effects associated with practolol administration. Br Med J 1975;i:598–561.

55 Jachuck SJ, Bird T, Stephenson J, Jackson FS, Clark F: Practolol induced antibodies and their relationship to oculocutaneous complications. Postgrad Med J 1977;53:75–77.

56 Pouliquen Y, Patney A, Foster CS, Goichot L, Savoldelli M: Drug-induced cicatricial pemphigoid effecting the conjunctiva. Light and electron microscopic features. Ophthalmology 1986;93:775–783.

57 Fiore PM, Jacobs IH, Goldberg DB: Drug-induced pemphigoid. A spectrum of diseases. Arch Ophthalmol 1987;105:1660–1663.

58 Partrey PS, Clement M, Vandenburg J, Wright P: Captopril-induced pemphigus. Br Med J 1980: 281:194.

59 Shimizu H; McDonald JN, Gunner DB, Black MM, Bhogal B, Leigh IM, Whitehead PC, Eady RA: Epidermolysis bullosa acquisita antigen and the carboxy teminus of type VII collagen have a common immunolocalization to anchoring fibrils and lamina densa of basement membrane. Br J Dermatol 1990;122:577–585.

60 Shimizu H, Ishida-Yamamoto A, Eady RA: The use of silver-enhanced 1-nm gold probes for light and electron microscopic localisation of intra- and extracellular antigens in the skin. J Histochem Cytochem 1992;40:883–888.

61 Shimizu H, Masunaga T, Ishiko A, Hashimoto T, Garrod DR, Shida H, Nishikawa T: Demonstration of desmosomal antigens by electron microscopy using cryofixed and cryosubstituted skin with silver-enhanced gold probe. J Histochem Cytochem 1994;42:687–692.

62 McGrath JA, Ishida-Yamamoto A, O'Grady A, Leigh IM, Eady RA: Structural variations in anchoring fibrils in dystrophic epidemolysis bullosa: Correlation with type VIII collagen expression. J Invest Dermatol 1993;100:366–372.

63 Yokata S: Effect of particle size on labelling density for catalase in protein A-gold immunocytochemistry. J Histochem Cytochem 1988;38:107–109.

64 Ishiko A, Shimizu H, Kituchi A, Ebihara T, Hashimoto T, Nishikawa T: Human autoantibodies against the 230-kD bullous pemphigoid antigen (BPAG1) bind only to the intracellular domain of the hemidesmosome, whereas those against the 180-kD bullous pemphigoid antigen (BPAG2) bind along the plasma membrane of the hemidesmosome in normal human and swine skin. J Clin Invest 1993;91:1608–1615.

65 Novikoff AB, Novikoff PM, Quintana N, Davis C: Diffusion artifacts in 3,3′-diaminobenzidine cytochemistry. J Histochem Cytochem 1972;20:745–749.

66 Bedane C, Prost C, Bernard P, Catanzano G, Bonnetblanc J, Dubertret L: Cicatricial pemphigoid antigen is different from the bullous pemphigoid antigen by its exclusive extracellular location. A study by indirect immunohistochemistry. J Invest Dermatol 1991;97:3–9.

67 Woodley DT, Burgeson RE, Lunstrum GP, Bruckner-Tuderman L, Reese MJ, Briggaman RA: The epidermolysis bullosa acquisita antigen is the globular carboxy terminus of the type VII collagen. J Clin Invest 1988;81:683–687.

68 Stanley JR, Woodley D, Katz S: Identification and partial characterisation of pemphigoid antigen extracted from normal human skin. J Invest Dermatol 1984;82:108–111.

69 Bernard P, Prost C, Autcouturier P, Durepaire N, Denis F, Bonnetblanc JM: The subclass distribution of IgG autoantibodies in cicatricial pemphigoid and epidermolysis bullosa acquisita. J Invest Dermatol 1991;97:259–263.

70 Shimizu H, McDonald JN, Kennedy AR, Eady RA: Demonstration of intra- and extracellular localization of bullous pemphigoid antigen using cryofixation and freeze substitution for postembedding immunoelectron microscopy. Arch Dermatol Res 1989;281:443–448.

71 Nieboer C, Boorsma DM, Woerdeman MJ: Immunoelectron microscopic findings in cicatricial pemphigoid: Their significance in relation to epidermolysis bullosa acquisita. Br J Dermatol 1983;108:419–422.

72 Honigsmann H, Stingl G, Holubar K, Wolff-Schreiner E, Konrad K, Wolff K: Auto-antibodies and immune complexes in immune dermatoses. Mapping of fine structural binding sites. Invest Dermatol 1976;66:263.

73 Prost C, Dubertret L, Wechsler J, Touraine R: A routine immuno-electron microscopic technique for localizing an auto-antibody on epidermal basement membrane. Br J Dermatol 1984;110:1–7.

74 Bernard P, Prost C, Lecerf V, Intrator L, Combemale P, Bedane C, Roujeau JC, Revuz J, Bonnetblanc JM, Dubertret L: Studies of cicatricial pemphigoid autoantibodies using direct immunoelectron microscopy and immunoblot analysis. J Invest Dermatol 1990;94:630–635.

75 Robin H, Prost C, Hoang-Xuan T: Pure ocular cicatrical pemphigoid is a unique entity: A direct immunoelectron microscopy study. Invest Ophthalmol Vis Sci 1996;37:4701.

76 Shimizu H, Masunaga T, Ishiko A, Matsumura K, Hashimoto T, Nishikawa T, Domloge-Hultsch N, Lazarova Z, Yancey KB: Autoantibodies from patients with cicatricial pemphigoid target different sites in epidermal basement membrane. J Invest Dermatol 1995;104:370–373.

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The Cellular Response in the Conjunctiva

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Cicatricial pemphigoid, linear IgA disease, certain paraneoplastic syndromes and progressive forms of drug-induced conjunctival cicatrisation share a common pathogenesis in that they are potentially mediated by anti-basement membrane antibody [1–11]. Based on the findings of animal and immunohistochemical studies [1–4, 6, 12–16], the following sequence of events can be proposed for the pathogenesis of the lesions in conjunctivitis mediated by anti-basement membrane antibody: circulating antibodies bind to the antigen of the basement membrane (resulting in the biopsy finding of linear deposits of immunoglobulin), complement fixation occurs, and an inflammatory infiltrate with subsequent scarring develops.

The understanding of these mechanisms is incomplete. The exact site(s) where the antigen interacts with the antigen-presenting cells as well as the site where the antibody production takes place are unknown, the factors that control the inflammatory response and those that mediate the repair processes are known only partially. The possibilities to study these effector mechanisms in *patients* are limited, and the work on animal models has only started recently [4, 17]. In the last years the studies on effector mechanisms have thus focused on the antibodies that mediate the immune response (see 'Immunological Investigations', pp. 102–110) and on the conjunctiva itself with its cellular changes – and more recently – its cytokine network.

Histopathology in Progressive Cicatrising Conjunctivitis

The predominant morphological features in ocular cicatricial pemphigoid (OCP) and other progressive forms of cicatrising conjunctivitis are *metaplastic epithelial changes, infiltration of the substantial propria by inflammatory cells,*

Fig. 1. T lymphocytes in normal conjunctiva. Note non-keratinised stratified columnar epithelium with goblet cells. A small number of T cells is found in the epithelium and the underlying substantia propria. Anti-CD3 antibody, immunoperoxidase reaction. Original magnification × 250.

Fig. 2. T lymphocytes in OCP with moderate conjunctival inflammation. In addition to squamous metaplasia of the epithelium and absence of goblet cells, note the extreme degree of T cell infiltration of the conjunctival stroma. Anti-CD3 antibody, immunoperoxidase reaction. Original magnification × 250.

and fibrosis of the substantia propria with new connective tissue [2, 18, 19]. This formation of connective tissue with a mononuclear infiltrate beneath the conjunctival epithelium represents the esssential process in OCP [20].

Morphological *epithelial changes* reflect the ocular surface condition and include squamous metaplasia and a reduction in goblet cell numbers (fig. 1, 2). A wide spectrum of these changes can be observed in patients with identical clinical findings [2]. Hence these epithelial changes do not reflect the inflammatory disease activity or the stage of disease with the amount of scarring. In early conjunctival disease, the conjunctiva may show subepithelial vascular proliferation accompanied by perivascular inflammation. In severely inflamed conjunctiva, the epithelium and stroma may undergo focal necrosis with infiltration by neutrophils [21].
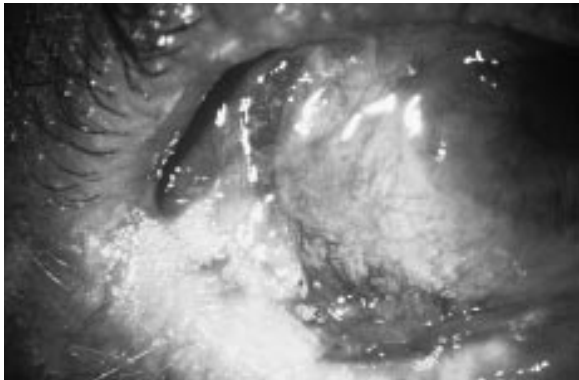
Fig. 3. OCP with severe conjunctival inflammation in a 73-year-old man. Note conjunctival swelling, ulceration and symblepharon formation.

The *cellular infiltrate* in the substantia propria of patients with OCP and other progressive forms of cicatrising conjunctivitis involves various cell types. Increased numbers of polymorphonuclear leucocytes, histiocytes, lymphocytes, plasma and mast cells have been described [2, 18, 22]. Immunopathologic studies gave evidence of an increase in the T helper/T suppressor cell ratio and increased numbers of Langerhans cells [23–25]. Abundant mature plasma cells contrast with a normal number of B lymphocytes [2].

Disease Activity and Cellular Infiltrate in Ocular Cicatrising Conjunctivitis

Progression of fibrosis generally occurs during or after manifest conjunctival inflammation [26, 27] and a correlation of disease activity with the cellular findings may be informative with regard to the mechanisms of chronic conjunctival scar tissue formation. The composition of the cellular infiltrate in the bulbar conjunctiva of OCP varies, resulting in changes mainly affecting neutrophil, macrophage, and T cell numbers (table 1) [25]. Severe ocular inflammation is characterised by an abundance of macrophages, Langerhans cells and neutrophils in the substantia propria (fig. 3, 4). Raised numbers of T lymphocytes, most marked in moderately inflamed conjunctiva (fig. 2), are generally found in affected conjunctiva. Abundant CD4+ cells, representing one of the T cell subsets, are mainly a feature of severely inflamed conjunctiva (fig. 5). Only about 5% of the T cells are activated as demonstrated by expression of the IL-2 receptor. Increased expression of MHC II molecules

Table 1. Synopsis of statistically significant changes in the subepithelial cellular infiltrate of 20 pemphigoid patients in comparison with controls (n = 12) [25, 28]

Severe inflammation	
Neutrophils	+ + +
Macrophages	+ +
Langerhans/dendritic cells	+ + +
T lymphocytes	+ +
CD4/CD8 ratio	1.0
MHC II molecule (HLA-DR) expressing cells	+ +
Actively proliferating fibroblasts	+
Moderate inflammation	
Neutrophils	+ +
Macrophages	+ +
T lymphocytes	+ + +
CD4/CD8 ratio	0.4
Activated T cells	+ +
MHC II molecule (HLA-DR) expressing cells	+ + +
Mild inflammation	
Macrophages	+
T lymphocytes	+ +
CD4/CD8 ratio	0.5
MHC II molecule (HLA-DR) expressing cells	+ +

+ Up to threefold increase over normal controls (mean value)

+ + Three- to tenfold increase over normal controls (mean value)

+ + + Ten-fold or higher increase over normal controls (mean value)

is found on macrophages, fibroblasts, and other cells (fig. 6–8). Actively proliferating fibroblasts may be present in inflamed conjunctiva [28], and studies in tissue cultures have shown that conjuctival fibroblasts of cicatricial pemphigoid patients are hyperproliferative when compared with normal controls [29].

Comment

Conventional histopathological study of the conjunctiva is not *diagnostic* for OCP or the other progressive forms of cicatrising conjunctivitis. The morphological epithelial changes described above only reflect the ocular surface
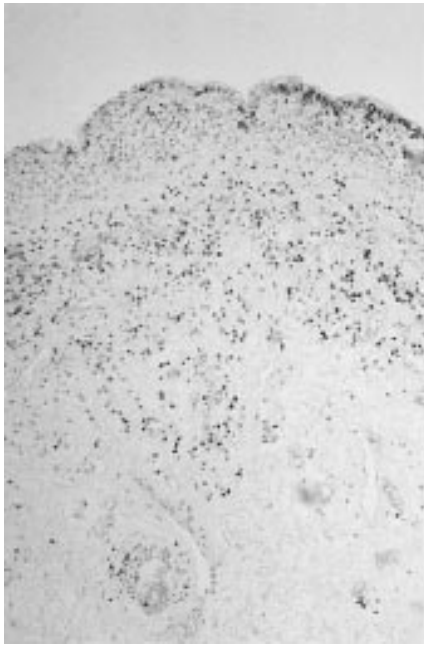
Fig. 4. Biopsy findings in the conjunctiva of the patient shown in figure 3. An abundance of neutrophilic granulocytes in the epithelium and substantia propria is present in severely inflamed conjunctiva. Antineutrophil-elastase antibody, immunoperoxidase reaction. Original magnification × 125.

condition, and other chronic conjunctival disorders show similiar characteristics in the composition of the cellular infiltrate [30–32].

Histopathology and cellular phenotyping by immunological methods, however, may help in the assessment of *disease activity* and in the *investigation of the mechanisms leading to conjunctival scar tissue formation*.

The inflammatory activity in OCP is accompanied by increased numbers of neutrophils, macrophages, CD4-positive cells and Langerhans cells in the conjunctival stroma (table 1). It is not clear yet whether these cellular findings represent early disease, but abundance of neutrophils are also found early in the experimental model of anti-basement membrane conjunctivitis [4].

Macrophages are essential in wound healing [33–35] and can promote the transition from inflammation to new tissue formation by secretion of growth factors [36]. A significantly increased number of macrophages is found in OCP, indicating potential promotion of scar tissue formation.

T cells are abundant in the conjunctival stroma of OCP patients [24, 25], but only a small number of them are activated (as identified with anti-IL-2-receptor). A relatively high CD4/CD8 ratio in severe conjunctival inflammation (early?) possibly reflects the role of the T cells in the recruitment of other inflammatory cells. The abundance of MHC class II expressing cells (HLA-
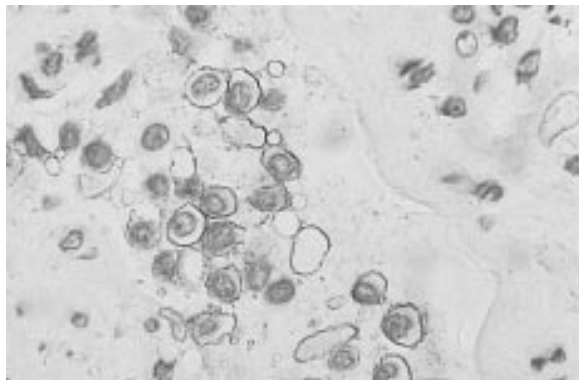
Fig. 5. OCP with moderate conjunctival inflammation. A cluster of CD4+ cells is shown. Note the characteristic ring-shaped staining pattern for T cells. Anti-CD4 antibody, immunoperoxidase reaction. Original magnification × 1,250.
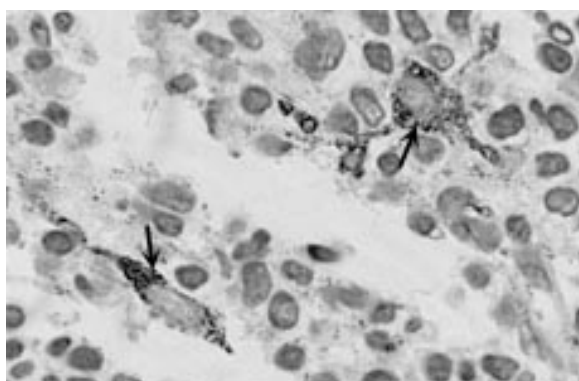
Fig. 6. OCP with moderate conjunctival inflammation. Two macrophages within a dense lymphocytic infiltrate are shown. Anti-CD68 antibody, immunoperoxidase reaction. Original magnification × 1,250.

DR+) indicates the potential to present local antigens to the CD4+ cells. The increase of Langerhans dendritic cells in severely inflamed conjunctiva may indicate that another cell type is implicated in local antigen processing for T cells. Activated T lymphocytes may contribute to the process of cicatrisation by recruiting and activating fibroblasts. This control can be achieved via lymphokines acting directly on fibroblasts or via macrophages or both [37]. T cells, however, are not essential for scar tissue formation, as shown in

Fig. 7. MHC class II molecule (human class II histocompatibility antigen) expression in normal conjunctiva. Anti-HLA-DR antibody, immunoperoxidase reaction. Original magnification × 312.
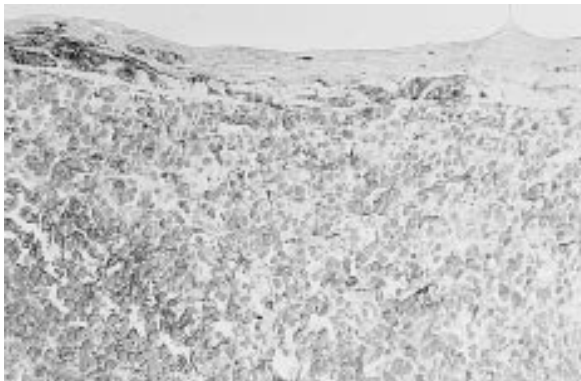
Fig. 8. MHC class II molecule expression in OCP with moderate conjunctival inflammation. Anti-HLA-DR antibody, immunoperoxidase reaction. Original magnification × 312.

experiments of T cell depletion [38] and in studies with nude rats, which have a profoundly impaired T cell system [39]. The role of T cells appears to be mainly in regulating the repair process [38] and this may explain why immunosuppressive therapy is frequently unable to stop the process of cicatrisation. Another explanation for this is the fact that fibroblasts themselves produce a variety of growth factors [4]; therefore once activated, they can sustain their own growth.

Although an increased number of plasma cells are present, only low numbers of B cells and also a low number of natural killer cells are found. This may indicate that these cells, when situated in the bulbar conjunctiva, do not play a major role in the pathogenesis of conjunctival cicatrisation. The disease process, however, also involves the forniceal and tarsal conjunctiva where biopsies are contra-indicated because of the possibility of promoting the disease process [2, 41, 42]. Crucial findings there may be missed. It is also important to note, that when biopsy specimens in this condition are investigated, the disease has already existed for some time and it is likely that there are secondary phenomena masking primary events.

The variability of the subepithelial cellular infiltrate with disease activity and the involvement of several cell lines (mainly neutrophil, macrophage, and T cells) provides the rationale for using broad-spectrum, rather than T cell-specific immunosuppressive agents in chronic progressive conjunctival cicatrisation.

The fact that other chronic conjunctival disorders show similar characteristics in the composition of the cellular infiltrate suggests that the clinical characteristics of different diseases are not primarily determined by cell morphology, but by differences in the secretory activity of these cells.

References

1   Mondino BJ, Ross AN, Rabin BS, Brown SI: Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 1977;83:443–450

2   Foster CS: Cicatricial pemphigoid. Trans Am Opthalmol Soc 1986;84:527–663.

3   Bean SF, Waisman M, Michel B, Thomas SI, Knox JM, Levine M: Cicatricial pemphigoid. Arch Dermatol 1972;106:195–199.

4   Roat MI, Alstadt S, Carpenter AB, Sundar Raj N, Thoft A: Antibasement membrane antibody-mediated experimental conjunctivitis. Invest Ophthalmol Vis Sci 1990;31:168–175.

5   Leonard JN, Haffenden GP, Ring NP, McMinn RMH, Sidgwick A, Mowbray JF, Unsworth DJ, Holborow EJ, Blenkinsopp WK, Swain AF, Fry L: Linear IgA disease in adults. Br J Dermatol 1982;107:301–316.

6   Leonard JN, Hobday CM, Haffenden GP, Griffiths CEM, Powles AV, Wright P, Fry L: Immunofluorescent studies in ocular cicatricial pemphigoid. Br J Dermatol 1988;118:209–217.

7   Anhalt GJ, Kim S, Stanley JR, Korman NJ, Jabs DA, Kory M, Izumi H, Ratrie III H, Mutasim D, Ariss-Abdo L, Iabib RS: Paraneoplastic pemphigus: An autoimmune mucocutaneous disease associated with neoplasia. N Engl J Med 1990;323:1729–1735.

8   Helm TN, Camisa C, Valenzuela R, Allen CM: Paraneoplastic pemphigus. A distinct autoimmune vesiculobullous disorder associated with neoplasia. Oral Surg Oral Med Oral Pathol 1993;75:209–213.

9   Bystrin JC, Hodak E, Gao SQ, Chuba VJ, Amorosi EL: A paraneoplastic mixed bullous skin disease associated with anti-skin antibodies and a B-cell lymphoma. Arch Dermatol 1993;129:870–875.

10 Pouliquen Y, Patey A, Foster CS, Goichot L, Savodelli M: Drug induced cicatricial pemphigoid affecting the conjunctiva. Light and electron microscopic features. Ophthalmology 1986;93:775–783.

11 Fiore PM, Jacobs IH, Goldberg DB: Drug-induced pemphigoid. A spectrum of diseases. Arch Ophthalmol 1987;105:1660–1663.

12 Bean SF: Cicatricial pemphigoid – immunofluorescent studies. Arch Dermatol 1974;110:552–555.

13 Griffiths M, Fukuyama K, Tuffanelli D, Silverman S: Immuno-fluorescent studies in mucous membrane pemphigoid. Arch Dermatol 1974;109:195–199.

14 Furey N, West C, Andrewa T, Paul PD, Bean SF: Immunofluorescent studies of ocular cicatricial pemphigoid. Am J Ophthalmol 1975;80:825–831.

15 Mondino BJ, Brown SI, Rabin BS: Autoimmune phenomena of the external eye. Ophthalmology 1978;85:801–817.

16 Frith PA, Venning VA, Wojnarowska F, Millard PR, Bron AJ: A conjunctival involvement in cicatricial and bullous pemphigoid: A clinical and immunopathological study. Br J Ophthalmol 1989;73:52–56.

17 Lazarova Z, Yee C, Darling T, Briggaman RA, Yancey KB: Passive transfer of anti-laminin 5 antibodies induces subepidermal blisters in neonatal mice. J Clin Invest 1996;98:1509–1518.

18 Bernauer W, Elder MJ, Leonard J, Wright P, Dart JK: The value of biopsies in the evaluation of chronic progressive conjunctival cicatrisation. Graefe's Arch Clin Exp Ophthalmol 1994;232:533–537.

19 Andersen SR, Jensen OA, Kristensen EB, Norn MS: Benign mucous membrane pemphigoid. III. Biopsy. Acta Ophthalmol (Copenh) 1974;52:455–463.

20 Duke-Elder S, MacFaul PA. System of Ophthalmology. London, Kimpton 1965, vol 8, pp 496–527.

21 Spencer WH: Ophthalmic Pathology. I. Philadelphia, Saunders, 1996, pp 88–89.

22 Hoang-Xuan T, Foster CS, Raizman MB, Greenwood B: Mast cells in conjunctiva affected by cicatricial pemphigoid. Ophthalmology 1989;96:1110–1114.

23 Sacks EH, Jakobiec FA, Wieczorek R, Donnenfeld E, Perry H, Knowles DM: Immunophenotypic analysis of the inflammatory infiltrate in ocular cicatricial pemphigiod. Further evidence for a T cell-mediated disease. Ophthalmology 1989;96:236–243.

24 Rice BA, Foster CS: Immunopathology of cicatricial pemphigoid affecting the conjunctiva. Ophthalmology 1990:97:1476–1483.

25 Bernauer W, Wright P, Dark JK, Leonard J, Lightman S: The conjunctiva in acute and chronic mucous membrane pemphigoid. An immunohistochemical analysis. Ophthalmology 1993;100:339–346.

26 Mondino BJ, Brown SI, Lempert S, Jenkins MS: The acute manifestations of cicatricial pemphigoid: Diagnosis and treatment. Ophthalmology 1979;86:543–552.

27 Elder BJ, Bernauer W, Leonard J, Dart JKG: Progression of disease in ocular cicatricial pemphigoid. Br J Opthalmol 1996;80:292–296.

28 Bernauer W, Wright P, Dark JK, Leonard J, Lightman S: Cytokines in acute and chronic mucous membrane pemphigoid: An immunohistochemical analysis. Graefe's Arch Clin Exp Ophthalmol 1993;231:563–570.

29 Roat MI, Sossi G, Lo CY, Thoft RA: Hyperproliferation of conjunctival fibroblasts from patients with cicatricial pemphigoid. Arch Ophthalmol 1989;107:1064–1067.

30 Foster CS, Rice BA, Dutt JE: Immunopathology of atopic kerato-conjunctivitis. Ophthalmology 1991:98:1190–1196.

31 Karma A, Tsakinen E, Kainulainen H, Partanen M: Phenotypes of conjunctival inflammatory cells in sarcoidosis. Br J Ophthalmol 1992;76:101–106.

32 Hoang-Xuan T, Rodriguez A, Zaltas MM, Rice BA, Foster CS: Ocular rosacea: A histologic and immunopathological study. Ophthalmology 1990;97:1468–1475.

33 Howes EL Jr: Basic mechanisms in pathology; in Spencer WH (ed); Ophthalmic Pathology. I. An Atlas and Textbook, ed. 3. Philadelphia, Saunders, 1985, vol 1, pp 14–51.

34 Leibovich SJ, Ross R: The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. Am J Pathol 1975;78:71–100.

35 Johnston BR: Monocytes and macrophages. N Engl J Med 1988;318:747–752.

36 Clark RAF: Cutaneous wound repair: A review with emphasis on integrin receptor expression; in Janssen H, Rooman R, Robertson JIS (eds): Wound Healing. Petersfield, Wrightson, 1991, pp 7–17.

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C

37 Regan MC, Barbul A: Regulation of wound healing by the T cell-dependent immune system; in Janssen H, Rooman R, Robertson JIS (eds): Wound Healing. Petersfield, Wrightson, 1991, pp 21–31.

38 Barbul A, Breslin RJ, Woodyard JP, Wasserkrug HL, Efron G: The effect of in vivo T helper and T suppressor lymphocyte depletion on wound healing. Ann Surg 1989;209:479–483.

39 Barbul A, Shawe T, Rotter SM, Efron JE, Wasserkrug HL, Badawy SB: Wound healing in nude mice: A study on the regulatory role of lymphocytes in fibroplasia. Surgery 1989;105:764–769.

40 Shipley GD, Keeble WW, Hendrickson JE, Coffey RJ Jr, Pittelkow MR: Growth of normal human keratinocytes and fibroblasts in serum-free medium is stimulated by acidic and basic fibroblast growth factor. J Cell Physiol 1989;138:511–518.

41 Wright P: Cicatrizing conjunctivitis. Trans Ophthalmol Soc UK 1986;105:1–17.

42 De la Maza MS, Tauber J, Foster CS: Cataract surgery in ocular cicatricial pemphigoid. Ophthalmology 1988;95:481–486.

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The Role of Cytokines in Chronic Progressive Conjunctival Cicatrisation

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Fibrosis results from the abnormal deposition of collagen and extracellular matrix secondary to pathophysiological changes in the fibroblasts and to the chemical signalling from other cells. The uniqueness of the fibrosis in chronic progressive cicatrising conjunctivitis is a function of the essential characteristics of the conjunctiva, the additional pathological cellular infiltrate and the cytokine profile of this admixture (see previous chapter). The exact aetiology of the fibrosis remains unknown, but we do have some of the few pieces of this puzzle.

Cytokines are polypeptides that act as intercellular messengers and information channels. The broad family includes the interleukins, the growth factors and the colony-stimulating factors (table 1). The cytokines usually influence cells by a local action (a paracrine effect). They may also respond to the self-production of a cytokine (an autocrine effect) or a distant effect via some fluid transport mechanism (an endocrine effect). In general, the fibrogenic cytokines are vital contributors to normal wound healing. However, in some instances an adaptive process results in dysregulation with the production of excessive scar tissue. This is analogous to the prostaglandins which are essential for homeostasis, but in excess they cause pain and inflammation.

Wound Healing

Healing of a wounded tissue involves reconstituting the former structure of that tissue. In neonates and in 'perfect repair', a tissue heals without scarring. This is uncommon but scar tissue per se is not essential for normal wound integrity [1]. The standard model of fibrosis is primary wound healing after

Table 1. The families of growth factors

Cytokine	Cells influenced by the factor
Fibroblast growth factor (FGF) *basic, acidic, FGF 3–6* *keratocyte growth factor*	fibroblasts, endothelium, myoblasts, neurones, epithelia, smooth muscle
Platelet derived growth factor (PDGF) *AA, BB, AB*	vascular smooth muscle, fibroblasts, myoblasts, glia
Transforming growth factor (TGF) *alpha and beta (1–5)*	fibroblasts, smooth muscle epithelia, fibroblasts
Epidermal growth factor	epithelia
Bone morphogenetic proteins	osteoblasts
Mullerian inhibiting substance	
Activins	mesenchyme, pituitary
Inhibins	mesenchyme, pituitary
Tumour necrosis factor (TNF) *alpha and beta*	fibroblasts, endothelium, T cells
Nerve growth factor	neuronal cells, Schwann cells

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surgical trauma, such as an incisional or excisional wound. This model is an excellent platform to understand the scarring response in chronic progressive cicatrising conjunctivitis although the primary stimulus is different: instantaneous wound versus chronic immune-related inflammation.

The stages of acute wound healing are:

(1) Acute inflammation. The trauma induces vascular leakage which deposits fibrinogen, fibronectin and platelets from the serum. Prostaglandins and leukotrienes are released from platelets and surrounding tissues.

(2) Coagulation of serum products.

(3) Cellular migration. This initially involves neutrophils followed by macrophages and epithelial cells. Phagocytic activity removes the necrotic tissue before repair begins.

(4) Granulation tissue formation. By approximately day 5, fibroblasts migrate and proliferate. They are followed by migrating endothelium which forms vessels – angiogenesis. Fibroblasts initially produce immature type III collagen along with elastin and mucopolysaccharides. The stimuli for migration and proliferation are various cytokines and extracellular matrix components such as fibronectin (vide infra).

(5) Mature scar formation. Eventually type I collagen replaces type III collagen and becomes increasingly cross-linked. Wound contraction and late remodelling occurs over months. The ultimate amount of collagen and extracellular matrix depends on the ongoing equilibrium between production and degradation.

The Time Course of Cytokine Involvement in Wound Healing

Cytokines are involved in wound healing as hyperacute, intermediate and late mediators. Within seconds of wounding, thrombin activates platelet release of the alpha granules which include preformed platelet factor 4, prostaglandins, leukotrienes, platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β) and other hyperacute mediators of vasoconstriction and inflammation. Platelet factor 4 is chemotactic for neutrophils, macrophages and fibroblasts and is released directly at the site [2]. PDGF from platelets also acts as a hyperacute chemoattractant. Therefore, the platelets have a prime role in the early events of wound repair. During the intermediate phase of repair, cytokines from inflammatory cells and fibroblasts support ongoing chemotaxis and trigger production of extracellular matrix and collagen. During the later phase of healing, cytokines and extracellular matrix provide secondary modulation of the key cytokines [1].

Platelet-Derived Growth Factor (PDGF)

PDGF is an erroneously named cytokine, initially discovered in the alpha granule of platelets, but also produced by activated macrophages, stimulated fibroblasts, smooth muscle cells and many malignant cells [1]. PDGF occurs as a dimer of either/or chain A or B; PDGF-AA, PDGF-BB and PDGF-AB. There are two types of PDGF receptors – Rα and Rβ and there is some evidence that these may be up- and down-regulated [3, 4].

In animal models, application of just one dose of 20 pmol of PDGF per wound accelerates wound healing by transiently recruiting and activating neutrophils, macrophages and fibroblasts and depositing extracellular matrix. In the first 3 weeks, the tensile wound strength is increased by 150% although thereafter the wounds are histologically and mechanically similar to normal [1, 5–7].

PDGF also has secondary actions: increased epithelial growth probably due to induction of keratinocyte growth factor and of TGF-alpha [1]; it is a stimulus for macrophages and fibroblasts to synthesize increased amounts of TGF-β1 [5]; it further modulates thrombospondin expression; stimulates collagenase and allows induction of the monocyte chemoattractant protein

gene in fibroblasts [6–11]. Recently, PDGF-BB has been used in humans to successfully accelerate healing in chronic leg ulcers [12].

Fibroblast Growth Factors (FGF)

The fibroblast growth factors (FGF) are a family of seven similar polypeptides and are referred to as FGF-1 (acidic), FGF-2 (basic), FGF-3 (int-2), FGF-4 (hst-1), FGF-5, FGF-6 (hst-2) and FGF-7 (keratinocyte growth factor) [13]. FGF-3 to FGF-6 are oncogene products, found essentially in tumour cells and are mitogenic and angiogenic. There are four receptor types (FGF R1–4) which may be highly specific for a given FGF or be non-specific [14].

FGF-1 and FGF-2 (acidic and basic) are similar and both are involved in wound healing. They are mitogenic to fibroblasts, smooth muscle, endothelia, some neural tissue and keratinocytes. They are chemoattractant for endothelia and encourage angiogenesis. FGF basic also modulates embryogenesis [13, 15].

Keratinocyte growth factor (KGF, FGF-7) is synthesized by fibroblasts and binds to a specific receptor, located almost solely on epidermal keratinocytes [16, 17]. During wound healing, there are initially increased levels of fibroblast mRNA for KGF, followed by stromal increases in KGF and then upregulation of KGF receptors within the epithelium [18]. This implies that this specific cytokine stimulus for epithelial healing is driven by the underlying fibroblasts. Further, excess KGF in mice results in grossly wrinkled skin. The epidermis is increased in thickness and there are features of metaplasia [19]. Both of these concepts have implications to the conjunctiva and cornea in ocular cicatricial pemphigoid (OCP).

Transforming Growth Factor-Beta (TGF-β)

The TGF 'superfamily' consists of TGF, the activins, inhibins, the mullerian inhibiting substance and the bone morphogenetic proteins. TGF-β is a family of five cytokines of which three are recognized in mammals (TGF-β1, 2 and 3). Each member is secreted as a precursor and is activated by cleavage of a terminal domain. TGF-β triggers its biological effects by binding to a complex, three part, receptor. The effects of the TGF-β family are varied. For epithelium and neuroepithelium, it is inhibitory and anti-inflammatory. For mesenchyme, it stimulates and regulates extracellular matrix and collagen deposition.

TGF-β is known to be produced by most mammalian cells. This includes B cells [20], T cells [21], macrophages [22], fibroblasts [23], bone [24], smooth

muscle [25], liver mesenchyme [26], mesangial cells of the kidney [27] and most other body organs [28]. The highest concentrations are found in platelets and this is important in wounding whereby TGF-β is initially released in large amounts [29].

TGF-β Chemistry

In mammals, the most abundant isoform is TGF-β1. Chemistry and modes of action of TGF-β1, 2 and 3 are broadly similar. The sequences of TGF-β are known: TGF-β1 [30], TGF-β2 [31], TGF-β3 [32]. 70–80% of the protein sequence of TGF-β1 is identical to TGF-β2 and TGF-β3 and 30% is identical to the bone morphogenetic proteins, activins and inhibins. There is almost 100% sequence conservation between mammals. Each TGF-β is synthesized and secreted as a latent precursor molecule. Cleavage forms active TGF-β, which is a dimer of 112 amino acids (25 kD) and an inactive latency-associated peptide [33]. Modulation of cleavage can be achieved with a subtilisin-like protease, by acidification, by reaction with transglutaminase and with thrombospondin [33–36]. This modulation is poorly understood but it may occur within the extracellular environment, or on the surface of cells [37]. It is probable that the conversion of TGF-β from 'latent' to 'active' is crucial in its regulation and localisation.

In particular, thrombospondin is able to achieve cleavage at physiological tissue concentrations and in the absence of cells [36]. Thrombospondin is an adhesion matrix protein which interacts with collagen, fibronectin, plasminogen and glycoproteins [38, 39]. In human wounds the tissue concentration of thrombospondin is maximal at the wound edge at days 2–5 and mainly absent by day 14 [40]. Its normal decline in wounded tissue after 10 days may help switch the TGF-β mediated scarring off. Delayed healing leads to ongoing production of cytokines such as PDGF and TGF-β which ensure further production of thrombospondin [41, 42].

TGF-β Receptors

The receptor complex is composed of three parts, named 'receptor' I, II and III. Receptor II is a trans-membrane protein of the serine/threonine kinase receptor family and both receptor I and II are required for transduction. Initially, TGF-β binds to the betaglycan receptor III component which regulates its presentation to receptor I and II. Betaglycan is a membrane-bound chondroitin/heparin sulphate proteoglycan and is essential for TGF-β2 receptor binding but not for TGF-β1 or 3. TGF-β1, 2 and 3 are bound equally strongly to receptor I and II and further enhanced by the betaglycan. Endothelial cells respond well to TGF-β1 and 3, but reduced levels of betaglycan correlate with the poor response to TGF-β2.

Biological Effects

TGF-β is a powerful immunosuppressor, plays a major role in day-to-day regulation in many tissues and is able to influence B cells, most T cells and macrophages [33]. Knockout mice that lack the gene for TGF-β1 die at 3 weeks due to a massive inflammatory infiltration of lymphocytes and macrophages in most tissues, including lung, heart, lymph nodes, glandular tissue, gastro-intestinal tract and striated muscle [43, 44].

TGF-β is also pivotal in the regulation of newly formed scar tissue via three interactions. First, it stimulates the synthesis of individual matrix components including proteoglycans, collagens and glycoproteins. Second, it blocks matrix degradation by decreasing the synthesis of proteases and increasing the synthesis of protease inhibitors. Third, it increases the synthesis of matrix receptors and facilitates cellular adhesion to the matrix components [33, 41]. It is clear that TGF-β promotes scarring and that these three different means of regulation enhance each other.

A third function of TGF-β is in embryogenesis due to its role in angiogenesis and matrix deposition. In mouse embryos, TGF-β is expressed in a unique spatial and temporal pattern particularly in mesenchymal and neural crest tissues [33].

TGF-β in Wound Healing

In normal wound healing, there is a direct correlation of the amount of scarring with the amount of TGF-β [45]. Injection of TGF-β into a newborn mouse results in formation of granulation tissue [46]. In tissue culture, TGF-β increases fibroblast production of mRNA for type I and type II collagen three fold whereas PDGF or interleukin-1 have no such effect [47]. In wounding, the main cells producing TGF-β are monocytes and fibroblasts [5–7, 10, 11]. However, the effects of TGF-β are unaltered when there is gross radiation-induced bone-marrow suppression and wound depletion of monocytes/macrophages. This compares to a total absence of effect of TGF-β when fibroblasts are impaired by megavoltage electron beam surface irradiation [48]. These data suggest that excessive levels of TGF-β are responsible for excess scarring and that fibroblasts are essential to its effects, whereas monocytes are not.

Topically applied TGF-β promotes healing of many wound types including incisional wounds [49], excisional wounds [50], partial thickness wounds [51], punch wounds [52] and gastro-intestinal wounds [53]. The effect is observed for a single dose given before or after wounding [49]. The effect is still apparent in the healing-impairment models such as with Adriamycin [54], steroid [55] and irradiation [48]. Further, anti-TGF-β antibodies block excess collagen deposition in wounding [56–58]. The exact role of the various isoforms in scarring is not yet clear; in wound healing in rodents, TGF-β1 and 2 promote scarring whereas TGF-β3 may inhibit it [58]. This contrasts with human

conjunctiva where TGF-β2 probably has no role in scarring (vide infra) and with the differences in the receptors between the three isoforms (vide supra).

TGF-β is not essential for adequate wound healing and an excess is deleterious. Therefore, for mesenchyme it would seem unnecessary. One explanation for its presence is that it promotes rapid healing which may have been an evolutionary advantage.

The Relative Importance of Each Cytokine in Acute Wounding

All three fibrogenic cytokines (PDGF, TGF-β, FGF) are able to accelerate wound healing by about 30% but each cytokine has its own specific function in the healing process [7].

PDGF has three key roles in wound healing. First, it is the most potent chemoattractant for wound macrophages and fibroblasts and this corresponds with its rapid release from platelets and later by cellular synthesis [1, 5–7, 10, 11]. Second, it is the main stimulant for deposition of glycosaminoglycans, fibronectin and thrombospondin. It is unable to stimulate collagen synthesis per se [59]. Third, it stimulates cells to produce other growth factors such as TGF-β1 and thrombospondin.

The main role of TGF-β is as the main stimulant for collagen synthesis and maturation. This is a direct effect, and TGF-β is the only cytokine that has been shown to be capable of directly stimulating excess collagen deposition. Extracellular matrix deposition is also stimulated by TGF-β, but much less than by PDGF [7]. TGF-β is chemoattractive for fibroblasts and macrophages; in comparison to other cytokines this effect is relatively weak. TGF-β is mainly produced by macrophages and fibroblasts, but the presence of macrophages is not a prerequisite for its effects. The transformation from an inactive to the activated form by cleaving and the regulation of this process by thrombospondin (stimulation) and decorin (inhibition) and further unknown mechanisms is unique [23, 26, 60, 61]. Presently, TGF-β seems to be the only cytokine that is capable of a significant autostimulation [5, 7]. Additional up-regulation of TGF-β is possible by fibroblasts via the production of thrombospondin [42].

The FGF family has two main roles in repair. First, is production of a dermal angiogenic response. It does not significantly enhance collagen or extracellular matrix production [7]. Second, is the promotion of epithelialisation by stimulation of basal epithelial keratinocytes by KGF which is produced in the dermis.

Hence, overall PDGF causes chemoattraction and lays down extracellular matrix, TGF-β enhances collagen deposition, FGF encourages vascularisation and KGF stimulates epithelial healing.

Diseases Postulated to be Due to Excess of the Fibrosing Cytokines

The key diseases postulated to be due to excess TGF-β are glomerulonephritis, diabetic nephropathy, pulmonary fibrosis, cirrhosis, post-angioplasty stenosis, scleroderma, intra-abdominal adhesions and proliferative vitreoretinopathy [62, 63]. For example, a rat model of anti-thymocyte-induced glomerulonephritis shows that the morphologically scarred glomeruli produce more mRNA for TGF-β1, secrete more TGF-β1 and produce more fibronectin and proteoglycans than normal glomeruli [23, 25, 26, 47, 61, 64–66].

Idiopathic pulmonary fibrosis accounts for 35% of all fibrotic lung diseases and is characterised by gradual, progressive fibrosis [67]. Histologically, there is infiltration of the alveoli and interstitium with macrophages followed by increased amounts of collagen and extracellular matrix associated with destruction of the lung architecture [68]. Therefore, it is similar to OCP. In the bleomycin-induced model of pulmonary fibrosis, the alveolar macrophages are almost the sole source of TGF-β. This model shows that the levels of TGF-β rise immediately before the production of mRNA for type I III collagen [69]. This correlates with the clinical response to steroids for which there is a moderate improvement early in the disease but no benefit in advanced disease [70]. A similar situation exists in the human pulmonary manifestations of systemic sclerosis [71] and in animal and human glomerulonephritis [63, 65].

Systemic sclerosis is very similar to OCP in that both have mononuclear infiltration of the stroma, increased deposition of collagen and extracellular matrix and increased expression of c-*myc* by the fibroblasts [72–74]. Systemic sclerosis has increased expression of TGF-β mRNA in the dermal fibroblasts and mononuclear cells and increased expression of TGF-β receptors by the fibroblasts [75, 76]. Neither disease has neovascularisation consistent with an absence of FGF in both diseases [72, 77]. In systemic sclerosis, PDGF is found only in macrophages, capillary endothelia and perivascular monocytes [77] but the fibroblasts are not stimulated by PDGF in vitro [78].

To date, there have been few therapeutic uses of TGF-β. Poor wound healing, particularly bone, is improved with administration of TGF-β [51]. Further, peri-operative application of TGF-β2 to retinal macula holes may function to 'seal the edges' by gliosis [79]. TGF-β1 has an unusual protective effect against hypoxic injury and may be beneficial in cerebrovascular and myocardial infarction [33].
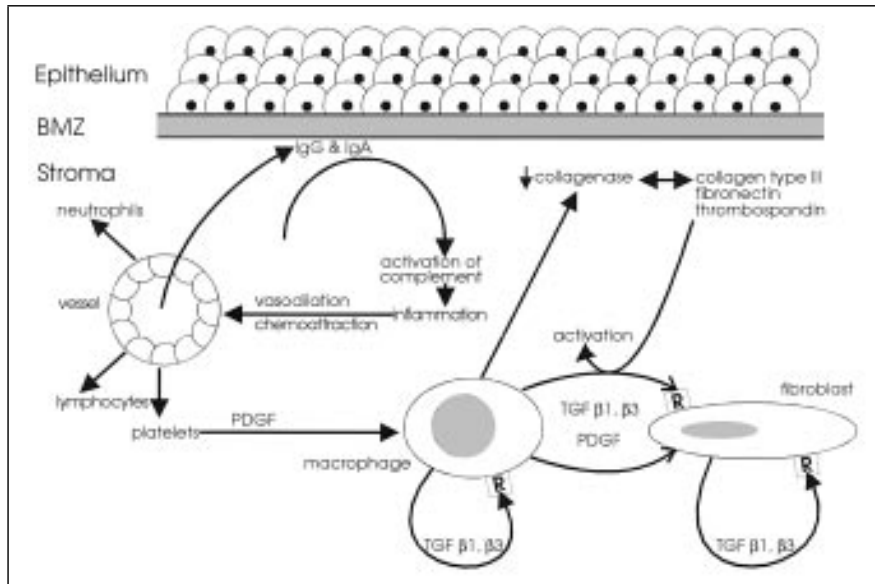
Fig. 1. A proposed model of the cellular and cytokine interactions that contribute to the fibrosis in OCP.

Cytokine Production in Ocular Cicatricial Pemphigoid

In vitro, OCP fibroblasts express the proto-oncogene c-*myc*, whereas normal conjunctival fibroblasts do not [74]. Experimentally, c-*myc* is expressed when quiescent fibroblasts are stimulated to proliferate by the application of TGF-β, PDGF tumour necrosis factor or interleukin-1 [80, 81], suggesting that in OCP, the fibroblasts could have been stimulated by one or more of these cytokines. Conjunctival fibroblasts from patients with OCP are also abnormal in their behaviour and morphology, and this abnormality is maintained throughout many passages in culture [74, 82, 83]. To explain this observation, Biesman et al. [83] have postulated that the fibroblasts undergo an alteration to the genome such that the original putative cytokine stimulus does not have to be present for the cells to remain abnormal.

The cytokine profiles in OCP were initially investigated by Bernauer et al. [84] who used immunohistochemistry to show an increased expression of TGF-β in acute OCP. This, together with the clinical observation that cicatrisation is most active during acute inflammation, suggested that the fibrosing cytokines such as TGF-β may have a pivotal role in the pathogenesis of the fibrosis. Laterly, it has been shown that in acute OCP there is up-regulation of
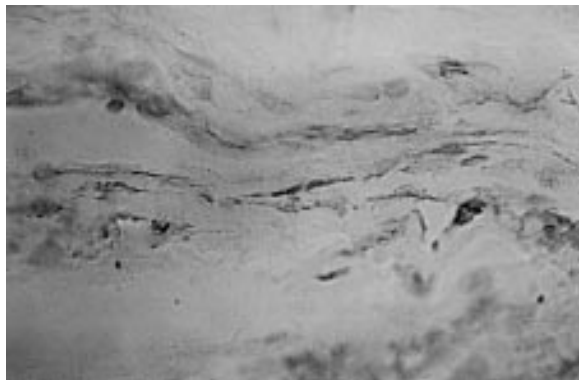
Fig. 2. Conjunctival stroma from acute OCP showing immunohistochemical staining of fibroblasts for TGF-β1. Uncounterstained. × 600.

Fig. 3. Conjunctival stroma from acute OCP showing immunohistochemical staining of fibroblasts for TGF-β receptor. Uncounterstained. × 400.

TGF-β1 and TGF-β3 mRNA by the conjunctival fibroblasts and macrophages together with up-regulation of TGF-β receptor by the fibroblasts and macrophages, and to a lesser extent, by the lymphocytes (fig. 2–5) [85]. There was also slight elevation of PDGF in the acute disease.

Thrombospondin was found weakly expressed by some normal conjunctival epithelium and stroma and this contrasts with OCP tissue where the epithelial and stromal staining was moderate or intense in almost all specimens [86]. Further, the enhanced staining in the subepithelial region was found only
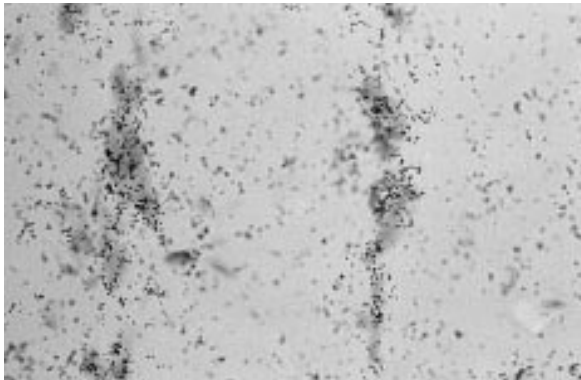
Fig. 4. In situ hybridisation showing positive signal for TGF-β1 mRNA overlying fibroblasts in the conjunctival stroma of acute OCP. Counterstained with haematoxylin. × 2,000.
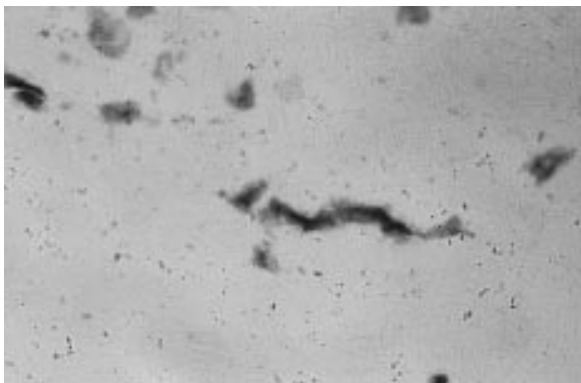
Fig. 5. In situ hybridisation showing negative control (sense probe) for TGF-β1 mRNA in the conjunctival stroma of acute OCP. There is only background staining Counterstained with haematoxylin. × 1,000.

in the acute and subacute cases of OCP. This paralleled the increased fibronectin deposition in this region although there is a much greater difference between the normal and OCP tissue for thrombospondin. The lack of enhanced staining in the subepithelial region of the chronic OCP cases and in normal conjunctiva implies that thrombospondin is actively secreted during inflammation and thereafter degraded. This is consistent with the known life-span in normal wound healing. The presence of thrombospondin is germane to the activation of latent TGF-β.

These results are consistent with the known actions of TGF-β (i.e. the stimulation of fibroblasts to produce excess collagen) and the clinical experience showing that deposition of scar tissue is maximal in acute OCP. Further, it supports the view that the conjunctival stroma is the source of the fibrosing cytokines and are against the hypothesis that the epithelium is the source of the TGF-β that facilitates the fibrosis. This is summarised in figure 1.

The Role of TGF-β2

There is no evidence for TGF-β2 involvement in any stage of OCP and this is consistent with clinical and laboratory data in glaucoma. TGF-β2 is the only TGF-β isoform found in the aqueous humour of normal patients and those with primary glaucoma [87–89]. Up to 61% of TGF-β2 in normal aqueous is activated [88, 89]. Therefore, after trabeculectomy for glaucoma, active TGF-β2 marinades the conjunctival stroma for many years. Yet, in primary glaucoma surgery there is good long-term drainage of aqueous because there is minimal conjunctival fibrosis [90–93]. Hence it is unlikely that TGF-β2 has a significant role in conjunctival fibrosis either after conjunctival surgery or in OCP.

Chronic Ongoing Fibrosis in the Absence of Inflammation

Clinically, there is a small subgroup of patients with OCP that have ongoing conjunctival fibrosis without overt clinical signs of inflammation. This begs an explanation. There are increased levels of fibrosing cytokines and upregulation of TGF-β receptors in chronic disease even though the conjunctiva is clinically 'white' and uninflamed. Such patients still have a significant cellular infiltrate even though it cannot be seen with a slit-lamp [94]. We propose the term 'white inflammation' to describe this conjunctiva. The chronic progression of fibrosis in clinically uninflamed eyes may simply be due to ongoing release of cytokines from the cellular infiltrate. However, fibroblasts from OCP conjunctiva continue to function abnormally long after the withdrawal of any cytokine influences and even after several passages in tissue culture [74, 82]. This may reflect secondary paracrine cytokine imprinting or idiosyncratic fibroblast behaviour. An identical phenomenon has been identified both clinically and in vitro in the fibroblasts in systemic sclerosis [95, 96]. Further, ocular fibroblasts in culture have differential responses to antiproliferative agents depending on whether they are 'activated' or not [97]. Ultimately, probably both the cytokine influences and the idiosyncratic fibroblast behaviour are significant.

The clinical problem is how to best manage such patients. If it is predominately due to cytokine stimulation, then the cytokines need to be selectively blocked or the conjunctiva needs to be depleted of the cellular infiltrate with

immunosuppressive agents. This would imply a role for immunosuppressive drugs in the absence of clinically detectable inflammation. If the fibroblasts have become abnormal and are permanently 'free-wheeling', then novel strategies will be needed to alter the cell genome.

At present, our knowledge about the fibrogenic cytokines is still embryonic but without a doubt, manipulation of their production or prevention of their interaction with various target cells will be a highly specific means of therapeutic intervention.

References

1   Duell TF, Kawahara RS: Growth factors and wound healing: Platelet-derived growth factors as a model cytokine. Annu Rev Med 1991;42:567–584.
2   Duell TF, Senior RM, Chang D, Griffin GL, Heinrikson RL, Kaiser ET: Platelet factor 4 is a chemotactic for neutrophils and monocytes. Proc Natl Acad Sci USA 1981;78:4584–4587.
3   Matsui T, Heidaran M, Miki T, Popescu N, La Rochelle W, Kraus M, Pierce J, Aaronson S: Isolation of a novel receptor cDNA establishes the existence of two PDGF receptor genes. Science 1989;243:800–804.
4   Abboud HE, Grandaliano G, Pinzani M, Knauss T, Pierce GF, Jaffer F: Actions of platelet-derived growth factor isoforms in mesangial cells. J Cell Physiol 1994;158:140–150.
5   Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, Senior RM, Duell TF: Platelet-derived growth factor and transforming growth factor-beta enhance tissue repair activities by unique mechanisms. J Cell Biol 1989;109:429–440.
6   Pierce GF, Vande-Berg J, Rudolph R, Tarpley J, Mustoe TA: Platelet-derived growth factor-BB and transforming growth factor beta 1 selectively modulate glycosaminoglycans, collagen, and myofibroblasts in excisional wounds. Am J Pathol 1991;138:629–646.
7   Pierce GF, Tarpley JE, Yanagihara D, Mustoe TA, Fox GM, Thomason A: Platelet-derived growth factor (BB homodimer), transforming growth factor-beta 1, and basic fibroblast growth factor in dermal wound healing. Neovessel and matrix formation and cessation of repair. Am J Pathol 1992;140:1375–1388.
8   Majack RA, Mildbrandt J, Dixit VM: Induction of thrombospondin messenger RNA levels occurs as an immediate primary response to platelet-derived growth factor. J Biol Chem 1987;262:8821–8825.
9   Chua CC, Geiman DE, Keller GH, Ladda RL: Induction of collagenase secretion in human fibroblast cultures by growth promoting factors. J Biol Chem 1985;260:5213–5216.
10 Pierce GF, Mustoe TA, Altrock BW, Duell TF, Thomason A: Role of platelet-derived growth factor in wound healing. J Cell Biochem 1991;45:319–326.
11 Pierce GF, Brown D, Mustoe TA: Quantitative analysis of inflammatory cell influx, procollagen type I synthesis, and collagen cross-linking in incisional wounds: Influence of PDGF-BB and TGF-beta 1 therapy. J Lab Clin Med 1991;117:373–382.
12 Robson MC, Phillips LG, Thomason A, Robson LE, Pierce GF: Platelet-derived growth factor BB for the treatment of chronic pressure ulcers. Lancet 1982;339:23–25.
13 Burgess WH, Maciag T: The heparin-binding (fibroblast) growth factor family of proteins. Annu Rev Biochem 1989;58:575–606.
14 Givol D, Yayon A: Complexity of FGF receptors: Genetic basis for structural diversity and functional specificity. FASEB J 1992;6:3362–3369.
15 Chen P, Carrington JL, Paralkar VM, Pierce GF, Reddi AH: Chick limb bud mesodermal cell chondrogenesis: Inhibition by isoforms of platelet-derived growth factor and reversal by recombinant bone morphogenetic protein. Exp Cell Res 1992;200:110–117.

3
C

16   Aaronson SA, Bottaro DP, Miki T, Ron D, Finch PW, Fleming TP, Ahn J, Taylor WG, Rubin JS: Keratinocyte growth factor. A fibroblast growth factor family member with unusual target cell specificity. Ann NY Acad Sci 1991;638:62–77.

17   Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AM, Aaronson SA: Determination of ligand-binding specificity by alternative splicing: Two distinct growth factor receptors encoded by a single gene. Proc Natl Acad Sci USA 1992;89:246–250.

18   Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT: Large induction of keratinocyte growth factor expression in the dermis during wound healing. Proc Natl Acad Sci USA 1992;89:8896–6900.

19   Guo L, Yu QC, Fuchs E: Targeting expression of keratinocyte growth factor to keratinocytes elicits striking changes in epithelial differentiation in transgenic mice. EMBO J 1993;12:973–986.

20   Kehrl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS: Transforming growth factor B is an important immunomodulatory protein for human B lymphocytes. J Immunol 1986; 137:3855–3860.

21   Kehrl JH, Wakefield LM, Roberts AB, Jakowlew S, Alvarez-Mon M, Derynck R, Sporn MB, Fauci AS: Production of transforming growth factor B by human T lymphocytes and its potential in the regulation of T cell growth. J Exp Med 1986;163:1037–1050.

22   Assoian RK, Fleurdelys BE, Stevenson HC, Miller DM, Madtes DK, Raines EW, Ross R, Sporn MB: Expression and secretion of type B transforming growth factor by activated human macrophages. Proc Natl Acad Sci USA 1987;84:6020–6024.

23   Vuorio T, Kahari VM, Black C, Vuorio E: Expression of osteonectin, decorin, and transforming growth factor-beta 1 genes in fibroblasts cultured from patients with systemic sclerosis and morphea. J Rheumatol 1991;18:247–251.

24   Seyedin SM, Thomas TC, Thompson AY, Rosen DM, Piez KA: Purification and characterisation of two cartilage-inducing factors from bovine demineralized bone. Proc Natl Acad Sci USA 1985; 82:2267–2271.

25   Majesky MW, Lindner V, Twardzik DR, Schwartz SM, Reidy MA: Production of transforming growth factor B1 during repair of arterial injury. J Clin Invest 1991;88:904–910.

26   Krull NB, Zimmermann T, Gresssner AM: Spatial and temporal patterns of gene expression for the proteoglycans biglycan and decorin and for transforming growth factor-beta 1 revealed by in situ hybridization during experimentally induced liver fibrosis in the rat. Hepatology 1993;18: 581–589.

27   Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E: Suppression of experimental glomerulonephritis by antiserum against transforming growth factor-β1. Nature 1990;346:371–374.

28   Thompson NL, Flanders KC, Smith JM, Ellingsworth LR, Roberts AB, Sporn MB: Expression of transforming growth factor-β1 in specific cells and tissues of adult and neonatal mice. J Cell Biol 1989;108:661–669.

29   Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB: Transforming growth factor-beta in human platelets. J Biol Chem 1983;258:7155–7160.

30   Derynck R, Rhee L, Chen EY, Van Tilburg A: Intron-exon structure of the human transforming growth factor-beta precursor gene. Nucleic Acids Res 1987;15:3188–3189.

31   de Martin R, Haendler B, Hofer-Warbinek R, Gaugitsch H, Wrann M, Schluessener H, Seifert JM, Bodmer S, Fontana A, Hofer E: Complementary DNA for human glioblastoma-derived T cell suppressor factor, a novel member of the transforming growth factor-beta gene family. EMBO 1987;6:3673–3677.

32   ten Dijke P, Hansen P, Iwata K, Pieler C, Foulkes JG: Identification of another member of the transforming growth factor type beta family. Proc Natl Acad Sci USA 1988;85:4715–4719.

33   Sporn MB, Roberts AB: Transforming growth factor-beta: Recent progress and new challenges. J Cell Biol 1992;119:1017–1021.

34   Barr PJ: Mammalian subtilsins: The long-sought dibasic processing endoproteases. Cell 1991;66:1–3.

35   Kojima S, Nara K, Rifkin DB: Requirements for transglutaminase in the activation of latent transforming growth factor-beta in bovine endothelial cells. J Cell Biol 1993;121:439–448.

36   Schultz-Cherry S, Murphy-Ullrich JE: Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. J Cell Biol 1993;122:923–932.

3
C

37 Dennis PA, Rifkin DB: Cellular activation of latent transforming growth factor-β requires binding to the cation-independent mannose-6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci USA 1991;88:580–584.

38 Jaffe EF, Ruggiero JT, Leung LL, Doyle MJ, McKeown-Longo PJ, Mosher DF: Cultured human fibroblasts synthesize and secrete thrombospondin and incorporate it into extracellular matrix. Proc Natl Acad Sci USA 1983;80:998–1002.

39 Mosher DF: Physiology of thrombospondin. Annu Rev Med 1990;41:85–97.

40 Raugi GJ, Olerud JE, Gown AM: Thrombospondin in early human wound tissue. J Invest Dermatol 1987;89:551–554.

41 Asch AS, Leung LL, Shapiro J, Nachman RL: Human brain glial cells synthesize thrombospondin. Cell Biol 1986;83:2904–2908.

42 Noble NA, Harper JR, Border WA: In vivo interactions of TGF-beta and extracellular matrix. Prog Growth Factor Res 1992;4:369–382.

43 Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T: Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. Nature 1992;359:693–699.

44 Kulkarni AB, Huh CH, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlson S: Transforming growth factors-β1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci USA 1993;90:770–774.

45 Whitby DJ, Farguson MW: Immunohistochemical localization of growth factors in fetal wound healing. Dev Biol 1991;147:207–215.

46 Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS: Transforming growth factor-beta: Rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vivo. Proc Natl Acad Sci 1986;83:4167–4171.

47 Westergren-Thorsson G, Antonsson P, Malmstrom A, Heinegard D, Oldberg A: The synthesis of a family of structurally related proteoglycans in fibroblasts is differently regulated by TFG-beta. Matrix 1991;11:177–183.

48 Cromack DT, Porras-Reyes B, Purdy JA, Pierce GF, Mustoe TA: Acceleration of tissue repair by transforming growth factor beta 1: Identification of in vivo mechanism of action with radiotherapy-induced specific healing deficits. Surgery 1993;113:36–42.

49 Mustoe TA, Pierce GF, Thomson A, Gramates P, Sporn MB, Duell TF: Accelerated healing of incisional wounds in rats by transforming growth factor-beta. Science 1987;237:1333–1336.

50 Quaglino D, Nanney LB, Ditesheim JA, Davidson JM: Transforming growth factor-beta stimulates wound healing and modulates extracellular matrix gene expression in pig skin: Incisional wound model. J Invest Dermatol 1991;97:34–42.

51 Jones SC, Curtsinger LJ, Whalen JD, Pietsch JD, Ackerman D, Brown GL, Schultz GS: Effect of topical recombinant TGF-beta on healing of partial thickness injuries. J Surg Res 1991;51:3344–3352.

52 Beck LS, Chen TL, Hirabayashi SE, DeGuzman L, Lee WP, McFatridge LL, Xu Y, Baes RL, Ammann AJ, Accelerated healing of ulcer wounds in the rabbit ear by recombinant human transforming growth factor-beta 1. Growth Factors 1990;2:273–282.

53 Mustoe TA, Landes A, Cromack DT, Mistry D, Griffin A, Duell TF, Pierce GF: Differential acceleration of healing of surgical incisions in the rabbit gastrointestinal tract by platelet-derived growth factor and transforming growth factor, type beta. Surgery 1990;108:324–329.

54 Lawrence WT, Norton JA, Sporn MB, Gorschboth C, Grotendorst GR: The reversal of an Adriamycin induced healing impairment with chemoattractants and growth factors. Ann Surg 1986;203:142–147.

55 Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Gramates P, Duell TF: Transforming growth factor-beta reverses the glucocorticoid-induced wound-healing deficit in rats; possible regulation in macrophages by platelet-derived growth factor. Proc Natl Acad Sci USA 1989;86:2229–2233.

56 Shah M, Foremen DM, Ferguson WJ, Control of scarring by neutralising antibody to transforming growth factor B. Lancet 1992;339:213–214.

57 Shah M, Foremen DM, Ferguson WJ: Neutralisation of TGF-beta 1, 2 reduces scarring in adult rodents. J Cell Sci 1994;107:1137–1157.

3
C

58 Shah M, Foremen DM, Ferguson WJ: Neutralisation of TGF-beta 1 and TGF-beta 2 or exogenous addition of TGF-beta 3 to cutaneous rat wounds reduces scarring. J Cell Sci 1995;108:985–1002.

59 Rossi P, Karsently G, Roberts AB, Roche NS, Sporn MB, de Crombrugghe B: A nuclear factor I binding site mediates the transcriptional activation of a type I collagen promoter by transforming growth factor-β. Cell 1988;52:405–414.

60 Yamaguchi Y, Mann DM, Ruoslahti E: Negative regulation of transforming growth factor-beta by the proteoglycan decorin. Nature 1990;346:281–284.

61 Hagedorn M, Esser P, Wiedemann P, Heimann K: Tenascin and decorin in epiretinal membranes of proliferative vitreoretinopathy and proliferative diabetic retinopathy. Ger J Ophthalmol 1993;2:28–31.

62 Border WA, Ruoslahti E: Transforming growth factor-β: The dark side of tissue repair. J Clin Invest 1992;90:1–7.

63 Border WA, Noble NA: Cytokines in kidney disease: The role of transforming growth factor-beta. Am J Kidney Dis 1993;22:105–113.

64 Connor TJ, Roberts AB, Sporn MB, Danielpour D, Dart LL, Michels RG, de Bustros S, Enger C, Kato H, Lansing M: Correlation of fibrosis and transforming growth factor-β type 2 levels in the eye. J Clin Invest 1989;83:1661–1666.

65 Okuda S, Languino LR, Ruoslahti E, Border WA: Elevated expression of transforming growth factor-β and proteoglycan production in experimental glomerulonephritis. Possible role in expansion of the mesangial extracellular matrix. J Clin Invest 1990;86:453–462.

66 Williams RS, Rossie AM, Chegini N, Schultz G: Effect of transforming growth factor B on postoperative adhesion formation and intact peritoneum. J Surg Res 1992;52:65–70.

67 Gaensler EA, Carrington CD: Open biopsy for chronic diffuse infiltrative lung diseases: Clinical, roentgenographic, and physiological correlations in 502 patients. Ann Thorac Surg 1980;30:411–426.

68 Carrington CB, Geansier EA, Coutur RE, Fitzgerald MX, Gupta RG: Natural history and treated course of usual and desquamative interstitial pneumonia. N Engl J Med 1978;298:801–809.

69 Khalil N, Bereznay O, Sporn MB, Greenberg AH: Macrophage production of transforming growth factor-β and fibroblast collagen synthesis in chronic pulmonary inflammation. J Exp Med 1989;170:727–737.

70 Khalil N, Greenberg AH: The role of TGF-β in pulmonary fibrosis; in: Clinical Applications of TGF-β. (CIBA Symposium 157) Chichester, Wiley, 1991, pp 194–207.

71 Broelemann TJ, Limper AH, Colby TV, McDonald JA: Transforming growth factor β1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc Natl Acad Sci USA 1991;88:6642–6646.

72 Fagundus DM, LeRoy EC: Cytokines and systemic sclerosis. Clin Dermatolol 1994;12:407–417.

73 Trojanowska M, Wu L, LeRoy EC: Elevated expression of c-*myc* proto-oncogene in scleroderma fibroblasts. Oncogene 1988;3:477–481.

74 Hunt LE, Vergnes JP, Roat MI: Altered proto-oncogene expression by conjunctival fibroblasts in cicatricial pemphigoid. Invest. Ophthalmol Vis Sci 1991;32:938.

75 Falanga V, Julien J, Altman R: Plasma and fibroblast receptor levels of transforming growth factor-beta in progressive systemic sclerosis. Arthritis Rheum 1988;31:S32.

76 Kulozik M, Hogg A, Lankat-Buttgereit J, Krieg T: Co-localisation of transforming growth factor β2 with α1 (1) procollagen mRNA in tissue sections of patients with systemic sclerosis. J Clin Invest 1990;86:917–922.

77 Moreland LW, Huang G, Gay R: Immunohistologic demonstration of platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) in scleroderma (SCL) skin. Arthritis Rheum 1990;33:S36.

78 Gay S, Jones RE, Huang G, Gey R: Immunohistologic demonstration of platelet-derived growth factor (PDGF) and sis-oncogene expression in scleroderma. J Invest Dermatol 1989;92:301–303.

79 Glaser BM, Michels RG, Kuppermann BD, Sjaarda RN, Penna R: Transforming growth factor-β2 for the treatment of full-thickness macular holes. Ophthalmology 1992;99:1162–1173.

80 Muller R, Bravo R, Burckhardt J, Curran T: Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. Nature 1984;312:716–720.

3
C

81 Liboi E, Pelosi E, Di Francesco P, Gallanari P, Petrini M, Sposi NM: The EL2 rat fibroblast line: Differential effects of growth factors (EGF, PDGF, FGF, TPA and TGFB) on cell proliferation and o-*fos* expression. Ann N Y Acad Sci 1987;511:318–328.

82 Roat MI, Sossi G, Thoft RA: Hyperproliferation of conjunctival fibroblasts from patients with cicatricial pemphigoid. Arch Ophthalmol 1989;107:1064–1067.

83 Biesman BS, Loess-Perez SM, Chandler JW, Cohen RS: Alterations in the ultrastructure of conjunctival fibroblasts from patients with ocular cicatricial pemphigoid. Invest Ophthalmol Vis Sci 1994; 35:170.

84 Bernauer W, Wright P, Dart JK, Leonard JN, Lightman S: Cytokines in the conjunctiva of acute and chronic mucous membrane pemphigoid: An immunohistochemical analysis. Graefe's Arch Clin Exp Ophthalmol 1993;231:563–570.

85 Elder MJ, Dart JK, Lightman S: The role of cytokines in conjunctival fibrosis in ocular cicatricial pemphigoid. Invest Ophthalmol Vis Sci 1995;36:1025.

86 Elder MJ, Alexander RA, Dart JK, Lightman S: The collagen and extracellular matrix composition of the conjunctiva in normal and ocular cicatricial pemphigoid patients. Invest Ophthalmol Vis Sci 1996;37:1023.

87 Cousins SW, McCabe MM, Danielpour D, Streilein W: Identification of transforming growth factor-beta as an immunosuppressive factor in aqueous humour. Invest Ophthalmol Vis Sci 1991;32: 2201–2211.

88 Jampel HD, Roche N, Stark WJ, Roberst AB: Transforming growth factor-β in human aqueous humour. Curr Eye Res 1990;9:963–969.

89 de Boer JH, Limpens J, Orenga-Nania S, de Jong PT, Heij EL, Kijlstra A: Low mature TGF-β2 levels in aqueous humour during uveitis. Invest Ophthalmol Vis Sci 1994;35:3702–3710.

90 Spencer WH: Histologic evaluation of microsurgical techniques; in Maumenee AE (ed): Contemporary Ophthalmology. St Louis, Mosby, 1972, pp 132–141.

91 Skuta L, Parrish RK: Wound healing in glaucoma filtering surgery. Surv Ophthalmol 1987;32: 149–170.

92 Lamping KA: Long-term evaluation of initial filtration surgery. Ophthalmology 1986;93:93–101.

93 Mills KB: Trabeculectomy: A retrospective long-term follow up of 444 cases. Br J Ophthalmol 1981;65:790–795.

94 Bernauer W, Wright P, Dart JKG, Leonard JN, Lightman S: The conjunctiva in acute and chronic mucous membrane pemphigoid: An immunohistochemical analysis. Ophthalmology 1993;100:339–346.

95 Peltonen L, Palotie A, Myllyla R, Krieg T, Oikarinen A: Collagen biosynthesis in systemic scleroderma: Regulation of post-transcriptional modifications and synthesis of procollagen in cultured fibroblasts. J Invest Dermatol 1985;84:14–18.

96 Botstein GR, Sherer GK, LeRoy EC: Fibroblast selection in scleroderma. An alternative model of fibrosis. Arthritis Rheum 1982;25:189–195.

97 Occleston NL, Alexander RA, Mazure A, Larkin G, Khaw PT: Effects of single exposures to antiproliferative agents on ocular fibroblast-mediated collagen contraction. Invest Ophthalmol Vis Sci 1994;36:3681–3690.

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The Eyelid Sequelae of Chronic Progressive Conjunctival Cicatrisation

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Cicatrising conjunctivitis is frequently associated with cicatricial entropion and with eyelashes that touch the globe. Both can potentially cause sight-threatening complications such as punctate epitheliopathy, corneal abrasions and keratitis (fig. 1) [1, 2]. In a study of 144 patients with ocular cicatricial pemphigoid, 10% ultimately developed microbial keratitis and the major risk factor was trichiasis [2]. A similar situation exists if cicatricial entropion has developed due to other causes [2, 3]. Further secondary mechanical irritation may provoke inflammation, inducing further conjunctival fibrosis and secondary squamous metaplasia which may aggravate the symptoms of mechanical irritation [1, 4].

This chapter discusses the physiology of normal eyelash formation and the pathophysiology of the lid changes due to cicatrisation. This is the prelude to the eyelash ablative procedures and surgical management of cicatricial entropion and trichiasis that is discussed in the chapter on 'Lid Surgery'.

Physiology of Normal Eyelash Formation

Eyelashes are formed by the hair follicles which are located anterior to the tarsal plate, deep to orbicularis oculi and adjacent to the inferior or superior marginal arterial arcades (fig. 2, 3) [5, 6]. Eyelash growth occurs in a cyclical manner, during which the follicles undergo growth, atrophy and then inactivity: anagen, catagen and telogen, respectively [5, 6]. Once growth has ceased, the hair is lost either due to mild trauma or due to recommencement of anagen; for example, the seasonal shielding of animal fur [6]. For eyelashes,
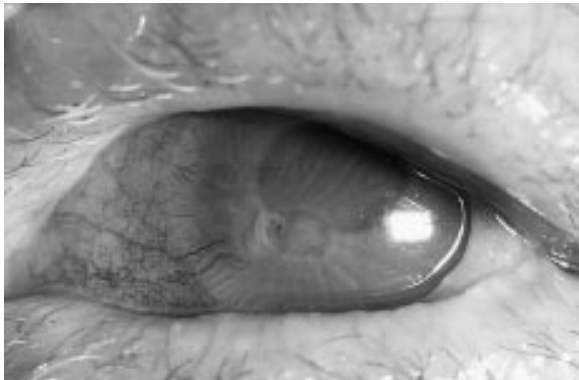
Fig. 1. Acute corneal perforation due to trichiasis. This was successfully treated by acute eyelash epilation, corneal glue and a contact lens. Corrective surgery was required after the cornea had healed.
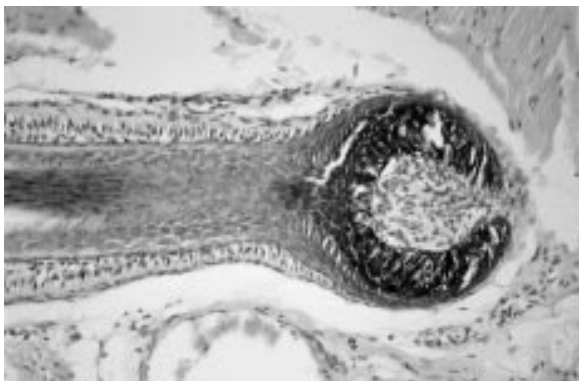
Fig. 2. Eyelash follicle during active growth showing the bulb and the sheaths of the eyelash.

the growth phase is 4–10 weeks and this is followed by a resting period of 4–9 months [7, 8]. This mechanism regulates the length of the eyelashes by stopping growth after they have reached the 'correct length'. In comparison, scalp hairs grow for 2–6 years and then rest for 3 months and therefore scalp hair grows longer [5]. Abnormally long eyelashes (ciliary trichomegaly) and eyebrows (supraciliary trichomegaly) represent a defect in the regulation of this hair cycle [9].
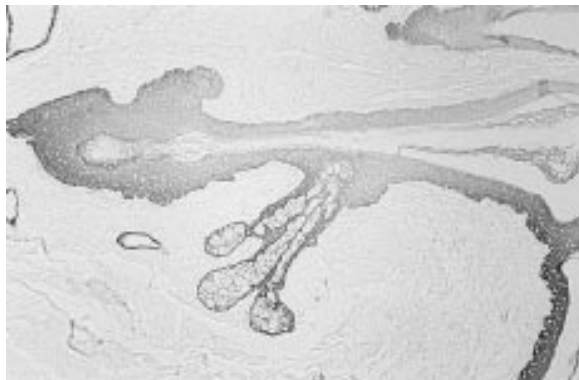
Fig. 3. An eyelash follicle with associated glandular structures. The epithelium of the adnexa is outlined using cytokeratin monoclonal antibodies.

Epilation of an eyelash during an inactive phase induces new growth while epilation during the growth phase fractures the hair above the bulb and reduces its rate and duration of growth [10, 11]. *The clinical importance is that it is better not to epilate a mature, non-growing eyelash unless there is actual eyelash-globe touch because this will rapidly induce further eyelash growth.* Permanent eyelash ablation requires destruction of all the germinal cells of the follicle (see 'Lid Surgery').

Mechanisms of Eyelash-Globe Contact

There are several mechanisms whereby eyelashes may touch the globe, and a distinction must be made between entropion with secondary eyelash-globe contact, misdirected eyelashes and metaplastic eyelashes. Unfortunately, the term 'trichiasis' is often used to describe any or all of the above conditions. If the eyelid anatomy is normal, eyelashes may be misdirected towards the globe ('aberrant' or 'misdirected' eyelashes) or eyelashes may exit from the posterior lid margin, 'metaplastic' eyelashes. In entropion there is a primary derangement of the lid architecture. The eyelash follicles reside within the anterior lamella of the lids and it is the orientation of the follicles that determines the eyelash direction.

Entropion
In cicatricial entropion, the posterior lamella is shortened and this redirects the anterior lamellar structures, including the follicles, towards the globe
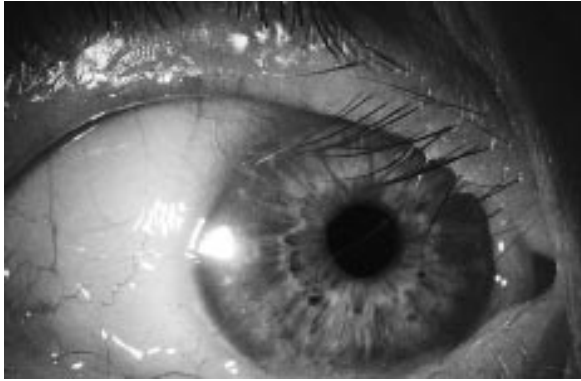
Fig. 4. Typical medial upper lid entropion associated with ocular cicatricial pemphigoid.
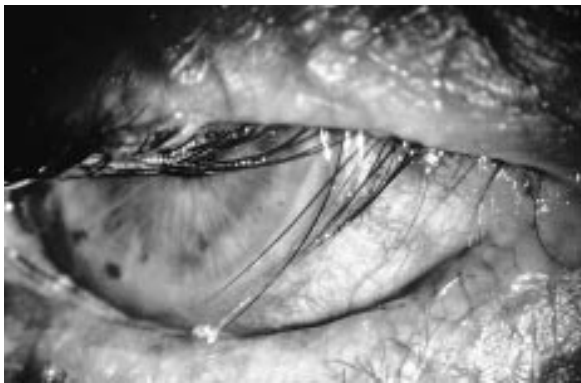
Fig. 5. Misdirected eyelashes involving the medial aspect of the upper eyelid.

Fig. 6. Fine, non-pigmented metaplastic eyelashes exiting from the posterior lid margin.

(fig. 4). In cicatrising conjunctivitis, entropion is the commonest cause of eyelash-globe contact although the lower lid may be affected by additional senile mechanisms that are unrelated to the cicatrisation [12]. Entropion also causes malpositioning of the meibomian gland orifices and allows the eye to come into contact with the keratinised epithelium of the skin. These aspects of entropion are particularly deleterious in any ocular surface disease such as that secondary to chronic progressive cicatrising conjunctivitis. However, surgical correction of entropion will not correct metaplastic or misdirected eyelashes which require either repeated epilation or an eyelash-ablative procedure such as cryotherapy (see 'Lid Surgery').

Aberrant or Misdirected Eyelashes

Misdirected eyelashes arise from essentially normal follicles that have a normal location but an abnormal orientation (fig. 5). The eyelashes exit from the anterior or middle aspect of the lid margin and course towards the globe, either obliquely or directly. These misdirected eyelashes and follicles occur either in isolation or in small clusters. In chronic progressive cicatrising conjunctivitis, the eyelid fibrosis is mainly located between the tarsal plate and the conjunctival epithelium whereas the follicles are located anterior to the tarsal plate. The exact aetiology of misdirected eyelashes is unknown but it is either due to localised lid margin fibrosis or to misdirected follicle regrowth during the hair cycle in the presence of inflammation.

Metaplastic Eyelashes

Metaplastic eyelashes are usually 'stunted and non-pigmented' and arise from the meibomian glands (fig. 6). Acquired metaplastic eyelashes occur after prolonged lid inflammation, such as occurs in chronic progressive cicatrising conjunctivitis [9, 13, 14]. Scheie [13] states that these eyelashes are due to 'meibomian glands [which] become modified and take on an atavistic hair-bearing function'. This contrasts with congenital distichiasis where the meibomian glands are absent or rudimentary [13, 15, 16]. The concept of acquired metaplastic eyelashes is that they are due to a transformation of the adnexal structures to form 'pseudo-follicles' that produce hairs. This metaplastic tendency of chronically inflamed adnexa can be further invoked by cryotherapy which induces metaplastic eyelashes in about 9% [17].

References

1 Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
2 Ormerod LD, Fong LP, Foster CS: Corneal infection in mucosal scarring disorders and Sjögren's syndrome. Am J Ophthalmol 1988;105:512–518.
3 Reacher MH, Munoz B, Alghassany A, Daar AS, Elbualy M, Taylor HR: A controlled trial of surgery for trachomatous trichiasis of the upper lid. Arch Ophthalmol 1992;110:667–674.
4 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.
5 Hamilton JB: In Montagna W, Ellis RA (eds): The Biology of Hair Growth. New York, Academic Press, 1958.
6 Spearman RIC: The structure and function of the fully developed follicle; in Jarrett A (ed): The Physiology and Pathophysiology of the Skin. London, Academic Press, 1977, vol 4, pp 1283–1299.
7 Pinkus F: Die normale Anatomie der Haut; in Jadassohn J (ed): Handbuch der Haut und Geschlechts-krankheiten. Berlin, Springer-Verlag, vol 1, Pt 1, 1927.
8 Munro DD: Disorders affecting the skin appendages; in Fitzpatrick TB (ed): Dermatology in General Medicine. New York, McGraw-Hill, 1971, Chapt 9, 299 pp.
9 Duke-Elder S, MacFaul PA: Adnexa; in System of Ophthalmology. St Louis, Mosby, 1974, vol 13, 375 pp.
10 Johnson E: Inherent rhythms of inactivity in the hair follicle and their control; in Lyne AG, Short BF (eds): Biology of the Skin and Hair. Sydney, Angus & Robertson, 1965, pp 491–505.
11 Johnson E, Ebling EJ: The effects of plucking hairs during different phases of the follicular cycle. J Embryol Exp Morph 1965;12:465.
12 Jones LT, Reeh MJ, Wobig JL: Senile entropion. Am J Ophthalmol 1972;74:327–329.
13 Scheie HG, Albert DM: Distichiasis and trichiasis: Origin and management. Am J Ophthalmol 1966;61:718–720.
14 Wright P, Collin JR: The ocular complications of erythema multiforme (Stevens-Johnson syndrome) and their management. Trans Ophthalmol Soc UK 1983;103:338–341.
15 Fox SA: Distichiasis. Am J Ophthalmol 1962;53:14–18.
16 Byrnes GA, Wilson ME: Congenital distichiasis. Arch Ophthalmol 1991; 109:1752–1753.
17 Wood JR, Anderson RL: Complications of cryosurgery. Arch Ophthalmol 1981;99:460–463.

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Keratopathy in Chronic Progressive Conjunctival Cicatrisation

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Chronic progressive conjunctival cicatrisation primarily affects the conjunctiva but often causes corneal disease with either ocular surface disease symptoms or a reduction in visual acuity. The pathophysiology of the corneal changes in chronic progressive cicatrising conjunctivitis is poorly understood, but insight can be gleaned from correlating the clinical information with histological and immunopathological data from various stages of the disease. Data are available on ocular cicatricial pemphigoid (OCP), but there is no literature documenting the clinical or pathological aspects of the corneal disease of chronic progressive cicatrising conjunctivitis associated with medication or paraneoplastic syndromes. The mechanisms would be expected to be similar, and some parallel can be made with non-progressive cicatrising diseases such as trachoma and Stevens-Johnson syndrome (pp. 11–31 in this volume).

Clinical Aspects of the Keratopathy in Ocular Cicatricial Pemphigoid

In a prospective natural history study of 66 patients with OCP, persistent epithelial defects, microbial keratitis and limbitis were the main factors associated with reduced visual acuity due to corneal pathology. There was no association with any of the systemic manifestations of OCP and the almost total loss of the inferior fornix (3 mm or less compared to normal of 11 mm) was still associated with an acuity of 6/18 or better in 58% of eyes. This suggests that the cornea can remain relatively transparent despite significant cicatrisation (fig. 1–4).
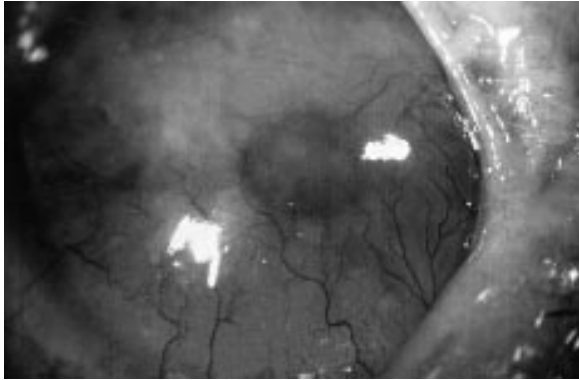
Fig. 1. OCP patient with a vascularised cornea but with relatively transparent epithelium and stroma.

Fig. 2. OCP patient showing the extensive keratinisation that is characteristic of end-stage disease.

Corneal Histology in Ocular Cicatricial Pemphigoid

Pathology specimens of corneas result from various interventions. Elective penetrating keratoplasty typically represents stable, non-inflamed cases with significant corneal opacification. Acute penetrating keratoplasty usually represents inflamed eyes with actual or impending perforation. Whole eyes represent end-stage disease with blind painful eyes. Therefore histological specimens can provide information on the spectrum of disease except for the very early changes.
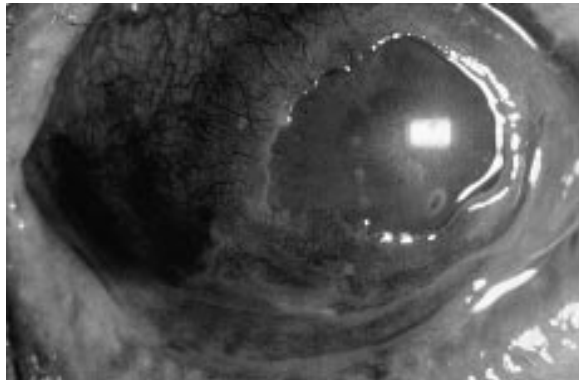
Fig. 3. OCP patient with marked conjunctival inflammation, vascularised peripheral corneal epithelium and a large central epithelial defect but normal stroma.
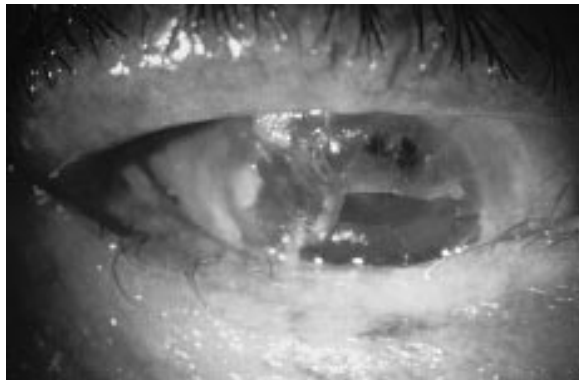
Fig. 4. OCP patient that required penetrating keratoplasty for inflammatory perforation 2 months previously. The graft has a persistent epithelial defect inferiorly and there is opaque epithelium superiorly.

For OCP, there are only 5 case reports of the corneal histology spanning 1885 to 1996 [1–5]. All of these concern whole eyes. This chapter presents data from these 5 cases and from 9 cases in the archives of the Pathology Department in the Institute of Ophthalmology, London.

Elective Penetrating Keratoplasty

Both cases showed that the corneal epithelium was hypertrophic, with some areas of acanthosis and keratinisation. In one case, Bowman's membrane was present and the underlying stroma was avascular but infiltrated with
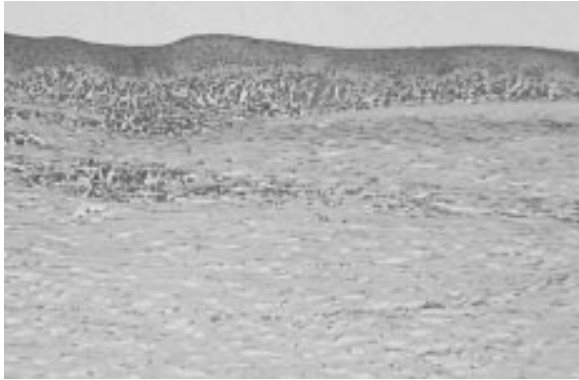
Fig. 5. There is a diffuse inflammatory infiltration beneath the corneal epithelium. Bowman's membrane is absent just to the left of centre and there is a stromal infiltration deep to this. Haematoxylin and eosin. × 200.
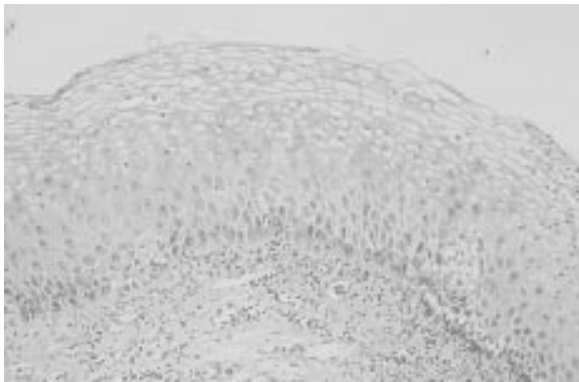
Fig. 6. The corneal epithelium is hypertrophic, Bowman's membrane is absent and there is an anterior stromal infiltration, mainly with lymphocytes. Haematoxylin and eosin. × 400.

lymphocytes. In the other, Bowman's membrane was absent in parts and there was stromal vascularisation (fig. 5, 6).

Acute Penetrating Keratoplasty

Both cases were for perforation; one due to uncontrolled inflammatory and the other due to acute streptococcal keratitis. The inflammatory case showed a heavy stromal infiltration with lymphocytes and Bowman's membrane was absent. The infected case showed that the epithelium was acanthotic, and keratinised with dyskeratosis and extensive neutrophil infiltration. Bow-
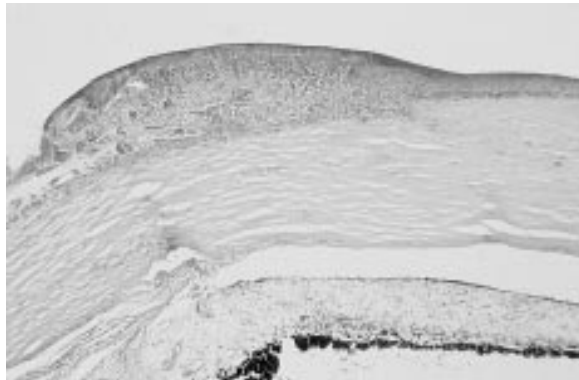
Fig. 7. The limbal region of the eye showing marked subepithelial infiltration, predominately with lymphocytes. This corresponds to the limbitis seen clinically. Haematoxylin and eosin. × 50.

man's membrane was present, but the underlying stroma contained severe acute and chronic inflammatory cell infiltrate.

Whole Eyes

Of the 10 cases (5 from the Institute, 5 in the literature), the key findings were thickened translucent epithelium (5/10), keratinised epithelium (7/10), areas of epithelial defects (3/10), Bowman's membrane absent (8/10), lymphocytic infiltration of the anterior stroma (6/10), stroma vascularisation (9/10) and extensive limbal infiltration with chronic inflammatory cells (4/10) (fig. 7).

Summary of the Histology

The corneal epithelium initially becomes hypertrophic, shows acanthosis and later keratinises. The epithelium can become opaque at any time. In the early phases, it can be peeled away exposing normal, transparent corneal stroma. Eventually, there is a localised lymphocytic infiltration and this precedes the destruction of Bowman's membrane. Thereafter, a fibrovascular subepithelial pannus develops. Later, there may be chronic active keratitis with diffuse lymphocytic infiltration without overt microbial keratitis. The later stages may be associated with a loss of epithelium. Descemet's membrane and the endothelium remain normal unless there is ulceration or perforation. The conjunctiva and particularly the limbus are infiltrated with inflammatory cells and this correlates with the poor clinical outcome associated with the development of the clinical sign of limbitis. A proposed sequence of events is given in table 1.

Table 1. Proposed stages of the keratopathy in OCP

Stage 1:	The corneal epithelium becomes abnormal with thickening and opacity; the underlying stroma remains normal
Stage 2:	There is focal destruction of Bowman's membrane; this is often associated with concomitant lymphocytic infiltration of the conjunctiva and/or the limbus
Stage 3:	There is anterior stromal vascularisation and a chronic keratitis develops with lymphocytic infiltration of the stroma
Stage 4:	There is extensive stromal scarring; the chronic keratitis may lead to corneal thinning with perforation; there may be chronic epithelial defects and microbial keratitis

Pathophysiology of the Ocular Cicatricial Pemphigoid Keratopathy

In health, the cornea retains its unique anatomical and physiological characteristics because of intrinsic factors and the maintenance of the external environment. Therefore, keratopathy can be induced or enhanced by abnormalities in any aspect of the external eye. This includes the tear film, blepharitis, the presence of pathogenic bacteria on the lid margin, architectural changes to the eyelids, trichiasis, poor lid mobility, iatrogenic disease, conjunctival inflammation of any cause, limbitis and dellen (see 'Management of Ocular Surface Problems', pp. 219–227).

In OCP, the conjunctival epithelium undergoes a sequence of changes from hypertrophy to metaplasia with parakeratosis and on to keratinisation [6, 7]. The globlet cell density is reduced [8]. Scanning electron microscopy of patients with active disease shows amorphous sheets of mucus-like material covering extensive areas of the surface [9]. These changes are not seen after adequate immunosuppression. However, active untreated disease retains normal microvilli, microplicae, elongated microvillus projections and tufted microvilli. The corneal implications of all of these changes are unknown.

The Role of the Tear Film

Tear film dysfunction can lead to keratopathy due to a deficiency in any or all of the layers or from the addition of toxic components. In OCP, the tear film break-up times and Schirmer's tests are normal until very late in the disease [10]. This implies a normal aqueous and basal mucus layer. However, there is a $5\times$ increased rate of tear evaporation and this is ascribed to a reduction in the lipid layer of the tear film consistent with meibomian gland and lid margin disease [11].

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A variety of inflammatory conjunctival diseases have abnormal products in the tear film and these may contribute to the keratopathy. In vernal kerato-conjunctivitis there are elevated tear film levels of major basic protein, Charcot-Leyden crystal protein, prostaglandins PGF and HETE, complement C3, factor b and C3 anaphylotoxin [12–15]. Prostaglandin PGF has been identified in trachoma [13] and leukotriene LTB_4 in acute allergic eye disease [16]. Compared to controls, OCP patients have statistically elevated levels of thromboxane A_2, PGE_2, 6-keto-PGF_{1a}, S-HETE, LTB_4 and LTC_4 [10]. Thromboxane A_2 causes platelet aggregation, PGF and HETE augment mediator release, LTB_4 causes chemotaxis of eosinophils and neutrophils and LTC_4 causes vasodilation [17]. PGE_2 causes vasodilation and may also act as a negative feedback inhibitor of T cell production, lymphokine production and macrophage activity [17]. While many of these mediators and products are non-specific, keratopathy is often seen in prolonged vernal keratoconjunctivitis, trachoma and thimerosal-induced epitheliopathy [18]. Therefore, it is possible that both a reduced lipid layer and the addition of untoward products in the tear film may contribute to the keratopathy.

The Corneal Epitheliopathy

The normal morphology of the corneal epithelium was traditionally thought to be a result of the conjunctiva modulating its morphology and physiology when it overlies the cornea [7, 19–23]. It is now accepted that corneal and conjunctival epithelia are derived from different lineages and that the conjunctiva is not the origin of normal corneal epithelium [24–27]. However, the origin of the diseased corneal epithelium in OPC is unknown and has been assumed to represent conjunctival overgrowth on to the cornea [28].

In OPC, the site of the primary pathology is the conjunctival basement membrane zone where there is binding of antibodies and activation of complement. This contrasts with the cornea where there is no laboratory evidence of antibodies directed towards the cornea-derived epithelial basement membrane. If there were, then subsequent complement fixation and acute and chronic inflammation should be clinically manifest with subepithelial infiltrates and other features of sterile keratitis. However, from the author's experience, and that of others, this does not occur when the cornea and its epithelium are otherwise normal [10, 29]. There is an analogy with Mooren's ulcer. This disease is due to binding of antibodies to the corneal epithelium ($\pm$ some stroma) and this manifests with gross limbal ulceration with corneoscleral guttering [30–35]. OCP does not behave in this manner. Therefore, the early keratopathy is not due to an antibody-mediated attack on the corneal epithelial basement membrane, and this is consistent with the known constitutional differences between these two basement membranes [36, 37].

This hypothesis has been explored by examining the expression of the family of intermediate filament proteins in normal and diseased corneas using immunohistochemistry [38, 39]. The results show that normal cornea-derived epithelium was cytokeratin 3 (CK 3)-positive and CK 19-negative, whereas normal conjunctiva was CK 3-negative and CK 19-positive. The examination of archive specimens showed that the entire corneal epithelium expressed CK 3 but not CK 19 in 11/16 chemical burns, 9/11 Stevens-Johnson syndrome, 4/5 OCP and 6/8 patients requiring limbal stem cell transplantation. Goblet cells (a sign of conjunctival derivation [27]) were not found in any of these cases but were found in 2/8 corneas that expressed CK 19 but not CK 3. CK 10 and 13 were additionally expressed by some cornea-derived epithelia, especially dysplastic and hyperplastic epithelia. This suggests that in these types of diseases, the abnormal epithelium is derived from the cornea rather than due to conjunctival overgrowth, except in severe cases. The abnormality of these cells may be due to their environmental conditions or result from the development of an altered clone of cells.

However, the *late* keratopathy may be associated with conjunctival encroachment onto the cornea. This provides an antigenic basement membrane that may invoke a response similar to that affecting the conjunctiva. This would lead to the focal destruction of Bowman's membrane with cellular infiltration and anterior stromal vascularisation, probably due to a type II hypersensitivity response. This is consistent with the histology.

The Overall Sequence of Events in the Keratopathy

The keratopathy in OCP is a spectrum due to a sequence of events. For the majority, the original cornea-derived epithelium probably remains in situ until very late. This epithelium may become dysfunctional because of a reduction in the tear film lipid, the presence of toxic tear film chemicals, a structurally abnormal conjunctiva and a disordered lid architecture [10]. This is reflected in the punctate keratopathy, persistent epithelial defects and microbial keratitis. This sequence is aided by the mechanical effects of trichiasis and the presence of pathogenic bacteria on the lid margins. The aqueous and mucin components of the tear film remain normal until very late in the disease [10]. After prolonged insult, the corneal epithelium may undergo a clonal change with changes in morphology which is seen clinically as opaque or rough epithelium. This may be exacerbated by the lymphocytic infiltration of the limbus. The cornea may vascularise due to a variety of causes and the tear film may contribute to this. The corneal epithelium is replaced by conjunctiva only at a very late stage. This further hastens the keratopathy due to antibody-mediated inflammation. The management of the corneal changes is detailed in 'Management of Ocular Surface Problems'.

References

1   Adam C: Untersuchungen zur Pathologie des Pempigus Conjunctivae. Z Augenheilk 1910;23:35–48.
2   D'Amico D: Sulla istogenesi del pemphigo conjunctale. Ann Ottal Clin Ocul 1925;53:111–125.
3   Smith EC, Myers EA, Lamb HD: Ocular and oral pemphigus. Arch Ophthalmol 1934;22:635–640.
4   Kapuscinski WJ: Sur Le pemphigus oculaire. Ann Ocul 1937;174:451–473.
5   Soudakoff PS, Whalman HF: Ocular pemphigus: Report of a case with the histologic findings in the cornea. Arch Ophthalmol 1953;36:231–236.
6   Anderson SR, Jensen OA, Kristensen EB: Benign mucous membrane pemphigoid. III. Biopsy. Acta Ophthalmol 1974;52:455–461.
7   Kinoshita S, Kiorpes TC, Friend J, Thoft RA: Goblet cell density in ocular surface disease: A better indicator than tear mucin. Arch Ophthalmol 1983;101:1284–1287.
8   Nelson JD, Wright JC: Conjunctival goblet cell densities in ocular surface disease. Arch Ophthalmol 1984;102:1049–1053.
9   Foster CS, Shaw CD, Wells PA: Scanning electron microscopy of conjunctival surfaces in patients with ocular cicatricial pemphigoid. Am J Ophthalmol 1986;102:584–591.
10   Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
11   Mondino BJ, Brown SI: Ocular cicatricial pemphigoid. Ophthalmology 1981;88:95–100.
12   Udell IJ, Gleich GJ, Allansmith MR, Ackerman SJ, Abelson MB: Eosinophil major basic protein and Charcot-Leyden crystal protein in human tears. Am J Ophthalmol 1981;92:824–828.
13   Dhir SP, Garg SK, Sharma YR, Lath NK: Prostaglandins in human tears. Am J Ophthalmol 1979;87:403–404.
14   Abelson MB: Lipooxygenase products in ocular inflammation. Invest Ophthalmol Vis Sci 1984;25(suppl):42.
15   Ehlers WH, Donshik PC: Allergic ocular disorders. A spectrum of diseases. CLAO J 1992;2:117–124.
16   Bisgaard H, Ford-Hutchison AW, Charleson S, Tauderf E: Production of leukotrienes in human skin and conjunctival mucosa after specific allergen challenge. Allergy 1985;40:417–423.
17   Goodwin JS, Ceuppers TB: Regulation of the immune response by prostaglandins. J Clin Immunol 1983;3:295–299.
18   Wright P, Mackie I: Preservative-related problems of soft contact lens wearers. Trans Ophthalmol Soc UK 1982;98:3–6.
19   Friendenwald JS: Growth pressure and metaplasia of conjunctiva and corneal epithelium. Doc Ophthalmol 1951;184:5–6.
20   Thoft RA, Friend J: Biochemical transformation of regenerating ocular surface epithelium. Invest Ophthalmol Vis Sci 1977;16:14–20.
21   Shapiro MS, Friend J, Thoft RA: Corneal epithelialization from the conjunctiva. Invest Ophthalmol Vis Sci 1981;21:135–142.
22   Tseng SC, Hirst LW, Farazdaghi M, Green WR: Goblet cell density and visualization during conjunctival transdifferentiation. Invest Ophthalmol Vis Sci 1984;25:1168–1176.
23   Danjo S, Friend J, Thoft RA: Conjunctival epithelium in healing of corneal epithelial wounds. Invest Ophthalmol Vis Sci 1987;28:1445–1449.
24   Thoft RA: The role of the limbus in ocular surface maintenance and repair. Acta Ophthalmol 1989;67(suppl 192):91–94.
25   Schermer A, Galvin S, Sun T-T: Differentiation-related expression of a major 64k corneal keratin in vivo and in culture suggests limbal location of corneal stem cells. J Cell Biol 1986;103:49–62.
26   Wei Z-G, Wu R-L, Lavker RM, Sun T-T: In vitro growth and differentiation of rabbit bulbar, fornix and palpebral conjunctival epithelia. Implications on conjunctival epithelial transdifferentiation and stem cells. Invest Ophthalmol Vis Sci 1993;34:1814–1828.
27   Wei Z-G, Sun T-T, Lavker RM: Rabbit conjunctival and corneal epithelial cells belong to two separate lineages. Invest Ophthalmol Vis Sci 1996;37:523–533.
28   Duke-Elder S: System of Ophthalmology. London, Kimpton, 1965, vol 8, pp 508–527.
29   Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.
30   Brown SI, Mondino BJ, Rabin BS: Autoimmune phenomenon in Mooren's ulcer. Am J Ophthalmol 1976;82:835–840.

31 Mondino BJ, Brown SI, Rabin BS: Autoimmune phenomenon in the external eye. Trans Am Acad Ophthalmol 1978;85:801–817.
32 Murray PI, Rahi AH: Pathogenesis of Mooren's ulcer: Some new concepts. Br J Ophthalmol 1984; 68:182–187.
33 Berkowitz PJ, Arentsen JJ, Felberg NT, Laibson PR: Presence of circulating immune complexes in patients with peripheral corneal disease. Arch Ophthalmol 1983;101:242–245.
34 Schaap OL, Felkamp TE, Breebaart AC: Circulating antibodies to corneal tissue in a patient suffering from Mooren's ulcer (ulcer rodens corneae). Clin Exp Immunol 1969;5:365–370.
35 van der Gaag R, Abdillahi H, Stilma JS, Vetter JC: Circulating antibodies against corneal epithelium and hookworm in patients with Mooren's ulcer from Sierra Leone. Br J Ophthalmol 1983;67: 623–628.
36 Cleujens JP, Havenith MG, Kasper M, Vallinga M, Bosman FT: Absence of type IV collagen in the centre of the corneal epithelial basement membrane. Histochem J 1990;22:688–694.
37 Marshall GE, Konstas AG, Lee WR: Collagens in ocular tissues. Br J Ophthalmol 1993;77:515–524.
38 Elder MJ, Dart JK, Hiscott P: Cytokeratin expression in the anterior structures of the human eye, the eyelids and adnexa. Invest Ophthalmol Vis Sci 1994;35:3149.
39 Elder MJ, Dart JK, Hiscott P: Intermediate filament expression by normal and diseased human corneal epithelium. Human Pathol 1997, in press.

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General Considerations in the Management of Chronic Progressive Conjunctival Cicatrisation

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The objectives of management of patients with chronic progressive cicatrising conjunctivitis involve attention to the local ocular surface and, if there are any, to the systemic features of disease. In the short term, patients require amelioration of their symptoms. In the long term, the objective is to prevent progression of cicatrisation, to minimise ocular morbidity and maximise visual function. These objectives may sometimes be met with topical ocular treatment and local measures alone. However, moderate to severe immune-mediated inflammatory ocular disease and/or systemic manifestations require a systemic approach that may include, besides steroids, also immunosuppressive agents [1–4]. Such systemic medication may be needed for several months or even for years. This long-term approach together with the fact that most patients are elderly contribute to a reduced tolerance to these drugs and enhanced side-effects.

Ocular symptoms in chronic progressive conjunctivitis may be caused by many mechanisms. Ocular surface disease and changes of the eyelids and adnexa are most frequently involved, but glaucoma and cataracts may also lead to complaints (table 1). At any stage, there may be episodes of conjunctival inflammation during which much of the fibrosis occurs [2, 4, 5]. With increasing cicatrisation, the symptoms, ocular morbidity and visual disability become more prominent and this needs to be prevented.

Table 1. Key local ocular considerations in chronic progressive cicatrising conjunctivitis

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Eyelids
   Staphylococcal lid margin disease
   Seborrhoeic lid margin disease
   Meibomian gland disease
   Entropion and trichiasis
Cornea
   Punctate epithelial keratitis
   Filamentary keratitis
   Persistent epithelial defect
Conjunctiva
   Inflammation
   Cicatrisation
Tear film
   Meibomian gland disease
   Keratoconjunctivitis sicca
   Goblet cell dysfunction
Iatrogenic disease
   Toxicity and preservatives of topical medication
   Glaucoma from topical or systemic steroids
Cataract
   Senile or iatrogenic
Glaucoma
   Due to predisposition or steroids

Local Ocular Considerations

Moderate to severe immune-mediated inflammatory ocular disease requires systemic medication with steroids and/or immunomodulatory or immunosuppressive agents. Before the inflammatory signs are attributed to the disease process itself, however, other aetiologies should be considered and the associated problems should be treated. These include:

(1) Blepharitis and lid margin disease are common and pathogenic bacteria are present in about 80% of cases. Lid margin culture is helpful in resistant cases. Topical antibiotics and steroids to the lids combined with hygiene and massage is useful, but failure of these measures may require oral tetracycline at 250 mg bd. This may be required for 2–6 months depending on the response [3].

(2) Punctate epithelial keratitis is often secondary to lid margin disease, conjunctival inflammation and topical medication toxicity and these need management. Lubricating ointment at night is often helpful.

(3) The preservatives in topical medication are particularly toxic in any compromised ocular surface, and the drug itself may also be harmful. Any patient requiring oral immunosuppression should be on the minimum necessary dose of topical medication. Frequently, we have been able to stop all topical medication, particularly after starting systemic immunosuppression.

(4) Keratoconjunctivitis sicca is uncommon until very late in the disease. All patients with clinically dry eyes should have an assessment of the meibomian gland function, the aqueous component of the tears and mucin layer function. Each should be managed separately. Temporary punctal occlusion should be offered with a view to permanent punctal occlusion provided that there is no symptomatic epiphora.

(5) Filamentary keratitis is especially associated with keratoconjunctivitis sicca and often responds to topical acetylcysteine, 5–10% four times per day.

(6) Persistent epithelial defects are a major risk factor for permanently decreased vision [6]. Any immune-related inflammation and all of the external disease factors need to be treated aggressively. Lagophthalmos or exposure due to the cicatrised lids must be considered and lubricating oinments may be required every 2 h. Failure of these measures should be dealt with by tarsorrhaphy, either laterally or centrally. If there is enough tissue elasticity, temporary lid closure may be induced with botulinum toxin.

(7) The management of trichiasis is covered in more detail elsewhere in this volume [pp. 207–218]. Essentially, if the eye is inflamed, then the lashes need epilating in the short term. Once the inflammation has been resolved for several months, consideration should be given to lash ablation or lid surgery. If there are a few isolated lashes, then electrolysis or argon laser thermoablation is recommended. If there are a large number of lashes, then cryotherapy is recommended for misdirected lashes, posterior lid margin lashes and trichiasis not otherwise associated with entropion. Two freezings of 25 s duration are required for the upper lid and 20 s for the lower lid using the Collin cryoprobe. If there is entropion, the lower lids should have an inferior retractor plication [7]. The upper lid entropion should be managed by a grey line split and anterior lamellar repositioning [8].

(8) Cataract surgery should be avoided until all conjunctival inflammation is resolved for a minimum of 4 months. Surgically, the fornices may be too shallow to retain a speculum and therefore lid sutures and a temporal approach through a clear cornea is recommended.

(9) Glaucoma occurs in about 14–26% of patients and this must be monitored carefully, particularly if they are on steroids [6, 9].

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References

1 Mondino BJ, Ross AN, Rabin BS, Brown SI: Autoimmune phenomena in ocular cicatricial pemphigoid. Am J Ophthalmol 1977;83:443–450.
2 Mondino BJ, Brown SI: Ocular cicatricial pemphigoid. Ophthalmology 1981;88:95–100.
3 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.
4 Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
5 Mondino BJ, Brown SI, Lempert S, Jenkins MS: The acute manifestations of ocular cicatricial pemphigoid: Diagnosis and treatment. Ophthalmology 1979;86:543–552.
6 Elder MJ, Bernauer W, Leonard J, Dart JK: Progression of disease in ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:292–296.
7 Elder MJ, Dart JK, Collin R: Inferior retractor plication surgery for lower lid trichiasis in ocular cicatricial pemphigoid. Br J Ophthalmol 1995;79:1003–1006.
8 Elder MJ, Collin JRO: Anterior lamellar reposition and grey line split for upper lid entropion in ocular cicatricial pemphigoid. Eye 1996;10:439–442.
9 Tauber J, Melamed S, Foster CS: Glaucoma in patients with ocular cicatricial pemphigoid. Ophthalmology 1989;96:33–37.

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Topical and Systemic Immunosuppression for Chronic Progressive Conjunctival Cicatrisation

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There is no known specific treatment for chronic progressive cicatrising conjunctivitis including cicatricial pemphigoid. Studies in the last few years have focused on the use of various types of immunosuppression, and immuno-modulation/-suppression is presently regarded as the method of choice for the treatment of active ocular disease.

As discussed before, it is important to first assess and manage all non-immune causes of conjunctival inflammation before considering immunosuppression. A classification of conjunctival disease activity as 'mild', 'moderate' or 'severe' has shown to be helpful for clinical and study purposes (see 'Monitoring of Activity and Progression').

Mild ocular disease is defined as conjunctival hyperaemia and mild stromal oedema. Eyes with *moderate* disease activity show extensive or marked conjunctival hyperaemia with stromal oedema and significant tissue thickening. There is no limbitis and there are no conjunctival ulcerations. *Severe* ocular disease: there is inflammation in all quadrants with severe ocular oedema. Limbitis and conjunctival ulceration may be present (fig. 1).

The drugs that are discussed in this chapter are listed in table 1. Table 2 summarises the monitoring of patients undergoing immunosuppression.

Mild Inflammation

Mild inflammation may be managed with topical steroids alone. However, these patients are predisposed to glaucoma, and management of any sub-

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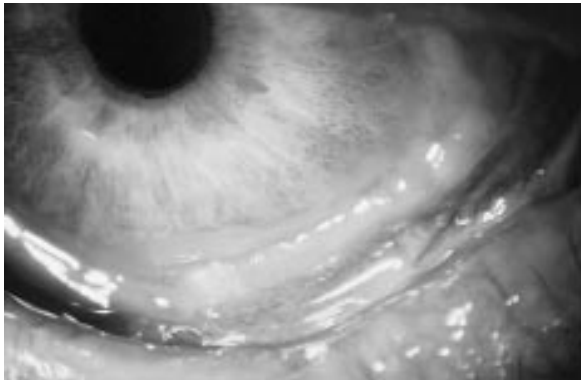
Fig. 1. Severe conjunctival inflammation with limbitis in a patient with OCP.

sequent cataract is surgically difficult. Therefore, the minimum dose should be used for the minimum time and ideally the steroids should be stopped within 4 months if possible. There is no evidence that topical steroids prevent progression of the disease and therefore there is no reason to maintain small doses of steroids for prolonged periods. It is the author's experience that the patients that respond well do so within a few weeks and that if there is no clinical response within 1 month, steroids should be stopped. Preservative-free medication is initially recommended in these inflamed eyes. If a patient requires systemic immunosuppression, then any topical steroids should be stopped once the oral medication has had a therapeutic effect. Occasionally, an exacerbation of inflammation that occurs while a patient is on systemic immunosuppression can be controlled with topical steroids. Again they should be stopped if there is no effect after 1 month. Other causes of redness should always be considered and should resolve during follow-up.

Moderate Inflammation

For moderate inflammation without evidence of rapid progression of fibrosis, topical steroids may be tried initially. It is also important that other causes of inflammation are treated aggressively. Trichiasis is a particularly common and potent cause of prolonged irritation. If topical immunosuppression has failed, then systemic immunosuppression is indicated. The drug of choice has traditionally been dapsone and latterly sulphapyridine [1, 2].

Table 1. Immunosuppressive agents, mechanisms of action and important side effects

Drug	Dapsone	Sulphapyridine	Cyclophosphamide
Trade name	Dapsone (BP, USP)	Sulphapyridine (BP)	Cycloblastin (Pharmacia) Cytoxan (Bristol-Myers, USA) Endoxan (ASTA Medica)
Mechanism	suppresses the migration of neutro-phils and stabilises lysosomal membranes of polymorphonuclear leucocytes	impairs neutrophils and blood monocytes and inhibits cyclo-oxygenase- and lipoxy-genase-dependent pathways	an alkylating agent that affects DNA replication and RNA transcription and hence produces broad immunosuppression
Side effects	chronic haematolysis nausea abdominal pain peripheral neuropathy hepatitis	nausea rash due to allergy headache mild leucopenia arthralgia drug fever neurotoxicity hepatotoxicity	alopecia haemorrhagic cystitis marrow suppression infection malignancy (especially bladder) teratogenicity short-term sterility

Neither drug is particularly effective if there is limbitis. Failure of dapsone or sulphapyridine may respond to the addition of azathioprine or by changing to cyclophosphamide [2]. Methotrexate may also help [3–6].

Dapsone

Dapsone is a sulfone that inhibits the myeloperoxidase enzyme system of polymorphonuclear leucocytes, suppresses the migration of neutrophils and sta-bilises lysosomal membranes [7, 8]. The clinical response is mainly due to inhibi-tion of neutrophil recruitment and function. In ocular cicatricial pemphigoid (OCP), partial or complete improvement of ocular inflammation occurs in 70–71% and is apparent rapidly; 55% within 4 weeks [9, 10]. Over a mean of 35 months, the disease is controlled in 45%. Severe inflammation responds poorly [2]. Side effects may be marked, the most significant of which is chronic haemo-lysis which occurs in all patients due to a dose-dependent reduction in red blood cell life (table 1). Ninety-five percent of patients have a 1.0–3.4 g/dl drop in hae-moglobin, but this reverses with cessation of the dapsone [9].

Table 2. Systemic immunosuppression for OCP

	Treatment	Pretreatment tests	Ongoing tests
Mild inflammation	topical steroids preservative free	intraocular pressure lens clarity	intraocular pressure lens clarity
Moderate inflammation	(1) sulphapyridine 500 mg twice/day (2) dapsone 75 mg once/day	full blood count urea and electrolytes screen for G6PD deficiency	full blood count urea and electrolytes ensure Hb > 11 g/l $2 \times$ monthly
Severe inflammation	(1) cyclophosphamide 1 mg/kg/day plus prednisolone 1 mg/kg/day (2) methotrexate 15 mg/week or (3) azathioprine 50 mg twice/day	blood pressure, $2 \times$ monthly full blood count, $1 \times$ monthly RBC sedimentation rate urea and electrolytes liver, $2 \times$ monthly calcium glucose, $2 \times$ monthly ? previous tuberculosis chest X-ray urine dipstick	full blood count area and electrolytes alter dose to achieve lymphocyte count at $0.5–1.0 \times 10^9$/l – check every $1 \times$ monthly

NB: Always consider the non-immune ocular surface and external eye disease problems as well.

Clinical Aspects of Dapsone Treatment

The starting dose is 75 mg/day and this can be increased by 25–50 mg/month up to a maximum of 150 mg if required. The haemoglobin must be assessed monthly and the dose reduced if anaemia develops. Occasionally, the chronic haemolysis depletes the total body stores of iron, folate or vitamin B_{12}. This may also cause anaemia and requires supplementation rather than a reduction in the dose of dapsone. An absolute contra-indication to dapsone is glucose-6-phosphate dehydrogenase deficiency as it causes acute, severe haemolysis, and it is mandatory for all patients to be screened for this before starting treatment [2, 9, 10]. If there has been an adequate response to dapsone and both eyes are free from inflammation then the drugs should be continued for one year. Thereafter it should be stopped and the situation reviewed.

Sulphapyridine

Sulphapyridine is a sulphonamide antibiotic with anti-inflammatory properties that has been shown to be safe, effective and to have a minimum of side

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effects in OCP (table 1) [11–13]. It is well absorbed orally and has no first-pass effect [14]. Elimination is by acetylation that depends on a genetically determined phenotype. The half-life in 'fast acetylators' is 6 h while that in 'slow acetylators' is 14 h and about 51–81% of patients are slow acetylators [15, 16]. This has clinical significance as these patients achieve much higher serum levels for the same oral dose. The anti-inflammatory effects are due to impairment of neutrophils and blood monocytes and inhibition of the cyclo-oxygenase- and lipoxygenase-dependent pathways [17, 18].

The main indications for sulphapyridine use have been the inflammatory dermatoses such as bullous pemphigoid [19, 20] and dermatitis herpetiformis [21–23]. These diseases also tend to respond to dapsone. The patients that clinically benefit most from these drugs have significant neutrophil infiltration, consistent with the known effects of sulphapyridine and dapsone on neutrophil function. Sulphapyridine also has a beneficial effect on rheumatoid arthritis, causing both clinical and biochemical improvements [24–27]. The objectives of treating this disease are similar to those of OCP. In the short term, patients require symptom control. In the long term, permanent tissue damage must be minimised; for example, joint destruction or extensive ocular cicatrisation.

Clinical Aspects of Sulphapyridine Treatment

The initial dose of sulphapyridine is 500 mg twice a day but this should be reduced to 500 mg once a day if there is nausea present for more than 1 week. Overall, about 55% of patients will have a good response and all of these will have done so within 2 months. The allergy presents as acute, widespread skin reaction in 15% after about 2 weeks and requires cessation of the drug [1]. Sulphapyridine can reduce the white blood count and full blood screens are recommended every 2 months.

Alternatives to Sulphapyridine

Sulphapyridine may not be easy to obtain in some countries. The alternative is sulphasalazine (Salazopyrin, Kabi Pharmacia, Milton Keynes, UK) which has been safely used for ulcerative colitis for 50 years [28–30]. Sulphasalazine is cleaved by colonic bacteria into sulphapyridine and 5-aminosalicylic acid [12]. The benefits in colitis are solely due to the 5-aminosalicylic acid that exerts a topical action on the colonic mucosa. The sulphapyridine is absorbed unaltered and reaches levels similar to oral sulphapyridine itself [14, 31]. Two grams of sulphasalazine is the molar equivalent of 800 mg of sulphapyridine [27]. In rheumatoid arthritis, oral administration of sulphasalazine or sulphapyridine results in the same beneficial anti-inflammatory response. Therefore, sulphasalazine can be substituted for sulphapyridine [24–26, 32]. Another alternative is sulphamethoxypyridazine at 500 mg twice a day.
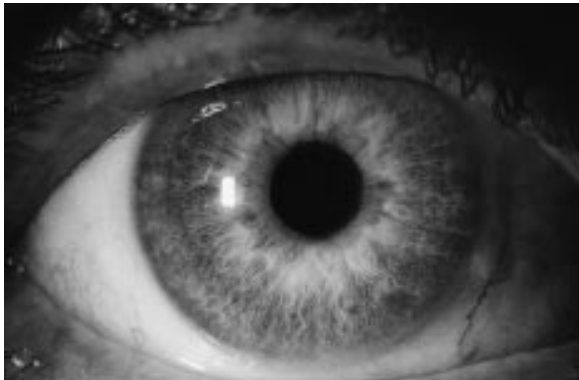
Fig. 2. The same patient as in figure 1. Two months after treatment with oral cyclophosphamide and high-dose prednisolone.

This is a sulphonamide with similar anti-inflammatory effects and is therapeutic in bullous skin diseases and OCP [33]. Unfortunately, it is difficult to obtain in many countries, costs approximately 1 US $/tablet and has the potential to induce Stevens-Johnson syndrome. The author's personal experience suggests that the clinical response is similar to sulphapyridine, but that it offers no further advantages.

Severe Inflammation

The drug of choice for severe inflammation is cyclophosphamide supplemented with an initial short course of high-dose oral prednisolone (fig. 1, 2). The rationale for this combined treatment regime is based on laboratory and clinical evidence. Severe conjunctival inflammation in OCP is usually immune-driven. Histologically, these acutely inflamed eyes are congested with neutrophils, macrophages and T lymphocytes. The majority have antibodies, complement and fibrinogen bound to the conjunctival basement membrane [34, 35]. Therefore, medical treatment for severe disease should reduce acute inflammation and impair delayed hypersensitivity and the cell-mediated immune system. This can be achieved with prednisolone and cyclophosphamide [36, 39]. Further, high-dose prednisolone on its own is comparatively ineffective at reducing conjunctival inflammation in OCP [9, 40, 41]. In one series, only 42% of patients had resolution of their inflammation and this required initial doses of 60–80 mg/day and a mean maintenance dose of 40 mg/day [40]. Side effects included peptic ulceration (33%), hypertension (25%), diabetes (25%),

myopathy (25%), osteoporosis (25%) and psychosis (25%). One third of these patients became legally blind.

Foster [9] reported that cyclophosphamide (2 mg/kg/day) and a 3-month course of high-dose prednisolone resolved the conjunctival inflammation in 100% of patients with symblepharon [9]. Further, it prevented recurrence and cicatrisation over 1 year of follow-up [2, 9]. However 100% developed some degree of anaemia, 83% developed alopecia, 8% developed haematuria and 8% developed leucopenia. A later retrospective study has suggested that cyclophosphamide was the most successful treatment in severe inflammation [2].

Mondino et al. [42] and Mondino [43] assessed conjunctival cicatrisation in a 24-month, three-armed trial of cyclophosphamide, cyclophosphamide and prednisone and placebo. Progression of conjunctival cicatrisation occurred in patients with very mild amounts of cicatrisation (their 'stage 1') in 25, 17 and 40% of eyes, respectively, and in severe amounts of cicatrisation (their stage 3) in 75, 25 and 73%. This study suggests that combined cyclophosphamide and prednisone was the most effective at preventing cicatrisation although no regimen completely prevents it. This has been confirmed by Elder et al. [44] who found that 22% of patients had progression of the cicatrisation while on cyclophosphamide.

Clinical Aspects of Cyclophosphamide

The clinical dose of cyclophosphamide is 1.5–2 mg/kg/day, initially together with oral prednisolone (1 mg/kg/day). Cyclophosphamide at these doses will cause a selective reduction in the lymphocyte count; the dose is adjusted monthly to achieve a lymphocyte count of $0.5–1.0 \times 10^9$ while retaining a total WBC over $2 \times 10^9/l$. A typical maintenance dose is 100 mg/day. The steroid dose should be reduced after an adequate clinical response is observed and stopped completely within 4 months. Oral steroids may increase the blood pressure, blood glucose and the risk of reactivating tuberculosis. Therefore, these aspects need to be considered and monitored monthly.

Cyclophosphamide is a nitrogen-mustard-derived alkylating agent that affects DNA replication and RNA transcription. The side effects are detailed in table 1 [36–38, 45]. Toxic cystitis may result because the by-products of cyclophosphamide accumulate in the bladder and therefore an increased fluid intake and emptying the bladder before bedtime is recommended. Because of the teratogenic effects, patients should not start a family until they are off the drug for about 6 months. The risk of developing malignancy becomes significant with more than 1 year of treatment. For OCP, cyclophosphamide should be continued for 1 year and thereafter reviewed. A successfully managed patient may then have the medication stopped or replaced with sulphonamides or dapsone. An alternative to cyclophosphamide is methotrexate 15 mg per

week or azothiaprine 100 mg per day. These are similar to cyclophosphamide in effects and side effects.

Other Potentially Useful Agents for Severe Inflammation

There is evidence that azathioprine and methotrexate are useful in moderate and severe disease, although cyclosporin A is not [9]. Azathioprine is a purine analog that is incorporated into DNA and RNA and clinically it is a relatively selective suppressor of CD4 + T cells and CD8 + T cells [36]. The oral dose is 1–2.5 mg/kg/day. In OCP, when compared to a non-treatment control group, progression occurred in 33% of patients with minimal cicatrisation (control group, 40%) and in 50% of patients with severe cicatrisation (control, 73%) [3]. A retrospective review showed that azathioprine alone controlled only 33% of patients compared to dapsone which controlled 45% [2]. Arthralgia occurred in 10%, leucopenia in 8% and hepatitis in 8%. Therefore, azathioprine is somewhat effective although it does have side effects.

Methotrexate antagonizes folic acid and hence blocks DNA but not RNA synthesis. It is a powerful immunosuppressant and can conveniently be given as a once-weekly, oral dose (15–35 mg per week). It may cause nausea, fever, headache, bone marrow depression, hepatitis and alopecia. It is therapeutic in pemphigus vulgaris and bullous pemphigoid and there is reasonable evidence that it is therapeutic in OCP [3–6]. Therefore, it may be a good alternative to cyclophosphamide.

Chronic Progression of Cicatrisation without Inflammation

A small subgroup of patients continue to have progressive cicatrisation without any clinical signs of inflammation. This phenomenon is not understood although bulbar conjunctival specimens from these patients show significant cellular infiltrates of acute and chronic inflammatory cells [35]. The optimal clinical management is unclear and there are no cytokine-specific drugs that just block the fibrosis. Despite this, the natural history studies suggest that, compared to other agents, cyclophosphamide is the most effective agent to impair the deposition of scar tissue [2, 9, 42, 43]. The decision to use such powerful immunosuppressive agents must be tempered by unequivocal evidence of progression and the potential harm of the side effects.

Conclusion

The key to the long-term management of these diseases is 'prevention'. This applies at all levels. For example, trichiasis must be managed and prevented from recurring. This provides symptomatic relief but also helps prevent

microbial keratitis. Management of active inflammation and prevention of further episodes helps minimise further cicatrisation, although no current treatment regime has been able to prevent this altogether. Cicatrisation, per se, is associated with the keratopathy and it is probable that prevention of the inflammation helps prevent the development of the keratopathy. Clinically, there may be minimal keratopathy even with very shallow fornices (i.e. 3 mm or less), but the corneal complications may suddenly become frequent and severe with minimal extra cicatrisation. This knife-edged phenomenon suggests that for every patient, every effort should be made to prevent further cicatrisation. Because of the longevity of the patients, the 'buffer zone' needs to remain as large as possible. Certainly, data from patients with severe inflammation treated with cyclophosphamide show that good visual acuity can be retained even in the presence of severe inflammation with limbitis if it is adequately managed [46]. Further, intraocular and extraocular procedures, such as cataract extraction and lid surgery, are comparatively safe if the immune-driven inflammation is quiescent although this has often required systemic immunosuppression. The cost of this 'aggressive' approach is the ever-present dangers of powerful medication in elderly patients.

The clinical studies have shown that a variety of drugs are effective at reducing the conjunctival inflammation and that these agents work by affecting specific cell lines. This contrasts to steroids which act by blocking specific mediators of inflammation and are comparatively ineffective in OCP. This suggests that the pathways of inflammation are more complex than those affected by steroids alone. In the future, improvements in our basic scientific and pharmaceutical knowledge should lead to more specific drugs with less side effects (see 'New Concepts: The Manipulation of the Wound Healing Response').

References

1   Elder MJ, Leonard J, Dart JK: Sulphapyridine – A new agent for the treatment of ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:549–552.
2   Tauber J, Sainz de la Maza M, Foster CS: Systemic chemotherapy for ocular cicatricial pemphigoid. Cornea 1991;10:185–195.
3   Lever WF: Treatment of pemphigus vulgaris with methotrexate. Arch Dermatol 1969;100:70–78.
4   Lever WF: Pemphigus; in Fitzpatrick TB (ed): Dermatology in General Medicine. New York, McGraw-Hill, 1970, pp 609–618.
5   Lever WF: Methotrexate and prednisone in pemphigus vulgaris. Arch Dermatol 1972;106:491–497.
6   Francis IC, McCluskey PJ, Walls RS, Wakefield D, Brewer JM: Ocular cicatricial pemphigoid. Aust NZ J Ophthalmol 1990;18:143–150.
7   Lang PG: Sulfones and sulfonamides in dermatology today. J Am Acad Dermatol 1979;1:479–492.
8   Booth SA, Moody CE, Dahl MV, Herron MJ, Nelson RD: Dapsone suppresses integrin-mediated neutrophil adherence function. J Invest Dermatol 1992;98:135–140.

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E

9   Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.

10   Rogers RS III, Seehafer JR, Perry HO: Treatment of cicatricial (benign mucous membrane) pemphigoid with dapsone. J Am Acad Dermatol 1982;6:215–223.

11   Ashworth M, Arthur M, Turner AD, Smith PR: A comparison of serum concentrations of sulphasalazine and some of its metabolites after therapy by the oral or rectal route. Pharmatherapeutica 1984;3:551–555.

12   Watkinson G: Sulphasalazine: A review of 40 years' experience. Drugs 1986;32(suppl):1–11.

13   Panayiotou BN: Pulmonary infiltrates and eosinophilia associated with sulphasalazine administration. Aust NZ J Med 1991;21:348–349.

14   Crotty B, Jewel DP: Drug therapy of ulcerative colitis. Br J Clin Pharmacol 1992;34:189–198.

15   Molin L, Larsson R, Karlsson E: Evaluation of the sulphapyridine acetylator phenotyping test in healthy subjects and in patients with cardiac and renal diseases. Acta Med Scand 1977;201:217–222.

16   Klotz U: Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-aminosalicylic acid. Clin Pharmacokinet 1985;10:285–302.

17   Hoult JR: Pharmacological and biochemical actions of sulphasalazine. Drugs 1986;32(suppl):18–26.

18   Punchard NA, Boswell DJ, Greenfield SM, Thompson RP: The effects of sulphasalazine and its metabolites on prostaglandin production by human mononuclear cells. Biochem Pharmacol 1992; 43:2369–2376.

19   Person JR, Rogers RS III: Bullous pemphigoid responding to sulfapyridine and the sulfones. Arch Dermatol 1977;113:610–615.

20   Venning VA, Frith P, Bron A, Millard P, Wojnarowska F: Mucosal involvement in bullous and cicatricial pemphigoid. A clinical and immunopathological study. Br J Dermatol 1988;118:7–15.

21   Costello MJ: Sulfapyridine in the treatment of dermatitis herpetiformis. Arch Dermatol 1947;56: 614–624.

22   Winkleman RK, Roth HL: Dermatitis herpetiformis with acantholysis or pemphigus with response to sulphonamides: Report of two cases. Arch Dermatol 1960;82:385–390.

23   Dabrowski J, Jablonska S, Chorzelski TP, Jarzabek-Chorzelska M, Maciejewski W: Electron microscopic studies in dermatitis herpetiformis in relation to the pattern of immune deposits in the skin. Arch Dermatol Res 1977;259:213–224.

24   Pullar T, Hunter JA, Capell HA: Sulphasalazine in the treatment of rheumatoid arthritis: Relationship of dose and serum levels to efficacy. Br J Rheumatol 1985;24:269–276.

25   Pullar T, Hunter JA, Capell HA: Which component of sulphasalazine is active in rheumatoid arthritis? Br Med J Clin Res Ed 1985;290:1535–1538.

26   Taggart AJ, Neumann VC, Hill J, Astbury C, Le-Gallez P, Dixon JS: 5-Aminosalicylic acid or sulphapyridine. Which is the active moiety of sulphasalazine in rheumatoid arthritis? Drugs 1986; 32(suppl):27–34.

27   Neumann VC, Taggart AJ, Le-Gallez P, Astbury C, Hill J, Bird HA: A study to determine the active moiety of sulphasalazine in rheumatoid arthritis. J Rheumatol 1986;13:285–287.

28   Truelove SC, Watkinson G: Comparison of corticosteroid and sulphasalazine therapy in ulcerative colitis. Br Med J 1962;ii:1708–1711.

29   Cowan GO, Das KM, Eastwood MA: Further studies of sulphasalazine metabolism in the treatment of ulcerative colitis. Br Med J 1977;ii:1057–1059.

30   Das KM, Dubin R: Clinical pharmacokinetics of sulphasalazine. Clin Pharmacokinet 1976;1: 406–425.

31   Hawthorne AB, Hawkey CJ: Immunosuppressive drugs in inflammatory bowel disease. A review of their mechanisms of efficacy and place in therapy. Drugs 1989;38:267–288.

32   Greenfield SM, Hamblin AS, Shakoor ZS, Teare JP, Punchard NA, Thompson RP: Inhibition of leucocyte adhesion molecule upregulation by tumor necrosis factor alpha: A novel mechanism of action of sulphasalazine. Gut 1993;34:252–256.

33   McFadden JP, Leonard JN, Powles AV, Rutman AV, Fry L: Sulphamethoxypyridazine for dermatitis herpetiformis, linear IgA disease and cicatricial pemphigoid. Br J Dermatol 1989;121:759–762.

34   Sacks EH, Jakobiec FA, Wieczorek R, Donnenfeld E, Perry H, Knowles DM: Immunophenotypic analysis of the inflammatory infiltrate in ocular cicatricial pemphigoid. Further evidence for a T-cell-mediated disease. Ophthalmology 1989;96:236–243.

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E

35 Bernauer W, Wright P, Dart JKG, Leonard JN, Lightman S: The conjunctiva in acute and chronic mucous membrane pemphigoid: An immunohistochemical analysis. Ophthalmology 1993;100:339–346.

36 Gilman AG, Goodman LS, Rall TW, Murad F: The pharmacological basis of therapeutics. New York, Macmillan, 1985.

37 Dantzig PI: Immunosuppressive and cytotoxic drugs in dermatology. Arch Dermatol 1974;110:393–406.

38 Boitard C, Bach JF: Long-term complications of conventional immunosuppressive treatment. Adv Nephrol 1989;18:335–354.

39 Roat MI, Sossi G, Thoft RA: Hyperproliferation of conjunctival fibroblasts from patients with cicatricial pemphigoid. Arch Ophthalmol 1989;107:1064–1067.

40 Hardy KM, Perry HO, Pingree GC: Benign mucous membrane pemphigoid. Arch Dermatol 1971;104:467–475.

41 Foster CS, Wilson LA, Ekins MB: Immunosuppressive therapy for progressive ocular cicatricial pemphigoid. Ophthalmology 1982;89:340–353.

42 Mondino BJ, Brown SI: Ocular cicatricial pemphigoid. Ophthalmology 1981;88:95–100.

43 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.

44 Elder MJ, Lightman SL, Dart JK: The role of cyclophosphamide and high dose steroid in ocular cicatricial pemphigoid. Br J Ophthalmol 1995;79:264–266.

45 Berkson BM, Lome LG, Shapiro I: Severe cystitis induced by cyclophosphamide, role of surgical management. JAMA 1973;225:605–606.

46 Elder MJ, Bernauer W, Leonard J, Dart JK: Progression of disease in ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:292–296.

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Lid Surgery: The Management of Cicatricial Entropion and Trichiasis

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The therapeutic modalities for lash-globe contact fit into two broad categories: lash ablation and surgical repositioning of the lid (also see 'The Eyelid Sequelae'). Lash ablation can be achieved by cryotherapy, electrotherapy, electrolysis, laser thermo-ablation, radiosurgery and radiotherapy, although all of these methods have shortcomings. The surgical methods usually require incision through the fibrous tissue and resuturing of the lid such that the entropion is permanently repositioned.

A distinction must be made between ocular disease due to non-progressive, non-inflamed cicatricial conditions such as infection, and that due to chronic progressive cicatrising conjunctivitis, because the outcomes of treatment are vastly different in these two groups. For example, ocular cicatricial pemphigoid (OCP) typically becomes activated after surgery and this may result in failure of the procedure. To achieve a successful outcome, such cases require either novel surgery that does not involve the conjunctiva, or the patients require adjunctive immunosuppression. The best results are obtained when both objectives are met. This involves shared care between ophthalmologists with oculoplastic, external eye disease and immunological skills.

Eyelash Ablation

Pathophysiology of Eyelash Ablation

The ultimate objective of eyelash ablation is to destroy all the cells that have the potential to replicate and form a new follicle, because follicles are not formed after birth in mammals. These germinal cells are located in the superficial root sheaths and in the base of the follicles [1–4]. Incomplete

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Table 1. Techniques of eyelash ablation

Method	Success at 12 months, %	Advantages	Disadvantages
Epilation	0	immediate benefit; non-inflammatory	temporary relief only
Cryotherapy	39	treats large areas	destroys meibomian glands; may reactivate disease; induces meta-plastic lashes (9%); depigmentation
Electrolysis	29	very selective; minimal anaesthesia; few complications	poor success rate
Laser thermo-ablation	50	very selective; minimal anaesthesia; few complications	

germinal cell ablation allows regeneration with subsequent recurrence of the trichiasis. Cellular destruction can be achieved via many different mechanisms: cold, heat, electricity and radiation, and these correspond to cryotherapy, electrolysis, laser thermo-ablation, radiosurgery and radiotherapy (table 1).

Cryotherapy

The pathological reaction to cryotherapy depends on the differences in sensitivity of the various tissues to freezing. When rabbit eyelids are frozen to $-15°C$ there is complete retention of the lash follicles but significant, permanent depigmentation of the melanocytes. At $-30°C$ the follicles and the meibomian glands are replaced by a dermal scar. [5]. Effective cryotherapy for trichiasis will always be accompanied by a hypopigmented epidermis because the melanocytes are more sensitive to cryotherapy than lash follicles and this is cosmetically important in dark-skinned people [6]. Clinical studies suggest that freezing the lids twice to $-20°C$ is satisfactory for permanent follicle destruction [7]. The cryoprobe should be applied to the skin because this reduces the direct thermal and mechanical conjunctival trauma and places the probe closest to the follicles.

Cryotherapy achieves a long-term success rate of 70–90% in non-cicatricial lid disease [7–9]; however, complications occur in up to 26% [9]. These include
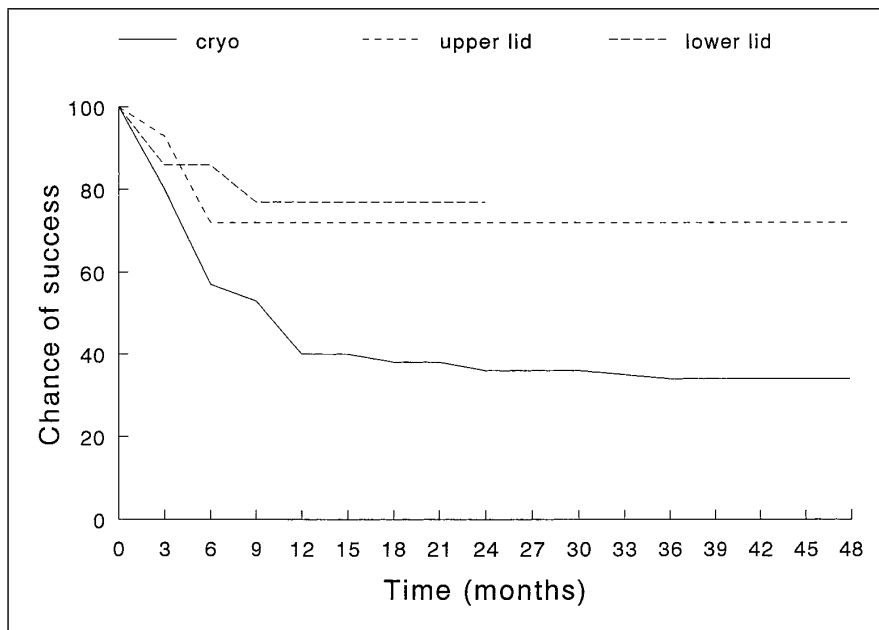
Fig. 1. The cumulative chance of success of cryotherapy, lower lid retractor plication and upper lid anterior lamellar repositioning. After Elder and Bernauer [12], Elder and Collin [36] and Elder at al. [31].

severe short-term lid oedema, lid depigmentation, destruction of meibomian glands, lid notching and induction of metaplastic lashes (in 9%). In trichiasis due to trachoma, cryotherapy is successful in 27–56% when assessed for long periods of time [10, 11].

For OCP in the absence of immunosuppression, marked progression of symblepharon and conjunctival scarring occurred in 77% of patients [9]. In a cohort of patients where the inflammation had been managed and resolved before surgery, the success rate declined to 40% at 1 year and remained at this level over the next 4 years (fig. 1) [12]. Complications included lid notching (8%), asymptomatic tarsal atrophy (4%) and an altered lid contour (4%) (fig. 2). Most patients developed significant short-term lid oedema but no patient developed new symblepharon, extended existing symblepharon or otherwise activated their disease. These data lend credence to the opinion that, in OCP, the least complications occur in eyes without conjunctival inflammation [13–15].

To ensure that cryotherapy is successful, the follicles must be adequately frozen and the only absolute method to ensure this is to use a thermocouple at the level of the follicles. Clinically, various authors have often relied on a

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Fig. 2. This lower lid shows tissue atrophy with an alteration of lid contour after cryotherapy. There has been complete lash ablation in the region of the cryotherapy.

specific duration of freezing based on previous correlations with a thermo-couple. However, this is dependent upon the type of probe used. For example, for an eyelid to reach $-20\,°C$ requires 45 s with the Cryomedics MT650 probe, 30 s with the Amoilette cryoprobe and 25 s with the Collin cryoprobe [7–9, 16]. The lower lid takes approximately 20% less time to reach the same temperature [7]. The other clinical method is to apply the cryotherapy until there has been adequate 'whitening' of the affected area. While this does compensate for individual variation, it relies on the assumption that the follicles have reached $-20\,°C$ because the skin has turned white. Neither method is entirely satisfactory. The laboratory evidence suggests that clinical failure is solely due to inadequate freezing of the follicles [5]. However, excessive freezing would be expected to increase the complications and some compromise is required.

Electrolysis

Electrolysis is attractive in that it may be done at the slit lamp, is highly selective and has a minimum of side effects [17, 18]. The actual technique requires that a needle is inserted deep into the hair follicle approximately 2.4 mm into the upper lid and 1.4 mm into the lower lid [19] and a current 2.0–3.5 mA applied for 10 s [10]. In humans, the success rate is about 29% [10]. Histology reveals focal necrosis of the follicle with only an occasional specimen showing perifollicular scarring, consistent with the clinical outcome [20].

Argon Laser Thermo-Ablation

Argon laser thermo-ablation offers selective follicle ablation with minimal anaesthesia. The technique involves treating each lash separately by repeatedly

applying argon laser burns to the hair root and follicle site to permanently ablate the germinal cells. Compared to cryotherapy, the histology shows that laser thermo-ablation is accompanied by much less inflammation, causes less scarring and does not destroy the adjacent meibomian glands [2]. These are all significant advantages in the treatment of cicatrising conjunctivitis.

The technique uses a spot size of 50–200 μm and about 10–12 applications are required to gently burn down to the follicle base. This procedure may be performed with topical anaesthetic drops but often infiltrative anaesthesia is required [21–25]. One treatment session is successful in approximately 50% of cases, and retreatment further improves the success for any given lash [21–25]. The only complications reported are of mild discomfort and lacrimation during the procedure. There are no reports of post-operative lid swelling, lid notching or deformity, meibomian gland destruction or induction of adjacent aberrant lashes.

Surgery for Cicatricial Entropion of the Lower Lid

Non-Progressive Disease
Pure cicatricial entropion can be divided into 'mild to moderate' disease where the lower lid is retracted 1.5 mm or less below the limbus and 'severe' where the lid is greater than 1.5 mm below the limbus [26]. For pure mild to moderate disease due to non-progressive disease, a tarsal fracture is recommended as the procedure of choice. This is achieved by incising the full width of the conjunctiva and tarsal plate down to the orbicularis approximately 2 mm from the lid margin. Both ends of each of three double-armed 4-0 catgut sutures are placed through the conjunctiva inferior to the tarsal incision and pass upwards and outwards through the orbicularis and skin where they are tied externally near the lash line. The sutures are tied to induce ectropion for the 14 days before their removal [26]. For severe disease, the posterior lamella must be lengthened with a free graft of donor sclera, mucous membrane, tarsal plate, hard palate mucosa or nasal or auricular cartilage. The tarsal plate is split horizontally as for a tarsal fracture and the free graft is held in place with interrupted sutures. Double-armed 4-0 catgut sutures are placed through the graft and the lid, tied externally and removed after 10 days [26].

Chronic Progressive Cicatrising Conjunctivitis
All of the above procedures involve operating directly on the conjunctiva. Unfortunately, in OCP this often triggers conjunctival inflammation and further cicatrisation which can lead to surgical failure [14, 15, 27, 28]. Therefore, there is potential benefit in any entropion procedure that does not involve

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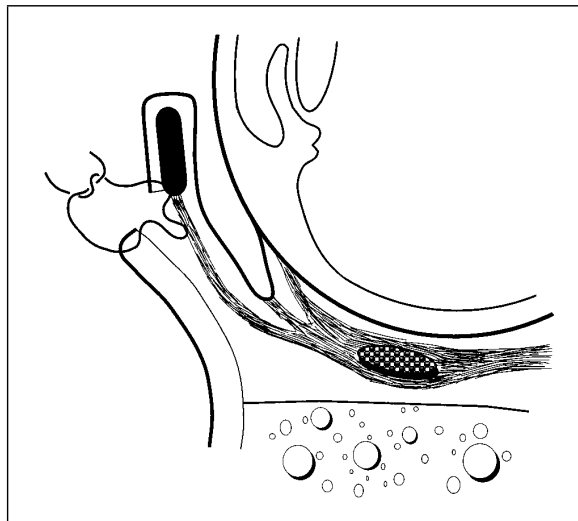
Fig. 3. The Jones procedure. The inferior lid retractors extend from the inferior rectus tendon, intermuscular septum and inferior oblique muscle forwards to the inferior aspect of the tarsal plate. The retractors are plicated with multiple 6-0 polyglycolic acid sutures that are brought out through the skin.

operating on the conjunctiva: for example, the lower lid retractor plication operation of Jones [29, 30]. The lower lid retractors are slips of fascia that extend mainly from the inferior rectus muscle to the inferior tarsal plate and function to adjust the vertical height of the lid with up- and down-gaze [29]. The cornea can rotate downwards about 55° (10 mm) during which the lower lid descends about 5 mm to avoid obscuring the visual axis. A lack of lower lid movement on vertical gaze suggests a defective retractor mechanism. If the fascia is shortened, then the enhanced tension on the tarsus helps to resist entropion. If the surgery is performed through the skin, a fibrous barrier also helps to prevent orbicularis overriding. These factors are the basis of the 'Jones procedure' of inferior retractor plication for lower lid entropion [29, 30].

Surgical Details of the Jones Procedure

A full width, horizontal skin incision is made 4–6 mm inferior to the lid margin. The orbicularis is split parallel to the skin incision, the inferior border of the tarsal plate is exposed and the orbital septum is divided. The slips of the lower lid retractors are exposed and this is confirmed by voluntary up- and down-gaze. Multiple, interrupted 6-0 polyglycolic acid sutures are placed through the retractors, the inferior tarsal plate, the orbicularis muscle

Table 2. Factors influencing upper lid
entropion surgery

Lid closure
Cicatricial rotation of the terminal tarsus
Thickening of the tarsus
Keratinisation of the tarsoconjunctiva
Type of trichiasis
Ongoing inflammation

and brought out through the skin (fig. 3). The amount of retractor tendon shortening (typically 6–10 mm) is individualised such that there is slight lid eversion at the end of surgery. The conjunctiva is not breached during the surgery.

A prospective study of patients with OCP showed a 77% chance of anatomical success at 2 years and a 54% chance of completely preventing lash-globe touch (fig. 1) [31]. The 18% difference in outcomes between the two groups is the result of metaplastic and misdirected lashes that touched the globe. The surgery did not cause clinical activation of conjunctival inflammation or other complications. Anatomical failure was primary in 12% and due to late cicatrisation in 6%. These data support the notion that any conjunctival inflammation must be resolved before surgery and that the procedure itself should avoid the conjunctival incisions.

Surgery for Cicatricial Entropion of the Upper Lid

The cicatricial entropion that occurs in the upper lid is essentially due only to posterior lamellar shortening and yet a large number of surgical techniques have been described including: bilamellar tarsal rotation [10, 32, 33], tarsal rotation and advance [26, 34], eversion splinting [35], tarsal advance [26], tarsal grooving [17] and posterior lamellar grafts of auricular cartilage [36]. This is because the cicatrisation may effect various types of anatomical deformity and tissue changes. These are major factors in the type of procedure (table 2).

Non-Progressive Disease
For mild to moderate entropion with adequate lid closure, bilamellar repositioning and anterior lamellar repositioning are essentially equally successful procedures for non-inflamed, non-progressive disease (see below) [10, 36]. Sometimes, cicatrisation selectively causes rotation of the terminal tarsus. This can be managed by incising the full width of the tarsal conjunctiva and

tarsus about 3 mm from the lid margin, externally rotating the distal portion and suturing this by passing sutures through the new external surface and through the underlying tarsus. Additional full-thickness sutures that pass through the skin at the level of the superior tarsal border are required to aid the eversion [26, 34]. If the tarsus is very thickened, then a simple anterior lamellar reposition or bilamellar reposition will be insufficient to correct the deformity and a wedge of tarsus must be excised. This is combined with an anterior lamellar repositioning and grey line split. Alternatively, the entire tarsus can be thinned or excised depending on the amounts of fibrosis in the tarsus and conjunctiva. If there is moderate keratinisation of the tarso-conjunctiva, then this area needs to be everted away from the globe with a Trabut type tarsal eversion. If there is extensive keratinisation, then mucous membrane grafting is required (see below). A localised area of trichiasis can be managed by excising the block of the anterior lamellar tissue that the follicles reside in. If there is inadequate lid closure, then the posterior lamella needs to be lengthened with a graft. Because the upper lid tarsal plate is larger and more 'structural' than the lower lid, the graft needs to be stiff such as auricular cartilage, hard palate or nasal septum. In these cases of severe cicatrisation, the terminal tarsus often needs to be externally rotated to prevent lash-globe touch.

Chronic Progressive Cicatrising Conjunctivitis

Most of the operations for upper lid entropion entail directly operating on the conjunctiva and in OCP this often promotes further fibrosis which can precipitate surgical failure [14, 15, 27]. Therefore, surgical avoidance of the conjunctiva is prudent in OCP [37]. For upper lid cicatricial entropion, if the anterior lamella is dissected away from the cicatrised tarsus and appropriately repositioned, then the follicles become aligned away from the globe. To ensure adequate surgical realignment of the entropion, the grey line typically needs to be split, because the lid margin structures are tightly bound [36]. This technique of 'anterior lamellar reposition and grey line split' has the added advantage that the surgery is performed on structures anterior to the tarsal plate thereby avoiding the conjunctiva (fig. 4). A study of this procedure in patients with OCP had a 1-year overall success rate of 62% [38] and this compares favourably to the tarsal rotation procedure in trachoma (68%) [10]. The technique was otherwise safe and complication free. However, 13% of eyes became significantly inflamed postoperatively and it is recommended that the surgery is only performed when the eye has been free from inflammation for at least several months. This may require systemic immunosuppression pre-operatively combined with frequent visits in the interim for epilation. Any conjunctival inflammation that occurs post-operatively needs to be treated aggressively.
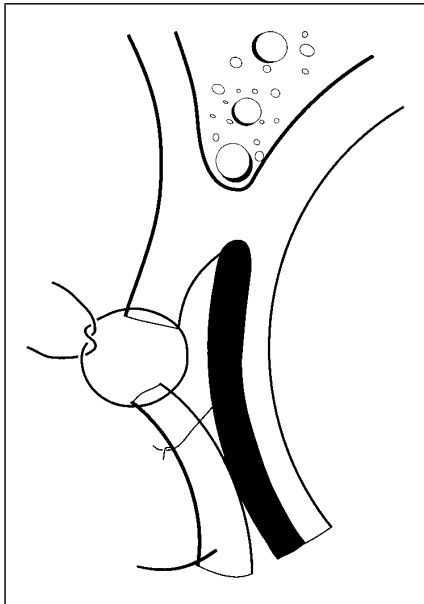
Fig. 4. The anterior lamellar reposition and grey line split for upper lid entropion. The anterior lamellar structures are dissected away from the cicatrised tarsus and repositioned superiorly. The grey line is split to ensure adequate surgical realignment of the entropion and resolution of the trichiasis.

Mucous Membrane Grafting

Mucous membrane grafting for entropion involves excising the tarsal cicatrix and transplanting buccal mucosa into the tarsal and forniceal defect [39–42]. Previously, the technique has had a poor prognosis, particularly in OCP, because it caused severe ocular inflammation and further cicatrisation [27, 39, 43]. However, in 1983, the modern technique of onlay grafts was described favourably for 4 patients with OCP [41]. Latterly, Shore et al. [42] described a series of 42 eyelids of 17 patients with medically controlled OCP (cyclophosphamide or dapsone). Cryotherapy was used to ablate the lashes and the mucous membrane grafting component aimed to improve the mechanical lid-globe relationship and increase the number of globlet cells [41, 44]. The outcomes were 'improved' in 69% but there were multiple complications including microbial keratitis (10%) and inflammation requiring increased systemic immunosuppression (29%). Clearly, the extensive risks need to be contrasted with the potential benefits for each individual patient.

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Conclusion

This chapter has examined the three main methods of eyelash ablation for which the success rates have been: cryotherapy 39%, electrolysis 29%, laser thermo-ablation 50%. Both electrolysis and laser thermo-ablation allow selective energy delivery and therefore have fewer complications. However, these methods are better suited to fewer numbers of lashes while cryotherapy is more suited to a large number. The survival curve analysis suggests that lash regrowth may occur up to 1 year after treatment but thereafter a successful result is likely to remain so for many years. This is consistent with the normal eyelash hair cycle and it can be concluded that adequate clinical trials need at least 1 year of follow-up.

The other alternative to lash ablation is to surgically reposition the lid so that the lashes are not touching the globe. This is potentially better for large numbers of lashes. For the lower lid, an inferior retractor plication has minimal side effects and gives a 77% chance of anatomical success at 2 years and a 54% chance of completely preventing lash-globe touch. It is important to plicate enough tendon to evert the lid completely at the time of surgery.

Upper lid trichiasis can be managed by bilamellar tarsal rotation and this is very successful in trachoma over long periods of time (77%). Its outcome in OCP is unknown but it would be expected to provoke conjunctival inflammation. This complication is uncommon with a grey line split and anterior lamellar reposition which has been successful in 61% and hence this is the procedure of choice in this disease.

For any lid intervention, inflammatory disease such as OCP has a very different response to the trauma of lash ablation and surgery and this must be planned for, both pre- and post-operatively.

References

1 Spearman RIC: The structure and function of the fully developed follicle; in Jarrett A (ed): The Physiology and Pathophysiology of the Skin. London, Academic Press, 1977, vol 4, pp 1283–1299.
2 Orentreich N: Scalp hair replacement in man; in Montagna W, Ellis RA (eds): The Biology of Hair Growth. New York, Academic Press, 1958, vol 9, chapt 6.
3 Johnson E: Inherent rhythms of inactivity in the hair follicle and their control; in Lyne AG, Short BF (eds): Biology of the Skin and Hair. Sydney, Angus & Robertson, 1965, pp 491–505.
4 Johnson E, Ebling EJ: The effects of plucking hairs during different phases of the follicular cycle. J Embryol Exp Morphol 1965;12:465.
5 Sullivan JH, Beard C, Bullock TD: Cryosurgery for treatment of trichiasis. Am J Ophthalmol 1976; 82:117–121.
6 Soll DB, Harrison SE: Basic concepts and an overview of cryosurgery in ophthalmic plastic surgery. Ophthalmic Surg 1979;10:31–36.
7 Johnson RJ, Collin JRO: Treatment of trichiasis with a lid cryoprobe. Br J Ophthalmol 1985;69: 267–270

8   Collin JRO, Coster DJ, Sullivan JH: Cryosurgery for trichiasis. Trans Ophthalmol Soc UK 1978; 98:81–83.

9   Wood JR, Anderson RL: Complications of cryosurgery. Arch Ophthalmol 1981;99:460–463.

10 Reacher MH, Munoz B, Alghassany A, Daar AS, Elbualy M, Taylor HR: A controlled trial of surgery for trachomatous trichiasis of the upper lid. Arch Ophthalmol 1992;110:667–674.

11 Rice CD, Kersten RC, Al-Hazzaa S: Cryotherapy for trichiasis in trachoma. Arch Ophthalmol 1989;107:1180–1182.

12 Elder MJ, Bernauer W: Cryotherapy for ocular cicatricial pemphigoid. Br J Ophthalmol 1994;78: 769–771.

13 Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.

14 Mondino BJ, Brown SI, Lempert S, Jenkins MS: The acute manifestations of ocular cicatricial pemphigoid: Diagnosis and treatment. Ophthalmology 1979;86:543–552.

15 Mondino BJ, Brown SI: Ocular cicatricial pemphigoid. Ophthalmology 1981;88:95–100.

16 Hecht SD: Cryotherapy of trichiasis with the use of the retinal cryoprobe. Ann Ophthalmol 1977; 9:1501–1503.

17 Fox S: Ophthalmic Surgery, ed 5. New York, Grune & Statton, 1976, pp 313–315, 330–349.

18 Spaeth GL: Ophthalmic Surgery, Principles and Practice. Philadelphia, Saunders, 1982, p 570.

19 Elder MJ: Anatomy and physiology of eyelash follicles and its relevance to lash ablation procedures. J Ophthalmic Plast Reconstr 1997;13:21–25.

20 Bartley GB, Bullock JD, Olsen TG, Lutz PD: An experimental study to compare methods of eyelash ablation. Ophthalmology 1987;94:1286–1289.

21 Berry J: Recurrent trichiasis: Treatment with laser photocoagulation. Ophthalmic Surg 1979;10: 36–38.

22 Awan KJ: Argon laser treatment of trichiasis. Ophthalmic Surg 1986;17:658–660.

23 Awan KJ: Laser photocoagulation-vaporization therapy of trichiasis. Ophthalmic Laser Therapy 1988;3:3–9.

24 Campbell DC: Thermo-ablation for trichiasis using the argon laser. Aust NZ J Ophthalmol 1990; 18:427–430.

25 Sharif KW, Arafat AF, Wykes WC: The treatment of recurrent trichiasis with the argon laser photocoagulation. Eye 1991;5:591–595.

26 Collin JRO: Entropion and trichiasis; in A Manual of Systematic Eyelid Surgery. New York, Churchill-Livingston, 1989, pp 7–26.

27 Rycroft B: The surgery of ocular pemphigus. Proc R Soc Med 1961;54:111–112.

28 Beyer CK: The management of special problems associated with Stevens-Johnson syndrome and ocular pemphigoid. Trans Am Acad Ophthalmol Otolaryngol 1977;83:701–707.

29 Jones LT: The anatomy of the lower eyelid. Am J Ophthalmol 1960;49:29–36.

30 Jones LT, Reeh MJ, Wobig JL: Senile entropion. Am J Ophthalmol 1972;74:327–329.

31 Elder MJ, Dart JK, Collin R: Inferior retractor plication surgery for lower lid trichiasis in ocular cicatricial pemphigoid. Br J Ophthalmol 1995;79:1003–1006.

32 Ballen PH: A simple method for the relief of trichiasis and entropion of the upper lid. Arch Ophthalmol 1964;72:239–240.

33 Reacher MH, Huber MJ, Canagaratnam R, Alghassany A: A trial of surgery for trichiasis of the upper lid for trachoma. Br J Ophthalmol 1990;74:109–113.

34 Trabut G: Entropion-trichiasis en Afrique du Nord. Arch Ophthalmol NS 1949;9:701–707.

35 Nyunt Win Y: Surgery for trachoma in Burma. Br J Ophthalmol 1976;63:113–116.

36 Kemp EG, Collin JR: Surgical management of upper lid entropion. Br J Ophthalmol 1986;70: 575–579.

37 Rosser PM, Collin JRO: Retractor plication for lower lid entropion in ocular cicatricial pemphigoid. Aust NZ J Ophthalmol 1993;21:93–97.

38 Elder MJ, Collin JRO: Anterior lamellar reposition and grey line split for upper lid entropion in ocular cicatricial pemphigoid. Eye 1996;10:439–442.

39 Duane A (ed): Fuchs' Textbook of Ophthalmology, ed 5. Philadelphia, Lippincott, 1917, p 203.

40 Friedman MW, Wright ES: The surgical treatment of ocular pemphigus. Am J Ophthalmol 1953; 36:237–240.

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E

41 McCord CD, Chen WP: Tarsal polishing and mucous membrane grafting for cicatricial entropion, trichiasis and epidermalization. Ophthalmic Surg 1983;14:1021–1025.

42 Shore JW, Foster CS, Westfall CT, Rubin PA: Results of buccal mucosal grafting for patients with medically controlled ocular cicatricial pemphigoid. Ophthalmology 1992;99:383–395.

43 Duke-Elder S, MacFaul PA: Adnexa; in Duke-Elder S (ed): System of Ophthalmology. St Louis, Mosby, 1974, vol 13, p 383.

44 Ralph RA: Goblet cell density in normal subjects and dry eye syndromes. Invest Ophthalmol 1975; 14:299–302.

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The Management of Ocular Surface Disease

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Introduction

Ocular surface disease (OSD) is a term introduced to describe disease resulting from failure of the mechanisms responsible for maintaining a healthy ocular surface. It is caused by a group of disorders of diverse pathologies. In chronic progressive conjunctival cicatrisation, OSD is the major cause of the symptoms and the corneal manifestations are almost the sole cause for the severe visual impairment. The objectives of management are to minimise the symptoms, restore and maintain normal visual function and to prevent further cicatrisation and secondary complications. These goals can only be achieved by the use of all of the skills and therapeutic measures from the 'corneal and external disease world' and those of the physicians to ensure safe immunosuppression and good general health. This chapter discusses the medical management of OSD whereas the surgical management of corneal disease is covered in the chapter on 'Corneal and Cataract Surgery' (pp. 228–239).

Manifestations of Ocular Surface Disease

The clinical signs of OSD are multiple and reflect the differing tissue responses and different aetiologies. Some manifestations are common to both the cornea and conjunctiva while others are tissue specific (table 1). The aetiologies of OSD are a function of the structures that are anatomically or physiologically related and each can 'play their part' in OSD.

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Table 1. Clinical manifestations of ocular surface disease

Cornea and conjunctiva
Punctate epithelial changes
  Localised
  Generalised
Epithelial defect
  Localised
  Generalised
  Acute
  Persistent
Loss of epithelial surface smoothness
Loss of epithelial transparency
Keratinisation
Loss of tear meniscus

Specific corneal manifestations
Subepithelial vascularisation
Filamentary keratitis
Increased tear break-up time

Specific conjunctival manifestations
Loss of surface area secondary to shrinkage
Inflammation of the substantia propria
  Increased vascularity
  Stromal oedema
  Limbitis

Management of Ocular Surface Disease

Specific Ocular Surface Disease Aspects of Chronic Progressive Cicatrising Conjunctivitis

Chronic progressive cicatrising conjunctivitis presents a constellation of unique problems. For example, the inflamed conjunctiva is particularly sensitive to medication of any sort and to preservatives in particular. The conjunctival shrinkage reduces the surface area for the production of mucins. This impairs tear film wetting which deteriorates further if there is keratinised or dysplastic epithelium. The architectural changes to the eyelids can impair the tear film distribution, which leads to relative or absolute dessication. Severe conjunctival shortening may prevent lid closure (nocturnal or diurnal lagophthalmos) or adequate therapeutic ptosis from botulinium toxin. The symblepharon and shallow fornices may prevent the use of therapeutic contact lenses.

Table 2. Ocular surface disorders that affect progression of disease in cicatrising conjunctivitis

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Inflamed conjunctiva
   Autoimmune
   Infectious (bacterial, viral, fungal, chlamydia)
   Toxic (especially topical medication with preservatives)
   Blepharoconjunctivitis

Limbitis with or without secondary dellen

Corneal epithelial metaplasia

Keratinised conjunctival and corneal epithelium

Blepharitis
   Meibomian gland disease
   Staphylococcal blepharitis
   Seborrheic blepharitis

Tear film disorders
   Reduced mucin layer
   Reduced aqueous
   Lipid layer dysfunction
   Abnormal products in the tear film

Eyelid abnormalities
   Entropion
   Trichiasis
   Cicatricial lid tethering leading to
      Impaired blinking
      Lagophthalmos
   Aberrant lashes
   Lid notching

Primary and Secondary Problems

Because of the complexity of the OSD in chronic progressive cicatrising conjunctivitis, it is important to determine the primary cause and all of the contributing factors (table 2). For example, a patient may have lower lid trichiasis causing inferior punctate epithelial keratitis and symptoms of discomfort. However, this may be secondary to tarsal conjunctival cicatrisation with entropion. The corneal manifestations may be particularly marked because the corneal epithelial reserves for microtrauma may be diminished due to tear film dysfunction or the addition of toxic products from the conjunctival inflammation. Therefore, the primary problem is progression of the cicatrisation (leading to trichiasis) which requires immunosuppression. The secondary

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problems are the conjunctival inflammation and the entropion which need correcting. In the short-term, the lashes can be epilated and ultimately corrected surgically. However, if the other issues are neglected, the entropion will worsen and the patient will remain at risk of corneal ulceration, scarring and microbial keratitis. The 'big picture' must also be considered and the best treatment is that which considers the 'whole patient' as well as the eye. For example, frequent eyelash epilation may be a better option than eyelid surgery for a very frail patient or when the side effects of immunosuppressive therapy are unacceptable.

A management plan of OSD can be formulated by using the aetiologies given in table 2 and this is the approach used in this chapter. The broad management principles are found in 'General Considerations' (pp. 192–195) and the management of eyelid abnormalities is found in the chapter on 'Lid Surgery' (pp. 207–218).

Inflamed Conjunctiva

Auto-immune inflammation of the conjunctiva causes direct abnormalities of the conjunctival epithelium and adds toxic and inflammatory cytokines to the tear film. Its management is that of topical and systemic immunosuppression (see 'Topical and Systemic Immunosuppression', pp. 196–206).

An eye with surface abnormalities is at increased risk of infection. This risk is further enhanced by the presence of potentially pathogenic eyelid bacteria in 80% of patients with ocular cicatricial pemphigoid or Stevens-Johnson syndrome [1]. The spectrum of infection ranges from 'simple' bacterial conjunctivitis to severe microbial keratitis. It is important to stress that any immunosuppressive therapy can blunt or prevent the host inflammatory response. This may lead to atypical corneal lesions, and microbial keratitis may present solely as persistent epithelial defects. Thus, the corneal infiltration and stromal melting that usually herald microbial keratitis may be absent in these patients. Epithelial defects that are unresponsive to the usual regime with lubrication and padding should therefore alert the clinician, and the threshold to undertake microbiological investigations should be low.

Conjunctival inflammation can be solely due to the toxic effects of topical medication, particularly those of the preservatives. For example, benzalkonium chloride reduces the epithelial surface microvilli, impairs epithelial mobility and healing, and is directly toxic. This can lead to punctate keratopathy, papillary and follicular conjunctivitis, poor surface wetting, corneal vascularisation and drug-associated pemphigoid [2, 3]. Further, these effects can be amplified by any other cause of OSD. Unexplained follicular conjunctivitis should always be considered to be due to drug toxicity until proven otherwise. Topical medication *of any sort* should be restricted and minimised in any

patient with OSD. This includes lubricant drops that typically contain preservatives unless specifically stated, whereas most ointments are preservative free. Preservative-free drops greatly reduce the toxicity problems but they have a short life, have special handling criteria, are expensive, and pose an increased risk of infection, particularly if there is reduced compliance for refrigeration and disposal. Therefore, an adequate therapeutic trial is required to demonstrate their need for an individual patient. Just as important is the question, *'does the patient need the drops at all?'*.

Limbitis and Dellen

Limbitis is a key prognostic clinical sign of impending keratopathy in ocular cicatricial pemphigoid and reflects extensive *overt or occult* conjunctival inflammation [4]. It requires cyclophosphamide and oral steroids in order to prevent keratopathy (see 'Immunosuppressive Therapy', pp. 196–206). Any elevated area of bulbar conjunctiva or limbus can lead to a poor distribution of tears over the adjacent cornea, leading to epithelial dessication. This is initially seen as punctate keratopathy and later as a frank epithelial defect with stromal thinning. Any deficiency in the tear film or the blinking mechanism will enhance the problem. The management is: to reduce the primary conjunctival or limbal problem; to provide adequate lubrication supplementation such as an ointment four hourly; to minimise eyelid abnormalities; to enhance the tear film; to exclude infection. A combination of measures is usually required.

Keratinised Conjunctival and Corneal Epithelium

Keratinisation of the ocular surface typically starts as localised areas involving the temporal conjunctiva of patients with severe OSD. With progression, new areas form, enlarge and eventually involve the corneal epithelium with devastating visual results. This is similar to the effects of trachoma and vitamin A deficiency. The broad management is to optimise all conditions affecting the ocular surface. Topical retinoic acid (0.02–0.5%) may often effect a reduction of areas of keratinisation [5–9]. A trial of drops once a day for 1 month is recommended, higher doses can induce pronounced conjunctival inflammation in OCP.

Blepharitis

The term 'blepharitis' is a 'catch-all' term for a variety of eyelid margin diseases, all of which can cause OSD and tear film disease. Chronic blepharitis can be subdivided into meibomian gland disease, seborrhoeic blepharitis and staphylococcal blepharitis [10]. Because of the increased prevalence and importance for OSD, each category must be actively managed or excluded at each consultation in the standard way [1, 10].

Tear Film Disorders

The mucin layer is derived from the goblet cells (50%) and directly from the corneal and conjunctival epithelium (50%) [11–14]. A deficiency of mucin leads to poor wetting and may be reflected in reduced tear film break-up times although this test may also be abnormal (< 10 s) in aqueous deficiency and lipid deficiency [15, 16]. Specific management of a reduction in mucin is not possible. However, any measures to promote the health of the ocular surface cells might be expected to improve mucin production. Prevention of further cicatrisation also provides the maximum surface area of tissue to produce mucin.

Primary deficiency of the aqueous is seen as a reduction in Schirmer's test, the marginal tear film and sometimes as an increased tear break-up time in a non-localised manner. In chronic progressive cicatrising conjunctivitis, aqueous deficiency can occur due to lacrimal duct cicatrisation, but this is uncommon until late in the disease [17]. The objectives of treating aqueous deficiency are to minimise the patient's symptoms and promote ocular surface health. These are achieved by combinations of: minimising evaporation by ensuring the lipid layer is adequate (see above); ensuring that the aqueous is able to wet the ocular surface due to adequate mucin and health of the ocular surface cells (see above); providing the minimum amounts of aqueous tear film supplements (these need to be proven to be therapeutic); considering minimising aqueous loss via the lacrimal drainage system by occluding the punctae; considering the use of ointments or lubricating oils at night and during the day.

A deficiency of the lipid layer is due to meibomian gland disease and this leads to increased aqueous evaporation by 4–5 times. This exacerbates aqueous deficiency and worsens tear film break up times. The management is that of meibomian gland disease.

Abnormal tear film products mainly arise from immune-driven conjunctival inflammation or staphylococcal blepharitis. It is difficult to assess the effect of such components on the ocular surface without sophisticated laboratory tests. In unexplained OSD the diagnosis is made on suspicion and supported if there are follicular conjunctival changes.

Prevention

The key concept in the management of ocular surface disease is *prevention* and special attention must be given to the known risk factors for corneal pathology: conjunctival cicatrisation, limbitis, persistent epithelial defect and microbial keratitis [4]. It also applies to all the aetiologies of surface diseases listed in table 2.

If lower lid forniceal shortening is used as an index of conjunctival cicatrisation, there is no statistical association with visual impairment due to corneal disease until there is extreme loss [4]. But when the lower fornix shallows to less than 3 mm, the corneal complications suddenly become frequent and severe. Because of the longevity of most of the patients concerned a 'buffer zone' as large as possible is advantageous, and therefore in time every effort should be made to prevent further cicatrisation.

Appearance of limbitis is a sign of paramount importance, because it usually precedes corneal complications, yet it is very treatable. Whereas it is poorly responsive to moderate immunosuppressive agents such as sulphapyridine or dapsone [18], cyclophosphamide and short-term high dose oral steroids have been 100% effective at resolving limbitis and retaining the visual acuity [19].

Persistent epithelial defects and microbial keratitis generally occur due to tear film dysfunction, lid margin disease, lid architecture abnormalities, lagophthalmos and/or trichiasis. Therefore, these conditions must be *actively and aggressively* sought at every consultation before there is ocular surface disease that develops into persistent defects or keratitis. This is the notion of a 'pre-emptive strike'.

The other spin-offs from immunosuppression of inflammation are that cataract extraction and lid surgery are comparatively safe. The cost of this 'aggressive' approach is the ever-present dangers of powerful medication in elderly patients.

The Future

Specific Causes of Ocular Surface Disease

As any of the chronic progressive cicatrising conjunctivitides become advanced, the conjunctival epithelium becomes hyperplastic, metaplastic and keratinizes. The cause is unknown. One possibility is that it is due to keratinocyte growth factor (KGF, FGF-7). This cytokine is known to be produced by fibroblasts and binds to specific receptors located on epidermal keratinocytes [20, 21]. KGF is produced in large amounts during wound healing [22]. Therefore it may be produced by activated conjunctival fibroblasts. Certainly, in mice, excess KGF results in grossly wrinkled and thickened skin that shows hyperplasia and metaplasia histologically [23]. KGF has the potential to contribute to the conjunctival and corneal epithelial disease in OCP and therefore selective blockade would be a major therapeutic advance. Ocular studies are awaited.

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Corneal Epitheliopathy

An abnormal corneal epithelium is the initial and main cause of reduced visual acuity in OCP. A similar phenomenon is observed in Stevens-Johnson syndrome, aniridia and thimerosal epitheliopathy, and there is evidence that the epithelium in some of these patients is mainly cornea-derived rather than conjunctiva-derived (see chapter on 'Keratopathy', pp. 182–191). This has clinical implications since a metaplastic corneal epithelium has the potential – as have the majority of metaplastic epithelia elsewhere in the body – to become phenotypically normal. In diabetic corneal epitheliopathy this has been achieved medically with aldose reductase inhibitors [24, 25]. Dramatic improvement in epithelial morphology is seen within days in patients treated for vitamin A deficiency [5, 7, 8]. Therefore, it may be possible to pharmaceutically or genetically manipulate the cell phenotype to achieve cells that are more transparent and flatter, and hence optically better. Another alternative is to provide a new population of healthy cornea-derived epithelial cells from donors either as 'limbal stem-cell' transplants or as keratoepithelioplasty buttons [26–28] (see 'Corneal and Cataract Surgery', pp. 228–239). Certainly, the more severe cases will have the cornea covered by conjunctiva-derived epithelium. If there is still an ongoing systemic antibody response to basement membrane, then this will directly induce an immune-driven keratitis. This can only be managed by reducing the immune drive with systemic medication and/or transplanting a new source of cornea-derived epithelial cells that will make a basement membrane that is not antigenic.

References

1 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.
2 Wright P, Mackie I: Preservative-related problems of soft contact lens wearers. Trans Ophthamol Soc UK 1982;98:3–6.
3 Takahaski N: Cytotoxicity of preservatives on cultured human conjunctival cells. Acta Soc Ophthalmol Jpn 1980;84:1171–1176.
4 Elder MJ, Bernauer W, Leonard J, Dart JKG: Progression of disease in ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:292–296.
5 Sommer A, Emran N: Treatment of corneal xerophthalmia with topical retinoic acid. Am J Ophthalmol 1983;95:349–352.
6 Wright P: Topical retinoic acid therapy for disorders of the outer eye. Trans Ophthalmol Soc UK 1985;104:869–874.
7 Tseng SC, Maumenee AE, Stark WJ, Maumenee IH, Jensen AD, Green WR, Kenyon KR: Topical retinoid treatment for various dry-eye disorders. Ophthalmology 1985;92:717–727.
8 Tseng SC, Farazdaghi M: Reversal of conjunctival transdifferentiation by topical retinoic acid. Cornea 1988;7:273–279.
9 Soong HK, Martin NF, Wagoner MD, Alfonso E, Mandelbaum SH, Laibson PR, Smith RE, Udell I: Topical retinoid therapy for squamous metaplasia of various ocular surface disorders. Ophthalmology 1988;95:1442–1446.

10   McCulley JP, Dougherty JM, Denean DG: Classification of chronic blepharitis. Ophthalmology 1982;89:1173–1180.
11   Dilly PN, Mackie IA: Surface changes in the anaesthetic conjunctiva in man with special reference to the production of mucus from a non-goblet-cell source. Br J Ophthalmol 1981;65:833–842.
12   Greiner JV, Weidman TA, Korb DR, Allansmith MR: Histochemical analysis of secretory vesicles in nongoblet conjunctival epithelial cells. Acta Ophthalmol 1985;63:89–92.
13   Nichols BA, Chiappino ML, Dawson CR: Demonstration of mucus layer tear film by electron microscopy. Invest Ophthalmol Vis Sci 1985;26:464–473.
14   Gipson IK: Evidence that the entire ocular surface epithelium produces mucins for the tear film. Invest Ophthalmol Vis Sci 1994;35(suppl):2589.
15   Lemp MA, Hamill JR: Factors affecting tear film break up time in normal eyes. Arch Ophthalmol 1973;89:103–105.
16   McCulley JP, Sciallis GF: Meibomian keratoconjunctivitis oculo-dermal correlates. CLAO 1983;9: 130–132.
17   Foster CS: Cicatricial pemphigoid. Tr Am Ophthal Soc 1986;84:527–663.
18   Elder MJ, Leonard J, Dart JK: Sulphapyridine – A new agent for the treatment of ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:549–552.
19   Elder MJ, Lightman SL, Dart JK: The role of cyclophosphamide and high dose steroid in ocular cicatricial pemphigoid. Br J Ophthalmol 1995;79:264–266.
20   Aaronson SA, Bottaro DP, Miki T, Ron D, Finch PW, Fleming TP, Ahn J, Taylor WG, Rubin JS: Keratinocyte growth factor. A fibroblast growth factor family member with unusual target cell specificity. Ann NY Acad Sci 1991;638:62–77.
21   Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AM, Aaronson SA: Determination of ligand-binding specificity by alternative splicing: Two distinct growth factor receptors encoded by a single gene. Proc Natl Acad Sci USA 1992;89:246–250.
22   Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT: Large induction of keratinocyte growth factor expression in the dermis during wound healing. Proc Natl Acad Sci USA 1992;89:6896–6900.
23   Guo L, Yu QC, Fuchs E: Targeting expression of keratinocyte growth factor to keratinocytes elicits striking changes in epithelial differentiation in transgenic mice. EMBO J 1993;12:973–986.
24   Mori K, Takahashi Y, Tsuduki S, Kador PF, Akagi Y: Significance of aldose reductase to experimental corneal epitheliopathy. Invest Ophthalmol Vis Sci 1994;35(suppl):3197.
25   Hosotani H, Yamada M, Tsubota K: Reversal of corneal epitheliopathy in diabetic patients by an aldose reductase inhibitor. Invest Ophthalmol Vis Sci 1994;35(suppl):3196.
26   Kenyon RK, Tseng SC: Limbal autograft transplantation for ocular surface disorders. Ophthalmology 1989;96:709–723.
27   Turgeon PW, Nauheim RC, Roat MI, Stopak SS, Thoft RA: Indications for epitheliokeratoplasty. Arch Ophthalmol 1990;108:233–236.
28   Thoft RA: Keratoepithelioplasty. Am J Ophthalmol 1984;97:1–6.

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Corneal and Cataract Surgery in Chronic Progressive Conjunctival Cicatrisation

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Progressive conjunctival cicatrisation is a particular challenge for the ophthalmologist who attempts to preserve or improve sight in this rare but devastating ocular condition [1]. This chapter addresses surgical approaches to the problem, with particular emphasis on the commonest and most studied variety, ocular cicatricial pemphigoid (OCP).

Epidemiology

Cataract is common in patients with OCP. This is because of the high average age of the patients [2] resulting in senile cataract and due to the frequent development of secondary cataract that is associated both with the use of topical and systemic steroids for the control of conjunctival inflammation and with the uveitis resulting from episodes of keratitis.

Corneal scarring is the commonest cause of permanent visual loss in OCP, developing as a consequence of the tear film deficiency, eyelid malposition, trichiasis and secondary corneal infection resulting from the chronic conjunctival disease. The chronic limbitis that is common in active pemphigoid is a little recognised cause of corneal opacity and often occurs before tear film deficiency and trichiasis have developed in OCP [3]. Sclerosing keratitis is a rare complication. Elective optical keratoplasty may be performed to attempt to improve vision, and emergency tectonic keratoplasty is often required to treat perforations due to microbial corneal ulceration or persistent epithelial defects with corneal 'melt'. A high proportion of patients with OCP will eventually become legally blind from the disease, although the prognosis is improved with systemic immunomodulation [2, 4].

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Literature Review

Cataract Surgery

Sainz de la Maza et al. [5] reported the results of cataract surgery in 20 patients (26 eyes) with OCP. All patients were receiving systemic immunosuppression (dapsone, azathioprine or cyclophosphamide) at the time of surgery. The surgical technique used was extracapsular cataract extraction with a large limbal incision and limited conjunctival peritomy, followed by posterior chamber lens implantation. At an average follow-up interval of 22 months, there was a mean visual improvement of 3.5 Snellen lines, with visual deterioration in two eyes (from age-related macular degeneration and from corneal ulceration). Keratopathy worsened in 3 patients, which the authors attributed to inadequate immunosuppression (because of drug intolerance). For comparison, they also described 2 case reports, where patients with bilateral conjunctivitis of unknown cause underwent cataract surgery without immunosuppression. Each case developed severe conjunctival inflammation and cicatrisation with keratopathy after cataract surgery, and OCP was subsequently diagnosed in each case. The authors concluded that cataract surgery can be safely performed in patients with OCP provided that all conjunctival inflammation is controlled with systemic immunosuppression in the peri-operative period.

Keratoplasty

Tugal-Tukan et al. [6] reported the results of penetrating keratoplasty in patients with OCP, Stevens-Johnson syndrome and toxic epidermal necrolysis. Nine eyes of 8 patients with OCP underwent penetrating keratoplasty, 4 for tectonic and 5 for optical reasons. In spite of aggressive pre-operative treatment including systemic immunosuppression, mucous membrane grafts and eyelash cryotherapy, there was a high rate of complications with generally poor visual results. Out of the 9 eyes, 5 developed persistent epithelial defects, leading to ulceration in 4 cases. One required a keratoprosthesis and 5 required a further tectonic regraft. Vision was improved in 3 eyes (best post-operative acuity was 20/200), was unchanged in 3, and was worse in 3. In the light of these results, the authors caution against pursuing visual rehabilitation through penetrating keratoplasty in most of these patients.

In a series of 46 consecutive cases of penetrating keratoplasty performed for corneal perforation in a variety of diseases, all grafts failed (n = 6) where there was melting or ocular surface abnormality [7]. This was due to further melting, which resulted in a second perforation or a descemetocele, and in spite of regrafting in 5 of the 6 eyes, the final outcome was that 2 eyes became phthisical, 2 required a conjunctival flap, and 2 were intact but with a cloudy graft.

Various procedures have been used in an attempt to create a more favourable ocular surface environment for penetrating keratoplasty and these have utilised a variety of autogenous tissues to improve the ocular surface. Both split and full-thickness buccal mucous membrane grafts may be beneficial where there is severe trichiasis not amenable to cryotherapy and lid rotation procedures, or entropion with marked keratinisation, although there is a high rate of long-term complications [8, 9]. Nasal mucosal grafts have the advantage, over buccal mucosa, of providing a source of goblet cells with the potential for improving the tear film [10, 11]. Split-thickness skin grafts have also been used to restore the integrity of the ocular surface [12].

While such autograft techniques may stabilise the corneal environment, they cannot replace the stem cells from which the corneal epithelium is believed to originate [13]. As it is likely that limbal corneal stem cells are destroyed as part of the disease process in chronic cicatrising conjunctivitis, this may explain the high rate of epithelial breakdown following keratoplasty in these patients [6] and indicate the need for effective techniques of corneal stem cell transplantation as well. This procedure remains experimental, although early results for eyes damaged by burns have been encouraging [14].

Keratoprosthesis

It is not surprising, given the poor results of keratoplasty in OCP and other types of cicatrising conjunctivitis, that attempts have been made to replace the scarred cornea with an artificial cornea or keratoprosthesis. In 1977, Cardona and DeVoe [15] reported an initial very high extrusion rate of keratoprostheses in OCP, but this was considerably improved with changes in technique and a modified keratoprosthesis. They preferred a through-and-through prosthesis with the optical cylinder placed through the upper lid for this group of patients. Girard et al. [16], presenting results of keratoprosthesis in 119 consecutive cases with various diagnoses, suggested that in Stevens-Johnson syndrome or OCP, it was advisable to resect the diseased conjunctiva and overlay the prosthesis with Tenon's capsule and buccal mucous membrane grafts, followed by a tarsorrhaphy which is re-opened after 4 months. This gave better results than placing the prosthesis through the upper eyelid. Acqua-vella et al. [17] reported results using a Cardona through-and-through keratoprosthesis, covered with periosteum (taken from the tibia) and placed through the upper lid. Nine of the 31 cases had OCP, although the outcome for these was not analysed separately. With an average follow-up of 35 months, 17 of the 31 patients achieved a visual acuity of 20/200 or better for 1–7 years, all having been worse than 20/200 pre-operatively. Twelve out of 31 achieved vision of 20/40 at some point, although at the end of follow-up this figure

was 5/31. As in other series, there was frequent extrusion or dislocation and a high rate of surgical revision (15/31). Other complications included retroprosthetic membrane and sterile vitritis, and the authors emphasised the ever-present potential for endophthalmitis, which was associated with loss of vision in 4 cases. Because through-the-lid placement made re-operation extremely difficult, the authors abandoned this as an initial procedure and recommended that the conjunctiva was closed over the keratoprosthesis for 4–6 weeks, then if cover was inadequate the conjunctiva was excised, once the underlying periosteum had healed, and a buccal mucous membrane graft was used to cover the optical cylinder, and at a third stage a small opening was made in this.

Kozarsky et al. [18] reported a series of 11 consecutive patients with alkali burns, OCP, or Stevens-Johnson syndrome who received ceramic keratoprostheses and described the technique in detail. First, all conjunctiva was excised, as well as the palpebral part of the lacrimal gland. If the cornea was thin, this was followed by a penetrating keratoplasty (12 mm into 11 mm). In phakic eyes, the iris was removed through a standard superior limbal incision, followed by intracapsular cryo-extraction of the lens and an anterior vitrectomy. In aphakic eyes, the iris was left in place to avoid bleeding into the vitreous, and an anterior vitrectomy was done through the keratoprosthesis opening. The keratoprosthesis cylinder was placed through a 3-mm corneal incision, and its ceramic skirt was sutured to the cornea. A piece of periosteum 3 cm × 5 cm, with a 3-mm opening for the cylinder, was then sutured to the globe over the keratoprosthesis, which was externalised through the lower third of the upper eyelid. Finally, the eyelids were sutured together after the margins had been excised. Although the visual acuity was initially improved to between 20/100 and 20/20 in 6 patients, this was achieved in the first few weeks after surgery, with a subsequent decline. Of the 4 patients with OCP, 2 had maintained some visual improvement at the most recent review, 1 developed phthisis secondary to retroprosthetic cyclitic membrane, and the other required evisceration following endophthalmitis.

Sletteberg et al. [19] in 1990 reported an improved retention time when two-piece rather than one-piece keratoprostheses were used. During the years 1974–1987, 27 two-piece keratoprostheses were implanted into 25 eyes of 22 patients, 7 of whom had OCP. Pre-operatively, none had vision better than light perception; post-operatively 18 out of 27 eyes achieved 6/60 or better at some stage, although 9 prostheses were lost or removed because of complications.

With the high incidence of complications associated with keratoprosthesis, particularly endophthalmitis, extrusion, retroprosthetic membrane and glaucoma, the current recommendation is that penetrating keratoplasty should be tried first, if there is even a small chance of success [20].

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**Moorfields Eye Hospital Series of Cataract Operations and
Corneal Grafts for Ocular Cicatricial Pemphigoid**

We have reviewed the results of patients with a diagnosis of OCP who
have undergone cataract, corneal graft or combined surgery at Moorfields
Eye Hospital over the past 10 years (table 1). Twelve eyes of 10 patients were
included, with a mean length of follow-up of 2.3 years (range 1 month to 10
years). All patients had stage III disease before and after surgery, using Foster's
staging system [2], apart from patient 1 who progressed from stage II to stage
III post-operatively. Of the cataract patients undergoing surgery for visual
reasons, the earliest had intracapsular extraction without an implant, the most
recent had phaco-emulsification through a small corneal incision. In 1 of these
cases (patient 5), corneal scarring prevented an adequate view for capsu-
lorhexis, and extra-capsular surgery was performed instead. This technique
was also used for a patient with an intumescent lens (patient 6).

Cataract extraction gave improved vision in 4 out of 7 eyes at some stage
following surgery. However, at most recent review only 2 eyes had maintained
visual improvement compared with pre-operatively. A common feature of the
patients who did not achieve or maintain visual improvement following cata-
ract surgery was progressive deterioration of the corneal surface. The length
of follow-up is longer than previously described [5] and suggests that although
medium term – and worthwhile – visual benefit can be achieved through
cataract surgery, this may eventually be overtaken by uncontrolled ocular
surface disease. The cataract techniques used here have employed a corneal
wound, unlike the limbal surgery previously described [5], and it is probable
that this does not carry the same risks for precipitating an exacerbation of
conjunctivitis in OCP as does surgery involving the conjunctiva.

Keratoplasty was performed on 7 eyes of 5 patients, in the first case
(patient 2) for persistent epithelial defect with the hope of visual improve-
ment, and in the remainder for perforation (table 1). In 2 cases, keratoplasty
was combined with cataract surgery. Although the indication for keratoplasty
was tectonic in 6 out of 7 cases, 3 patients gained worthwhile visual
improvement from the procedure, although this was not maintained at the
most recent review. None of the grafts failed during the review period; the
main complication, as for cataract surgery, was progressive deterioration
of the corneal surface, and further penetrating keratoplasty was required
for 2 eyes developing a descemetoceles in the first graft (fig. 1). Two eyes
also developed glaucoma, which in one was not adequately controlled by
topical levobunolol. Cyclodiode laser with oral prednisolone cover was
successful in lowering the intra-ocular pressure without exacerbation of the
conjunctival disease in this patient.

Table 1. Surgery in OCP

Patient No. and Op. (a, b, c)	Age/sex	Operation	Indication	Follow-up	VA pre	Best VA	Final VA	Medication pre	Medication post	Comments
1	70 F	R ICCE, no implant	VA	10 years	CF	6/6 at 4 years	HM	topical steroid	sulphapyridine cyclosporin	progessive surface disease
3a	72 F	L ECCE+IOL	VA	4 years	6/36	6/12 at 6 months	(see 3b)	topical steroid	prednisolone cyclophos	progressive surface disease
4	79 F	L ECCE, no IOL	VA	3 years	PL	HM at 2 years	PL	prednisolone	prednisolone	postop. inflammation, progressive surface disease
5a	70 M	R phaco+IOL	VA	18 months	CF	6/18 at 7 months	(see 5b)	prednisolone, cyclophos	prednisolone cyclophos	progressive surface disease
6b	81 F	R RCCE+IOL	intumescent lens	11 months	HM	HM	(see 6c)	topical steroid	topical steroid	progressive surface disease
7	80 F	R phaco+IOL	VA	1 month	6/60	CF	CF	artificial tears	topical steroid	macular degeneration
5b	70 M	L ECCE+IOL	VA	1 year	6/60	6/60	6/60	nil	prednisolone	view too poor for phaco
8	83 F	R phaco+IOL	VA	2 months	6/60	6/12 at 2 months	6/12	azathioprine	prednisolone	
2	84 F	L PK previous PK, ECCE	VA, epithelial defect	2 years	HMS	6/24 at 8 months	CFS	nil	topical steroid	graft opacified
3b	73 F	R PK, ECCE, IOL	perforation	3 years	PL	6/36 at 2 years	2/60	cyclophos	prednisolone, cyclophos	
6a	81 F	R PK	perforation	12 months	HM	HM	(see 6b, c)	topical steroid	topical steroid	
6c	82 F	R PK	descemetocele	6 months	HM	6/60 at 3 months	PL	prednisolone	prednisolone	chronic surface disease, microbial keratitis, glaucoma
9	78 M	L PK, ICCE, no IOL	perforation	3 years	PL	PL	PL	prednisolone	prednisolone	progressive surface disease, glaucoma
10a	66 F	L PK	perforation	(see 10b)	CF	CF	CF	prednisolone	prednisolone, cyclophos	melt: needed regraft after 5 months
10b	66 F	L PK	descemetocele	20 months	CF	CF	CF	prednisolone cyclophos	prednisolone, cyclophos	

Cataract cases then keratoplasties are shown; patients are numbered chronologically. CF = Counting fingers; cyclophos = cyclophosphamide; ECCE = extracapsular cataract extraction; HM = hand movements; ICCE = intracapsular cataract extraction; IOL = intra-ocular lens; PK = penetrating keratoplasty; phaco = phaco-emulsification; PL = perception of light; VA = visual acuity.

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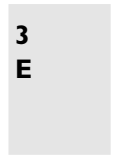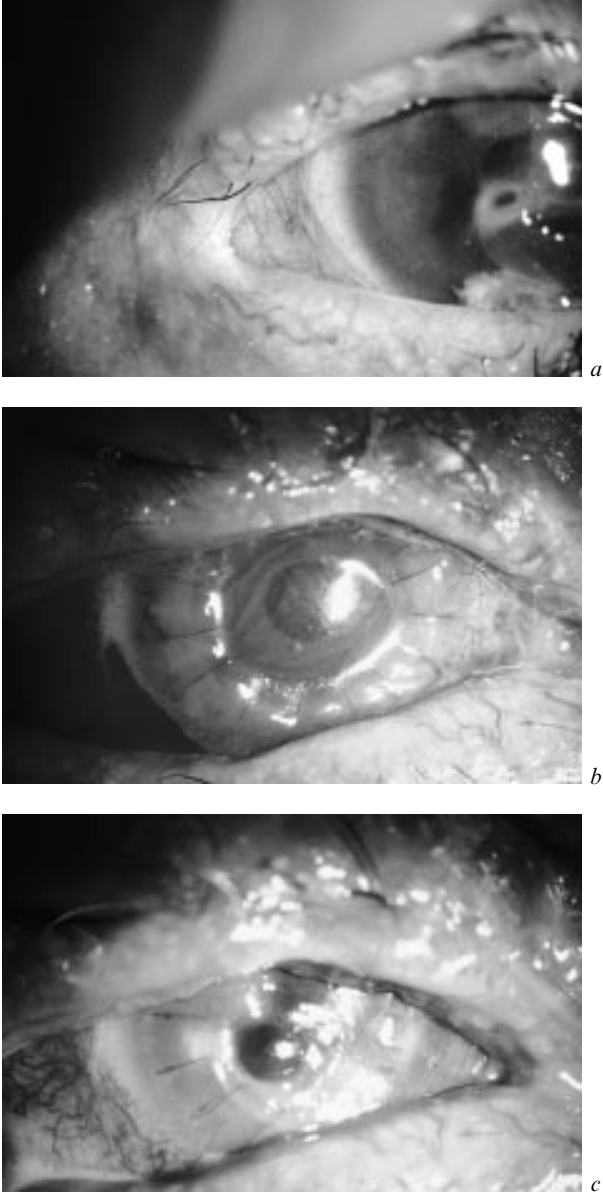
Fig. 1. Shows patient No. 10, before the first keratoplasty (*a*), shortly after keratoplasty (*b*) and with descemetocele in graft 5 months later (*c*).

Principles of Surgical Management

Immunosuppression

A minority of patients with OCP have very slowly progressive disease, without clinical evidence of inflammation, and do not require systemic immunosuppressive therapy [3]. In this group, cataract surgery, via a corneal rather than a conjunctival incision, can be carried out without the need for prophylactic systemic immunosuppression; in the event of post-operative conjunctival inflammation occurring, the regime for control of acute inflammation, described below, can be instituted. Most cases of OCP have more rapidly progressive disease in which the only treatment that has been shown to be effective is systemic immunosuppression. As surgery is liable to activate the disease process in these patients, this should not be considered until the conjunctival inflammation is fully controlled. Cyclophosphamide, dapsone, sulphapyridine and azathioprine have all been shown to be effective [2, 21–24]; of these, cyclophosphamide probably has the most consistent effect. Control of the disease with these agents takes 2–3 months. If rapid control is required for the management of fulminating disease, or for tectonic keratoplasty, this can be achieved by combining cyclophosphamide with high-dose oral prednisolone (usually 1 mg/kg up to a dose of 80 mg daily). Any emergency corneal surgery should be delayed, using cyano-acrylate glue for perforations, until clinical signs of inflammation have resolved. Long-term immunosuppression, after surgery, is required in most patients, if the visual benefit of the operation is to be maintained.

Treatment of Surface Disease

Treatment of ocular surface disease with correction, as far as possible, of all the factors contributing to a normal ocular surface is critical if normal wound healing is to occur and to reduce the problem of persistent corneal epithelial defect after surgery.

Trichiasis and entropion lead to corneal abrasion, persistent epithelial defects and microbial keratitis, to all of which the eye is more susceptible in the post-operative period. These problems must be corrected before surgery and management continued during the post-operative period. Aberrant lashes should currently be treated with cryotherapy and misdirected lashes, by upper lid anterior lamellar reposition and lower lid retractor plication [25].

The tear film should be optimised to obtain the most favourable environment for wound healing. *Conservation of the tear film* by punctal occlusion is helpful when this has not occurred spontaneously and can be carried out temporarily or permanently; it may result in more frequent bacterial conjunctivitis or tearing. Herrick plugs (Lacrimedics, Rialto, CA, USA) are easily

inserted into both superior and inferior canaliculi, quickly and painlessly, and are currently the choice in our department for temporary occlusion. However, these may migrate spontaneously and their continued effect can only be confirmed by gentle irrigation of the canaliculus. Permanent occlusion is carried out by cautery of the punctum, and the proximal 3–5 mm of the canaliculus, using a hot wire cautery after giving a local anaesthetic injection; reversal is impracticable afterwards. *Augmentation of the tear film* with lubricants is required for many patients with advanced disease. Conventional tear film replacements are satisfactory for some patients but, in those with a very reduced natural tear secretion, preservative toxicity is common when tear replacements are used frequently. The effects of toxicity, inferior punctate keratoconjunctivitis and filamentary keratitis are difficult to distinguish from those of the underlying disease and usually take 3–6 weeks to resolve when the patient is transferred to non-preserved preparations. Despite the short retention time, unpreserved balanced salt solution is favoured by many patients. In patients with very dry eyes, and poor wetting due to keratin, hydrophobic liquid paraffin eye drops (Parolein) may be helpful. Lubricant ointments are more effective than drops in maintaining a stable ocular surface in very dry eyes. *Keratin* on the conjunctiva causes discomfort and reduces the surface wetting and may respond to retinoic acid 0.05% drops once daily.

Technique of Cataract Surgery

It is particularly important to avoid stimulating further conjunctival scarring in OCP. For this reason, we have utilised corneal incisions for cataract surgery for many years. From currently available techniques, we prefer phaco-emulsification through a small sutureless corneal incision as this reduces the disruption of the ocular surface to a minimum and is ideally suited to approaching eyes with symblepharon which can restrict access to the peripheral cornea, particularly adjacent to the upper and lower lids, but usually allows entry in the temporal quadrant. Other factors which may restrict the choice of a cataract wound site are areas of peripheral corneal thinning following keratitis, but this has not usually precluded the choice of a corneal, as opposed to a conjunctival wound. Safe phaco-emulsification demands a reasonable view of the anterior chamber and may be impracticable if there is excessive corneal opacity. However, the use of 2% hydroxypropylmethylcellulose (HPMC for intra-ocular use) on the cornea during surgery, and the employment of paraxial rather than co-axial illumination on the operating microscope can dramatically improve the view in the presence of irregular astigmatism and opacity, allowing phaco-emulsification to proceed. When central corneal scarring is too advanced to permit phaco-emulsification then a corneal section extracapsular extraction can be carried out with a wound, enlarged to allow

'open sky' surgery if needed, followed by conventional wound closure with 10-0 nylon. It is important to maintain hydration of the cornea throughout surgery to prevent the development of corneal epithelial defects in the peri-operative period; the liberal use of 2% HPMC on the cornea during surgery is helpful. OCP is not a contra-indication to lens implantation, which can be combined with any of these procedures. The authors have not had to employ a limbal or scleral cataract wound, which might be required in cases with extensive peripheral corneal thinning, but this has been shown to be safe if appropriate immunosuppression is used in the peri-operative period [5].

Technique of Keratoplasty

Because of the poor prognosis for keratoplasty in OCP, the main indication is tectonic, to repair corneal perforations that cannot be managed conservatively with cyano-acrylate glue. In this context there is no other therapeutic option for maintaining globe integrity. Many of these patients have the potential for secondary visual benefit. However, this procedure is usually undertaken on a severely diseased eye and is complicated by early failure to epithelialise and late persistent epithelial defect and corneal stromal melt. Although perforation was successfully treated by keratoplasty in our patients (2 required a second graft to achieve this), only 1 of these (patient 3) had maintained even limited visual benefit at the most recent review.

Elective surgery must be preceded by careful preparation of the ocular environment as described above. If there is significant lens opacity, cataract extraction with lens implantation may be performed at the same time as penetrating keratoplasty. A single combined procedure has the advantage of providing good visualisation for cataract surgery, as well as of minimising trauma to the ocular surface. Even in the absence of significant cataract it is often more practical to carry out clear lens extraction while access to the lens is good rather than delay when later surgery may be technically much more demanding.

Penetrating keratoplasty has been required in all the cases performed at this centre despite our preference for lamellar keratoplasty where possible in high-risk situations. This has usually been because of the presence of microbial keratitis in perforated eyes precluding the use of a lamellar graft or, in the sighted eyes because of corneal endothelial decompensation or deep stromal opacity. Conventional techniques of penetrating keratoplasty have been employed but using grafts that are no larger than needed, to conserve host tissue where possible. Interrupted corneal sutures must be used to facilitate the management of the early suture loosening that is common in this situation.

Lamellar grafting has the advantages of eliminating the risk of endothelial rejection and of retaining host tissues that are probably more resistant to

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corneal melting than grafted tissue. Since the development of cyano-acrylate glue, viscoelastics, micrometer diamond knives, and the use of full-thickness donor material with deep lamellar dissection, the technique has become simpler and should be considered whenever a keratoplasty is needed in these high-risk cases. The only absolute contra-indications to lamellar keratoplasty are the presence of uncontrolled infection and corneal endothelial decompensation; deep corneal opacity is a relative contra-indication.

Corneal stem cell grafts are being increasingly experimented with at this centre for the management of patients with corneal disease co-existing with stem cell failure, a common problem in OCP. These have been unsuccessful so far in patients with dry eyes (3 cases including case 3b; table 1) in whom failure to epithelialise has been the principal post-keratoplasty complication. Because these patients have scarred and irregular corneas with or without co-existing infection, either a lamellar or penetrating keratoplasty has been used but from an eccentrically cut donor to include approximately 30% of donor limbus (a keratolimbal allograft). This may be placed centrally in the host within the confines of the cornea which may provide some degree of immune privilege. These techniques hold out some promise for the future.

Keratoplasty has been seldom successful for visual rehabilitation because of the formidable problems of corneal epithelialisation in the poor ocular environment that accompanies OCP. Tectonic keratoplasty may be necessary to prevent loss of the globe following perforations but has so far rarely resulted in useful vision although retention of the globe is mandatory for keratoprosthesis. The principal problems in maintaining keratoplasties in OCP are due to the poor tear film and corneal stem cell failure, both of which result in persistent failure to epithelialise. Newer techniques such as nasal mucosal grafts and submandibular gland autotransplantation, combined with effective immunosuppression, may improve the environment for corneal grafts sufficiently to achieve a stable epithelial surface after surgery [11, 26]. This will enable corneal transplantation to be offered in OCP for a visual, not just a tectonic, result.

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References

1 Bernauer W, Broadway DC, Wright P: Chronic progressive conjunctival cicatrisation. Eye 1993;7: 371–378.
2 Foster CS: Cicatricial pemphigoid. Trans Am Ophthalmol Soc 1986;84:527–663.
3 Elder MJ, Bernauer W, Leonard J, Dart JKG: Progression of disease in ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:292–296.
4 Elder MJ, Lightman S, Dart JKG: Role of cyclophosphamide and high dose steroid in ocular cicatricial pemphigoid. Br J Ophthalmol 1995;79:264–266.
5 Sainz de la Maza M, Tauber J, Foster CS: Cataract surgery in ocular cicatricial pemphigoid. Ophthalmology 1988;95:481–486.

6   Tugal-Tutkun I, Akova YA, Foster CS: Penetrating keratoplasty in cicatrising conjunctival diseases. Ophthalmology 1995;102:576–585.
7   Nobe JR, Moura BT, Robin JB, Smith RE: Results of penetrating keratoplasty for the treatment of corneal perforations. Arch Ophthalmol 1990;108:939–941.
8   Shore JW, Foster CS, Westfall CT, Rubin PA: Results of buccal mucosal grafting for patients with medically controlled ocular cicatricial pemphigoid. Ophthalmology 1992;99:383–395.
9   Heiligenhaus A, Shore JW, Rubin PAD, Foster CS: Long-term results of mucous membrane grafting in ocular cicatricial pemphigoid. Ophthalmology 1993;100:1283–1288.
10 Naumann GOH, Lang GK, Rummelt V, Wigand ME: Autologous nasal mucosa transplant in severe bilateral conjunctival mucus deficiency syndrome. Ophthalmology 1990;97:1011–1017.
11 Bialasiewicz AA: Nasal mucosa grafting in ocular pemphigoid. Ophthalmology 1991;98:273.
12 Mauriello JA, Pokorny K: Use of split-thickness dermal grafts to repair corneal and scleral defects – A study of 10 patients. Br J Ophthalmol 1993;77:327–331.
13 Tseng SCG: Concept and application of limbal stem cells. Eye 1989;3:141–157.
14 Coster DJ, Aggarwal R, Williams KA: Surgical management of ocular surface disorders using conjunctival and stem cell allografts. Br J Ophthalmol 1995;79:977–982.
15 Cardona H, DeVoe AG: Prosthokeratoplasty. Trans Am Acad Ophthalmol Otolaryngol 1977;83: 271–280.
16 Girard LJ, Hawkins RS, Nieves R, Borodofsky T, Grant C: Keratoprosthesis: A 12-year follow-up. Trans Am Acad Ophthalmol Otolaryngol 1977;83:252–267.
17 Acquavella JV, Rao GN, Brown AC, Harris JK: Keratoprosthesis. Results, complications and management. Ophthalmol Otolaryngol 1982;89:655–660.
18 Kozarsky AM, Knight SH, Waring GO: Clinical results with a ceramic keratoprosthesis placed through the eyelid. Ophthalmology 1987;94:904–911.
19 Sletteberg O, Hovding G, Bertelsen T: Keratoprosthesis. II. Results obtained after implantation of 27 dismountable two-piece prostheses. Acta Ophthalmol 1990;68:375–383.
20 Dohlman CH: Keratoprostheses; in Albert DM, Jakobiec FA (eds): Principles and Practice of Ophthalmology. Philadelphia, Saunders, 1994.
21 Tauber J, Sainz de la Maza M, Foster CS: Systemic chemotherapy for ocular cicatricial pemphigoid. Cornea 1991;10:185–195.
22 Elder MJ, Leonard JN, Dart JKG: Sulphapyridine – A new agent for the treatment of ocular cicatricial pemphigoid. Br J Ophthalmol 1996;80:549–552.
23 Dantzig PI: Immunosuppressive and cytotoxic drugs in dermatology. Arch Dermatol 1974;110: 393–406.
24 Mondino BJ: Cicatricial pemphigoid and erythema multiforme. Ophthalmology 1990;97:939–952.
25 Elder MJ, Dart JKG, Collin JR: Inferior retractor plication surgery for lower lid entropion with trichiasis in ocular cicatricial pemphigoid. Br J Ophthalmol 1995;79:1003–1006.
26 MacLeod AM, Robbins SP: Submandibular gland transfer in the correction of dry eye. Aust NZ J Ophthalmol 1992;20:99–103.

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Management of Glaucoma in Patients with Chronic Progressive Conjunctival Cicatrisation

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The management of glaucoma in patients with conjunctival cicatrisation is fraught with problems, both with respect to topical medical therapy and surgical therapy. Topical medications may affect the conjunctiva in a variety of ways, either inducing immediate symptomatic disease and worsening of the cicatrisation, or subclinical changes that may result in a long-term worsening of the process which could have been avoided. Likewise, surgery involving the conjunctiva invariably has a profound adverse effect on the cicatrising condition.

Treating Glaucoma

The principles of management of glaucoma in patients with conjunctival cicatrisation apply to patients with all types of cicatricial conjunctival disease. Essentially, when possible, any insult to the conjunctiva has to be avoided, this including both topical and surgical therapy. Each individual case should be assessed in its own right but, if appropriate, topical antiglaucoma therapy should be stopped and surgery avoided, at least in the first instance. If the patient is elderly, argon laser trabeculoplasty should be considered [1–4]. If surgery is deemed necessary, the type of surgery must be selected with great care. If subclinical conjunctival change is suspected (e.g. following long-term therapy with multiple topical agents) a standard trabeculectomy is indicated, although it is controversial as to whether adjunctive therapy with 5-fluorouracil is indicated. It has been shown recently that the adverse conjunctival effect of therapy with multiple antiglaucoma drugs (including a sympathomimetic)

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can be reversed by both stopping therapy with the sympathomimetic and commencing treatment with topical steroid (fluorometholone) for 1 month [5]. This reversal may increase the success rate of trabeculectomy in such patients [5]. When progressive 'immunological' cicatrisation occurs in association with glaucoma, topical therapy should be stopped and the intraocular pressure controlled with either laser or surgical modalities. Unfortunately, no studies have been published relating solely to patients with cicatricial conjunctival disease and glaucoma. Clinical experience, however, has shown that trabeculectomy is destined to fail in such patients and that the surgery has a detrimental effect on the conjunctival cicatrisation. Furthermore, when surgery is indicated, the use of adjunctive agents (5-fluorouracil or mitomycin C) are contra-indicated, only serving to make the postoperative cicatricial response worse. Insertion of a silicone drainage tube should be considered [6, 7] although such procedures are not without their complications and in many cases cyclodestructive procedures should be considered. In particular, partial cyclo-ablation with semiconductor diode laser transscleral cyclophotocoagulation shows promise and may well become the first choice in the management of these difficult cases [8–10].

References

1   Wise JB, Witter SL: Argon laser therapy for open-angle glaucoma: A pilot study. Arch Ophthalmol 1979;97:319–322

2   Wise JB: Long-term control of adult open-angle glaucoma by argon laser treatment. Ophthalmology 1981;88:197–202.

3   Schwartz AL, Love DC, Schwartz MA: Long-term follow up of argon laser trabeculoplasty for uncontrolled open-angle glaucoma. Arch Ophthalmol 1985;103:1482–1484.

4   The Glaucoma Laser Trial Research Group: The Glaucoma Laser Trial (GLT) 2. Results of argon laser trabeculoplasty versus topical medicines. Ophthalmology 1990;97:1043–1413.

5   Broadway DC, Stürmer J, Grierson I, Hitchings RA: Reversal of the adverse conjunctival effect of topical antiglaucoma medications prior to filtration surgery. Arch Ophthalmol 1996;114:262–267.

6   Molteno ACB, Straughan JL, Ancker E: Long tube implants in the management of glaucoma. S Afr Med J 1976;50:1062–1066.

7   Hitchings RA, Lavin MJ, Calthorpe M: Glaucoma drainage tubes – their role in glaucoma management. Int Ophthalmol 1989;13:151–157.

8   Hennis HL, Stewart WC: Semiconductor diode laser transscleral cyclophotocoagulation in patients with glaucoma. Am J Ophthalmol 1992;113:81–85.

9   Hawkins TA, Stewart WC: One-year results of semiconductor transscleral cyclophotocoagulation in patients with glaucoma. Arch Ophthalmol 1993; 111:488–491.

10   Ulbig MW, McHugh D, McNaught A, Hamilton P: Contact diode laser cyclo-photocoagulation for refractory glaucoma. A pilot study. Ger J Ophthalmol 1994; 3:212–215.

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New Concepts: Manipulation of the Wound-Healing Response

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Chronic progressive conjunctival cicatrisation is a dynamic process involving a complex series of events resulting in an exaggerated and abnormal wound-healing response. The severity and extent of clinical disease are closely related to the degree of conjunctival scarring, as described in preceding chapters. Many of the ocular complications of chronic conjunctival cicatrisation are secondary to disruption of the ocular surface and tissues caused by scarring. If the degree of scarring is reduced or eliminated at any stage in its evolution, then the severe complications of cicatrisation may be avoided. In addition to conjunctival cicatrisation, ocular scarring processes are involved in either the pathogenesis or failure of treatment of many of the major blinding or visually disabling conditions in the world today.

This review focuses on the various stages involved in conjunctival scar formation, and outlines the possible ways in which these processes may be modulated. We describe several new methods, some of which are currently being investigated in our laboratory, of manipulating the scarring response. The recent development of new modulating agents reflects an increasing understanding of the cellular and molecular biological mechanisms involved in wound healing.

Molecular and Cellular Events in Conjunctival Scarring

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The wound-healing response consists of a series of ordered events involving interactions between different cell types, the extracellular matrix (ECM) and a number of chemical mediators, and has been the subject of several excellent reviews [1–4].

Conjunctival scarring is initiated either directly by trauma or in response to an inflammatory process such as auto-immune disease. Although the initial conjunctival insult may be different, the healing pathways following traumatic injury and inflammation are similar. Much of the work on scarring has been done using surgical models, and therefore recent developments in the use of modulating agents have focused on this limb of the wound-healing pathway.

We hope to outline in this chapter the sequence of events involved in conjunctival scar formation. In addition we describe the current and potential methods of modifying the various components of the healing response, as illustrated in figure 1.

Inflammatory Response and Haemostasis

Conjunctival trauma (A) (e.g. surgical incisions) results in both connective tissue and blood vessel damage. This leads to the release of blood cells, plasma proteins and extracellular matrix fragments (B) into the damaged site, which in turn activates the clotting system and results in clot formation at the damaged area (C). Blood loss is reduced initially by the formation of haemostatic plugs from the aggregation of platelets. This process is stimulated by the presence of various factors including thrombin, adenosine diphosphate, fibrinogen, collagen, thrombospodin and von Willebrand factor VIII.

In the case of persistent inflammation (D) (e.g. cicatricial pemphigoid), a similar cascade of events is initiated with vasodilatation and leakage of plasma proteins (E) into the local environment.

Next, the inflammatory phase of the wound-healing response occurs (F). Polymorphonuclear cells move into the damaged area followed by lymphocytes and macrophages. The inflammatory reaction is further enhanced by activation of platelets and the blood-clotting cascade resulting in the release of a variety of growth factors (G), proteases, arachidonic acid metabolites, 5-hydroxytryptamine and lectins. Initiation of the classic complement pathway also helps in the chemo-attraction of neutrophils, monocytes and macrophages. Neutrophils, monocytes and tissue macrophages help eliminating infection by phagocytosing bacteria, and participate in the decontamination of the wound including scavenging of tissue debris. In addition, these cells secrete various growth factors which stimulate cell migration, proliferation and the production of matrix and matrix-degrading enzymes (G).

Activation of the Fibroblast

The formation of granulation tissue is heralded by the migration of fibroblasts, epidermal cells, macrophages and other inflammatory cells to the wound site, accompanied by the ingrowth of blood vessels (H).
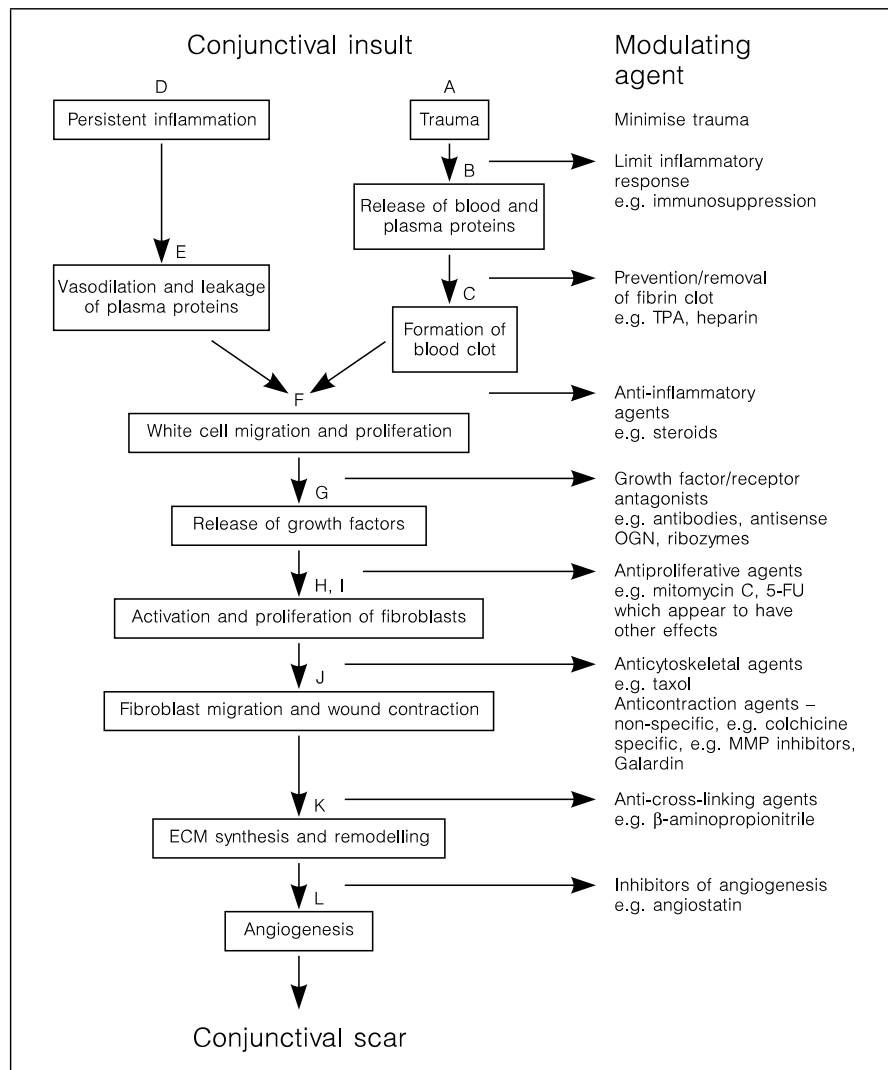
Fig. 1. The molecular and cellular events leading to conjunctival scarring, and modulating agents of the wound-healing response. OGN = Oligonucleotides.

However, it is the fibroblast that is the key cell involved not only in the formation of granulation tissue but in many other aspects of the wound-healing response. Normally, the fibroblast exists within the subconjunctival connective tissue as a quiescent undifferentiated mesenchymal cell, the fibrocyte. Although it was previously believed that blood-derived cells can

repopulate areas by transforming into fibroblasts, there is no good evidence of this. Instead, it is primarily the activation and recruitment of local fibrocytes which are found in low numbers throughout connective tissue, that is responsible for the scarring response [3] – without the conjunctival fibroblast, conjunctival scarring would not be significant. The fibrocyte converts to the active fibroblast which is then capable of carrying out the various cellular functions responsible for wound healing when stimulated by different factors in the environment.

There are a range of patient risk factors that seem to influence the activation of fibroblasts and need to be taken into account when considering antiscarring treatments. Studies at Moorfields Eye Hospital by Broadway et al. [5] upon patients undergoing glaucoma filtration surgery, showed that patients receiving topical treatments prior to surgery exhibited a greater degree of failure compared to those that received none, even though the increase in the local fibroblast population was relatively small. Subsequent studies in our laboratory using short, single applications of antiproliferative agents suggested that the fibroblasts populating the conjunctivae of these patients were more 'activated' and were capable of carrying out a proportion of their scarring response prior to inhibition by these agents, compared to 'non-activated' cells which were inhibited to a greater degree [6]. Broadway et al. [7] then showed clinically that removal of topical treatment prior to surgery (called reversal therapy) correlated with an increased surgical success rate. These findings suggest that the degree of cellular 'activation' prior to initiation of a scarring response is an extremely important factor in the modification of the degree of cicatrisation occurring following injury.

As the fibroblast is the key cell involved in the wound-healing response, carrying out a number of crucial functions, work in our and many other laboratories has concentrated on understanding the mechanisms underlying the regulation of fibroblast function. These functions include proliferation at the wound site (I), migration to and contraction of the wound (J), the synthesis of new extracellular matrix components and finally remodeling (K) of this new matrix to produce a scar.

Fibroblast Proliferation

Proliferation of fibroblasts is necesary so that sufficient numbers of cells are produced to allow the various wound-healing processes to occur in a relatively short time. This process peaks within the first two weeks after the initial insult [8–10], and is regulated by a number of growth factors. Work in our laboratory investigating the stimulation of proliferation of Tenon's capsule fibroblasts by growth factors including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor-β_1 (TGF-β_1) and

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insulin-like growth factor 1 (IGF-1) showed that $TGF\beta_1$ was the most potent factor, stimulating proliferation at much lower concentrations (10^{-12} M) and to a greater degree (2–3 times greater) than the other growth factors. These cells then eventually disappear as the healing process resolves. The mechanisms of this resolution are still unknown, although apoptosis has been suggested as one possible mechanism [11].

Fibroblast Migration and Wound Contraction

The migration of fibroblasts through their surrounding extracellular matrix to the wound site is stimulated by a number of factors including complement component C5a [12], leukotriene, extracellular matrix components such as fibronectin [13], elastin and collagen fragments [14, 15], growth factors such as platelet-derived growth factor (PDGF), TGF-β and FGF [16]. These factors are derived locally from damaged tissue, blood, inflammatory cells such as macrophages and lymphocytes, aqueous humour and from the fibroblast themselves [17]. Our laboratory has previously investigated the role of several growth factors on the migration of corneal fibroblasts and has more recently investigated the effects of different concentrations of various growth factors over a range of concentrations (10^{-7}–10^{-12} M) including EGF, basic FGF, TGF-β_1, and IGF-1. We found that all four growth factors stimulated migration but that TGF-β_1 maximally stimulated this process at much lower concentrations (10^{-12} M) compared to the other growth factors (10^{-10}–10^{-8} M) [3].

The contraction of collagen-containing tissues, such as the conjunctiva, is regarded as resulting from the tractional forces exerted by cells migrating upon their substratum [18, 19] as shown in figure 2a. We and many other groups have been using an in vitro model of collagen contraction described by Bell et al. [20] in which fibroblasts are entrapped within a three-dimensional collagen matrix, which they re-organise and contract over a period of several days (fig. 2b). Although this contractile process has been shown to be dependent upon several factors including cell number, an intact actin cytoskeleton, collagen concentration, protein synthesis and attachment of the cells to their surrounding matrix [21–26], the exact mechanisms underlying collagen contraction are currently unclear [27].

Extracellular Matrix Production and Remodelling

Scarring is a result of the deposition and contraction of the ECM and is defined as abnormal collagen organization (relative to the surrounding tissue) following an injury. However, the normal healing response involves careful remodelling of the ECM. It is a dynamic process involving both the synthesis and degradation of matrix proteins (K). If this does not occur optimally, then either too much or too little scar tissue is laid down, which may cause clinical

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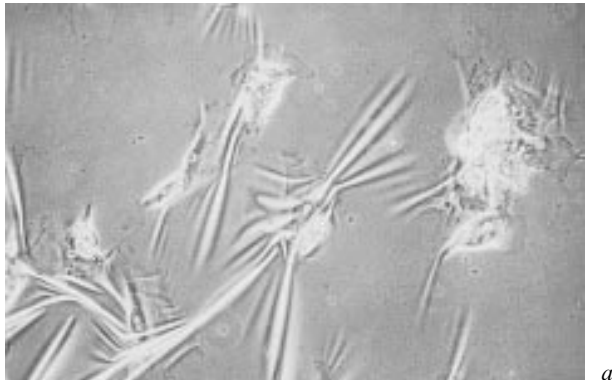
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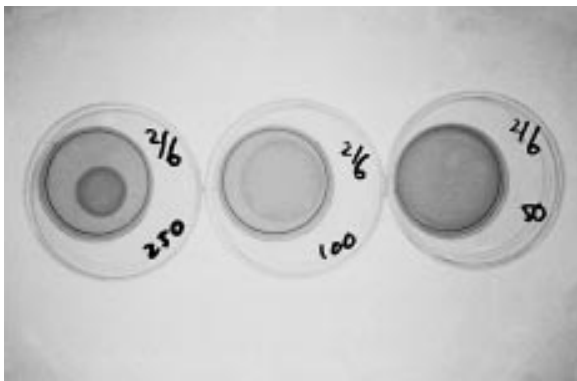
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Fig. 2. *a* Fibroblasts on a silicon sheet producing tractional lines as they migrate across sheet and cause contraction. *b* Collagen gels. In vitro model of wound contraction consisting of a three-dimensional collagen matrix in which fibroblasts are seeded. Fibroblasts cause the collagen gels to contract over a period of days.

disease. Remodelling can occur up to several years after an initial insult, although most of this activity takes place in the first few months after injury. It is dependent on the effects of various inhibitory and stimulatory factors affecting the synthesis and degradation of ECM.

Fibroblasts produce a number of ECM components including fibronectin, glycosaminoglycans and tropocollagen which is ultimately enzymatically cross-linked to form collagen. Initially, a fibrin scaffold is produced at the wound site, followed by the production of type III collagen which is then finally replaced by type I collagen. Type I collagen is the major component of scar tissue and its production is a prominent feature of both the acute and late phases of response to injury in human and experimental studies. Type I collagen

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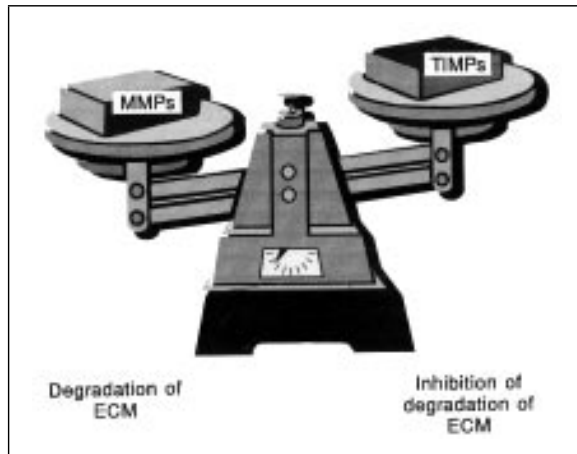
Fig. 3. Extracellular matrix degradation showing the balance between two systems.

is laid down in large parallel bundles lying perpendicular to the basement membrane providing tensile strength to the mature wound [28] and a visible scar. The production of extracellular matrix is, as for migration and proliferation, regulated by growth factors. We have found, using ^{3}H-proline uptake, that TGF-β_1 again was the most potent stimulator causing 2–3 times more collagen production than the other factors at a concentration of 10^{-6} M.

Following ECM synthesis, the new connective tissue produced during the wound-healing process is then enzymatically remodelled continuously for several months [29] with continued synthesis and breakdown of the ECM. The enzymes involved in the degradation of the ECM are primarily the matrix metalloproteinases (MMPs) which are a large family of enzymes consisting of several members that are regulated by the tissue inhibitors of matrix metallo-proteinases (TIMPs) [30, 31]. The actual degree of ECM present at the wound site is dependent upon the balance between the activity of the MMPs and TIMPs (fig. 3), which in turn are regulated by several chemical mediators.

Modulation of the Wound-Healing Response

The processes regulating scarring, as illustrated above, are both numerous and complex. The modulation of the healing and the scarring response can occur at various points which are represented in figure 1. Although many antiscarring therapies have been available for several years, it is only recently
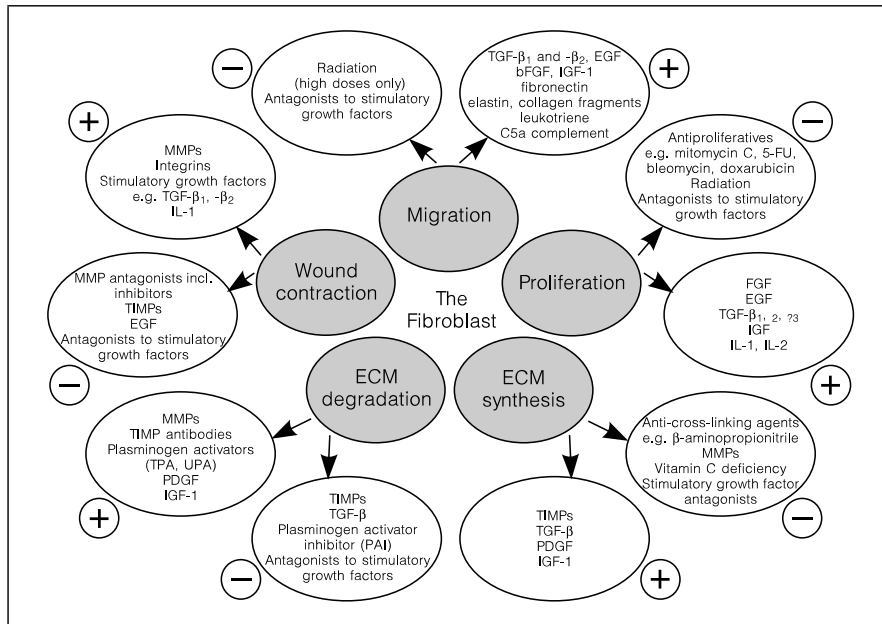
Fig. 4. Modulation of individual fibroblast functions.

that expansion in our knowledge of the mechanisms underlying these processes not only in adult but also in the development of the early foetus in the womb (which does not scar significantly), that the refinement of existing therapies and the development of potentially new therapies not only for ocular scarring but also scarring at other sites throughout the body have been achieved. These advances in the understanding of the scarring response, agents available for modifying the scarring response, their modes of action, and new and potential methods for modifying the scarring response are discussed below. In addition to the effects of modifying agents on the fibroblast itself (fig. 4), other important considerations are the length, degree and types of stimulation (e.g. growth factors) present in the surrounding wound area. This stimulation can modify the overall profile of healing despite fibroblast modulation with the same treatment.

Many agents are currently and potentially available for the modulation of the scarring response, several of which are highlighted below.

Immunosuppressive and Anti-Inflammatory Agents
Steroidal and other immunosuppressive drugs are believed to alter the healing response by depressing the local inflammatory response. This is prima-

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rily via their actions on neutrophils, lymphocytes and monocytes. Corticosteroids, in particular, impair inflammatory cell chemotaxis, inhibit angiogenesis, and decrease fibroblast proliferation and matrix synthesis [32]. Roth et al. [33] demonstrated a significant reduction in final intraocular pressure in a randomised trial of topical steroid treatment following glaucoma filtration surgery. However, the effect of non-steroidal anti-inflammatory drugs (NSAIDs) is less well established, with little benefit being demonstrated by Migdal and Hitchings [34] on trabeculectomy outcome following antiprostaglandin administration.

Systemic chemotherapy is extremely important in auto-immune diseases. However, the side effects of systemic treatment are considerable, and patients need careful management. Cyclophosphamide and dapsone have been shown to be important immunosuppressives in the management of chronic conjunctival cicatrisation [35, 36].

Fibrinolytic Agents

Prevention of clot formation may be achieved with local administration of heparin. However, once a blood clot has formed, fibrinolysis may be achieved using local administration of tissue plasminogen activator (TPA). TPA has been shown to be particularly useful in glaucoma filtration surgery in dogs [Bedford, pers. commun.; 37], where often, blockage of the filtering system occurs in the early days following surgery, because of a severe fibrinous reaction.

Agents Affecting Growth Factors and Growth Factor Receptors

As has been discussed earlier in this chapter, and shown by many other studies, the processes involved in the scarring response are under the control of a number of growth factor proteins (fig. 5). Consequently, these growth factors themselves as well as the growth factor receptors through which their effects are mediated, present themselves as potential targets for therapeutic intervention. One growth factor family in particular, the TGFβ family, has been shown to play a pivotal role not only in scarring in the eye but also at other sites throughout the body.

Figure 6 illustrates various mechanisms in which the actions of growth factors such as TGF-β may be antagonised. Several of these potential antiscarring therapies are now entering clinical trials.

The effects of neutralising antibodies to TGF-β and receptor proteins have been convincingly demonstrated as reducing scarring [38, 39]. Adult rat cutaneous wounds in particular heal with less scar tissue when treated with antibodies to TGF-β_1 and β_2 [40]. In addition to antibodies, recent work has shown the importance of receptors such as the mannose 6-phosphate/IGF

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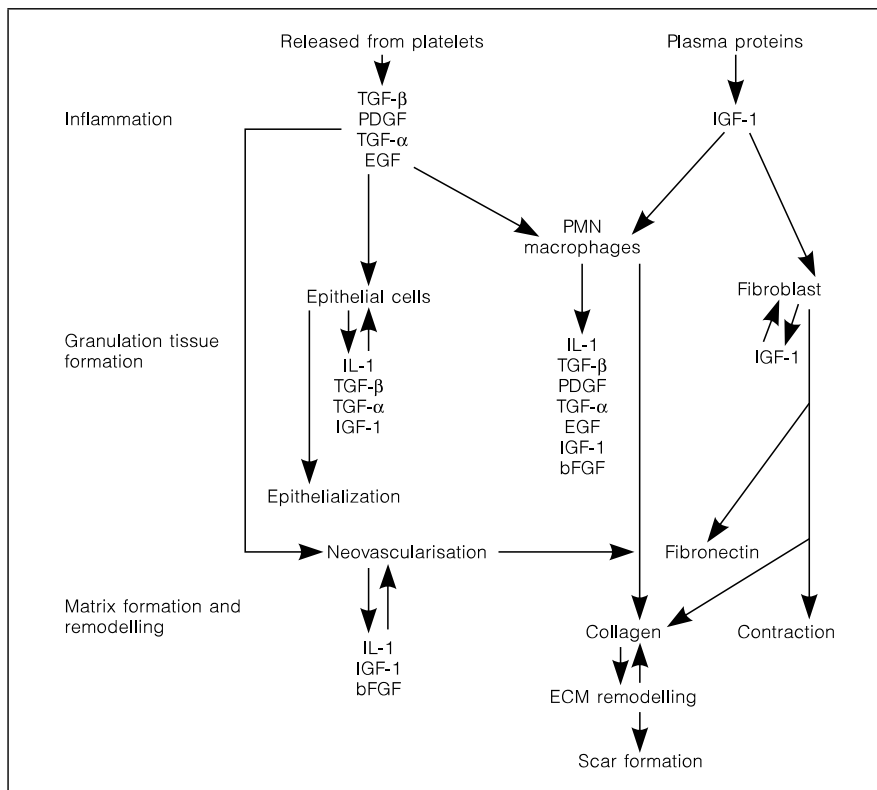
Fig. 5. The role of growth factors in the wound-healing response.

type II receptor. Latent TGF-β on binding to this receptor is activated to mature TGF-β [41]. Ferguson's group [pers. commun.] has demonstrated that the addition of the sugar mannose-6-phosphate (which competitively binds to the mannose 6-phosphate/IGF type II receptor) to a cutaneous wound reduces the scarring response by inhibiting activation of latent TGF-β.

Antisense oligonucleotides are synthetic molecules that bind to specific intracellular messenger RNA strands (mRNA). These mRNA strands code for the production of specific proteins [42–44]. By binding to the mRNA molecules, antisense oligonucleotides stop transcription of the mRNA, and hence synthesis of the protein. Ribozymes are RNA molecules that have recently been demonstrated to have catalytic activity on other RNA molecules [45–47]. They are able to enzymatically cleave specific bonds. Hence, if mRNA of a specific protein is cleaved by a ribozyme, inhibition of transcription and protein production occurs. Like antisense oligonucleotides, ribozymes can be

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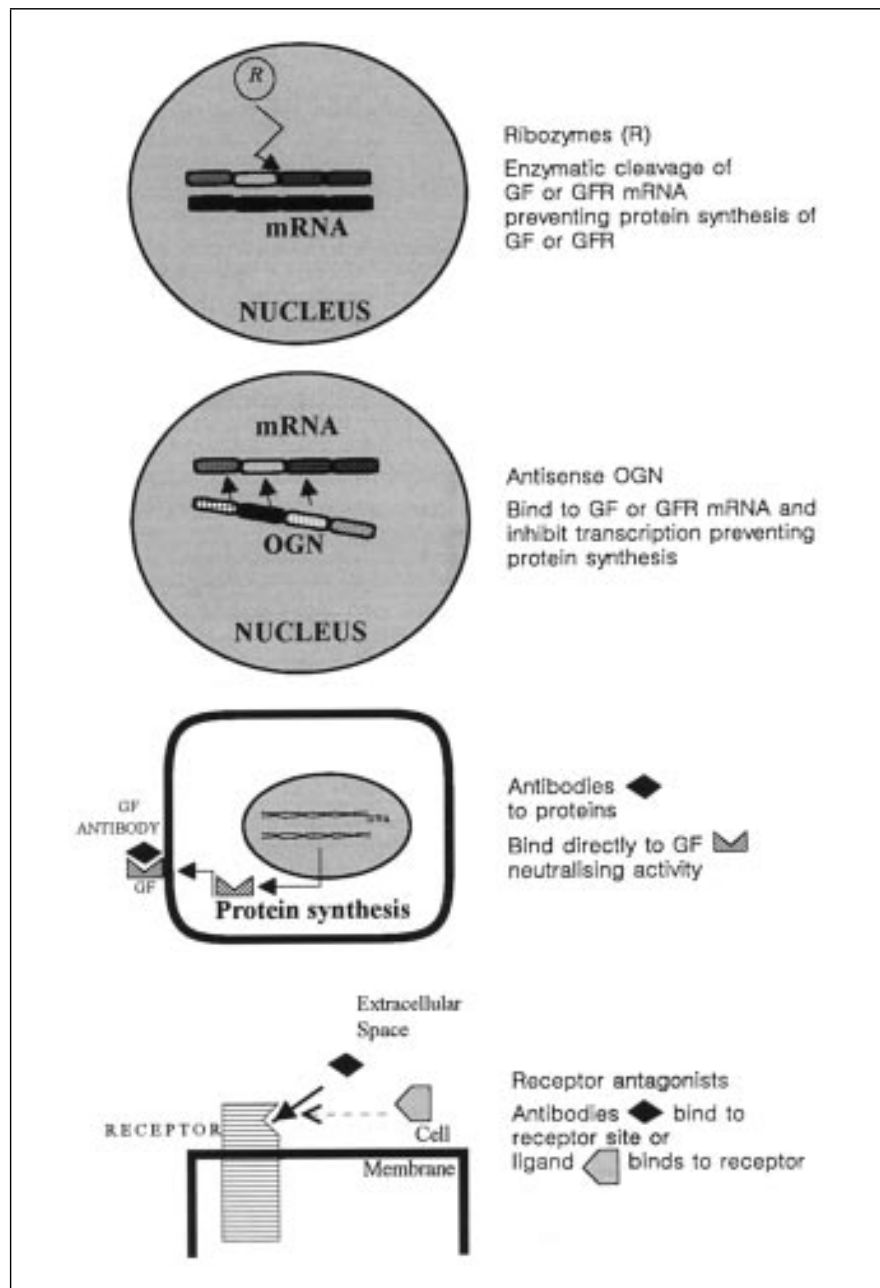
Fig. 6. Antagonists of growth factor (GF) and growth factor receptors (GFR) using TGF-β as an example. OGN = Oligonucleotides.

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designed to 'attack' any message, but its significant advantage compared to antisense technology is that the effects of this molecule are not concentration and time dependent. This is because the ribozyme is able not only to destroy a particular message but also has the capacity to re-attach to and destroy other messages of the same sequence many many times. The use of ribozymes as RNA-enzyme-directed gene therapies [48, 49] has been clearly shown. Their future in the management of ocular diseases is still to be investigated, although our group is in the midst of studying a ribozyme directed against mRNA of TGF-β_1 and the TGF-β II receptor proteins. Like antisense oligonucleotides, ribozymes may represent an exciting new mode of specific, focal and titratable treatment.

Antimigration/Anticytoskeletal Agents

A great deal of research has been carried out on methods to directly or indirectly modulate various aspects of fibroblast function. The majority of these methods have been used in modulating the healing response of the conjunctival fibroblast following glaucoma surgery [2, 50] attempting to target specific cell functions such as migration. Examples are colchicine, cytochalasin b and particularly taxol (affecting the cytoskeleton of the cell) which were all shown to inhibit the migration of fibroblasts to rabbit aqueous humour [51]. Taxol has been shown to prolong to survival of filtration surgery in an aggressive model of filtration surgery [52]. However, many of these 'specific' agents in fact have effects on other fibroblast functions: for instance, taxol also inhibits cell proliferation.

A particularly vital function of the fibroblast in the scarring response, the mechanisms of which are not currently fully understood, is its ability to contract ECM. We have been using type I collagen gels populated with ocular fibroblasts as an in vivo model of wound contraction. The fibroblasts rapidly contract these collagen gels when stimulated with growth factors, but upon 5-min exposures to 5-fluorouracil or mitomycin C, this process is significantly inhibited [6] without extensive cell death. The fact that this contractile process is not dependent upon cell proliferation and that the cells appeared to remodel their surrounding ECM during this process suggested to us that the MMPs may be involved in this process of contraction. Our work on this process of collagen contraction has shown that the MMPs are essential for the contraction of the ECM and can be inhibited using MMP inhibitors (e.g. Galardin™-MPI and BB-94). This mechanism may be a ubiquitous requirement not only in ocular tissues but at other sites throughout the body and also between different species. These findings may have therapeutic implications as these MMP inhibitors are specific, non-toxic and act reversibly enabling control of the degree of contraction that occurs.

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Antiproliferative Agents

Much antiscarring research has centred on the use of agents that suppress cell division (antiproliferatives). In 1984, Blumenkranz and colleagues [53, 54] established that various antiproliferative drugs, in particular 5-fluorouracil inhibited the proliferation of fibroblasts during a period of continous drug exposure in cell culture and prevented epiretinal scarring in the eye in an animal model. Stimulated by this initial research, a regimen involving subconjunctival injections of 5-fluorouracil was developed at Miami, culminating in the multicentre 5-fluorouracil filtering surgery trial [55]. Following these studies, extensive investigation has also been carried out looking at the effects of 5-fluorouracil and many other antiproliferative agents on ocular fibroblasts in culture in an attempt to find an optimal agent [56–63], and also to develop an optimal delivery system that does not require multiple injections [64–69]. However, largely unnoticed Chen et al. [70] and Chen [71] had been using single applications of another antiproliferative agent, mitomycin C, for more than a decade. One of the reasons for the initial reluctance to use this regimen was the lack of knowledge as to how a single application of these agents could suppress fibroblast proliferation adequately over the period of several weeks, during the period of maximal fibroblast proliferation. We have shown that several antiproliferative agents have long-term effects on the proliferation of ocular fibroblasts, even when the time of exposure is as short as 5 min [72, 73]. Given the appropriate concentration and agent, effective suppression of proliferation can be achieved for periods of up to 36 days without significant cell death [72, 74]. To our surprise, this long-term suppression even occurred with 5-fluorouracil, as the inhibitory action on DNA synthesis through thymidylate synthetase should have been rapidly reversible. However, like many other drugs, 5-fluorouracil has secondary effects including interference with RNA synthesis and these may explain the longer-term actions. We have also subsequently shown that this long-term suppression of proliferation occurs in an in vivo experimental model of glaucoma filtration surgery. It appears to be titratable in terms of length of action [75, 76] and is also focal in that only the fibroblasts in the treated areas are affected [77, 78].

On a more molecular level, our work has also shown that Tenon's capsular fibroblasts exhibit an increase in RNA levels of collagen, lysyl oxidase and TGF-β_1 when exposed to increasing concentrations of TGF-β_1. We have also found that the 'suppressed' growth arrested fibroblasts are still able to respond to stimulatory factors such as TGF-β_1 by upregulating levels of collagen and TGF-β_1 itself. The levels of lysyl oxidase were, however, much lower, although there was still an increase in RNA levels with higher concentrations of TGF-β_1. So cells, although growth-arrested appear to be still able to respond to exogenous stimulation. Based on these studies, we have performed pilot studies

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in vivo on filtration surgery drainage blebs induced by mitomycin C. When the blebs are injected with TGF-β_1 a healing response is generated which begins to reverse the effects of the mitomycin C. Thick scar tissue develops surrounding the bleb and the bleb begins to opacify and shrink compared to a control injection of carrier only. We are currently investigating the cellular and molecular mechanism of this scarring. However, what this pilot experiment confirms is that the local suppression of fibroblasts could possibly be reversed by growth factors in the wound environment.

The use of these new single, short exposure regimens has now seen an exponential increase in popularity over the last few years because of its effectiveness and ease of application. However, although we know we can adequately suppress fibroblast proliferation in a relatively titratable manner with practical single application treatments, we still need to know more about the individual healing responses, and what functions the suppressed fibroblast is able to carry out under these conditions.

Agents Affecting Extracellular Matrix Synthesis and Degradation

Examples of agents affecting ECM synthesis include lathyrogenic agents such as β-aminopropionitrile which prevents collagen cross-linking by inhibiting the enzyme lysyl oxidase [79]. There is experimental and clinical evidence that this may work [80–82]. Direct antagonism of MMPs and TIMPs with chemical inhibitors, neutralising antibodies, antisense oligonucleotides and ribozymes may in future be possible. In addition, inhibitors and antagonists of growth factors which have different effects on MMPs and TIMPs, offer further potential modes of therapy.

Angiogenesis Inhibitors

Angiogenesis is important in the formation of granulation tissue. Most of the advances that have been made in understanding the process of angiogenesis, are from its study in tumour progression. Studies have revealed that there are important regulators of endothelial growth control [83–85], and from these, inhibitors of angiogenesis have been isolated, such as inhibitors of the growth factors vascular endothelial growth factor, vasoproliferative factor and fibroblast growth factor. New inhibitors are constantly being discovered, e.g. angiostatin, urokinase receptor antagonists, scatter factor inhibitors [86]. Their use in modulating the wound-healing response is still to be established.

β-Irradiation

One physical agent that has been shown to be effective in preventing conjunctival scar tissue formation is irradiation. At Moorfields Eye Hospital, since 1980, most patients with congenital glaucoma have received β-radiation

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at the time of glaucoma filtration surgery with favourable results [87, 88]. Specifically, single doses of irradiation do not cause thin cystic blebs, as seen with mitomycin C, and have the advantage that the treated area can be controlled very precisely. A single application of β-irradiation leads to long-term inhibition of fibroblast proliferation [73]. It has long been used in the treatment of pterygium [89, 90] and in certain European centres, irradiation was the method of choice for cicatricial pemphigoid. However, high doses of irradiation result in severe complications such as scleral necrosis and ocular infection [91].

New Developments and the Future

In summary, we now have a huge variety of methods to suppress fibroblast function. Modulation of scarring using the single exposures to antiproliferative agents are at present the most practical method of achieving fibroblast suppression after glaucoma filtration surgery, but the applications of these modulating agents to treat established conjunctival cicatrisation is still to be assessed. From basic research we now understand a great deal more about how these treatments work in vitro and in vivo. We have also illustrated several new developments in both the understanding and potential modification of the scarring response. These include highlighting the importance of the state of activation of local tissue fibroblasts; the stimulatory factors that can have profound effects on the healing response, even when mediated by growth arrested cells; the potential therapeutic uses of agents which modify growth factors and the cellular responses to them; and the uses of MMP inhibitors in reducing scar tissue formation and contraction. A deeper understanding of the basic cellular and molecular biology of the healing process, variations in different individuals and circumstances, the effects of modulating agents on the fibroblast and application of developments from other areas in wound healing research are essential. Only then will we be able to achieve totally safe but effective control of the scarring process with retention of tissue function, not just in the conjunctiva but in the whole human body.

References

1   Clark RAF, Henson PM: The Molecular and Cellular Biology of Wound Repair. New York, Plenum Press, 1988.
2   Tahery MM, Lee DA: Review: Pharmacologic control of wound healing in glaucoma filtration surgery. J Ocul Pharmacol 1989;5:155–179.
3   Khaw PT, Occleston NL, Schultz GS, Grierson I, Sherwood MB, Larkin G: Activation and suppression of fibroblast activity. Eye 1994;8:188–195.

4

4   Kirsner RS, Eaglstein WH: The wound healing process. Wound Healing 1993;11:629–640.

5   Broadway DC, Grierson I, Hitchings RA: Adverse effects of topical anti-glaucomatous medications on the conjunctiva. Br J Ophthalmol 1993;77:590–596.

6   Occleston NL, Alexander RA, Mazure A, Larkin G, Khaw PT: Effects of single exposures to antiproliferative agents on ocular fibroblast mediated collagen contraction. Invest Ophthalmol Vis Sci 1994;35:3681–3690.

7   Broadway DC, Grierson I, O'Brien C, Hitchings RA: Adverse effects of topical anti-glaucomatous medication regimens. I. Effect on the cell profile of the conjunctiva. Arch Ophthalmol 1994;112: 1437–1445.

8   Ross R, Odland G: Human wound repair. II. Inflammatory cells, epithelial mesenchymal interrelations and fibrogenesis. J Cell Biol 1968;39:152–168.

9   Miller MH, Grierson I, Unger WI, Hitchings RA: Wound healing in an animal model of glaucoma fistulizing surgery in the rabbit. Ophthalmic Surg 1989;20:350–357.

10   Regan EF: Scleral cautery with iridectomy – An experimental study. Trans Am Ophthalmol Soc 1963;61:219–231.

11   Desmoulière A, Redard M, Darby I, Gabbiani G: Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. Am J Pathol 1995;146:56–66.

12   Postlethwaite AE, Snyderman RK, Kang AH: Generation of a fibroblast chemotactic factor in serum by activation of complement. J Clin Invest 1979;64:1379–1385.

13   Liotta LA, Stetler-Stevenson WG: Metalloproteinases and cancer invasion. Semin Can Biol 1990; 1:99–106.

14   Postlethwaite AE, Seyer JM, Kang AH: Chemotactic attraction of human fibroblasts to type I-II-III collagens and collagen-derived peptides. Proc Natl Acad Sci USA 1978;75:871–875.

15   Postlethwaite AE, Kang AH: Collagen and collagen peptide-induced chemotaxis of human blood monocytes. J Exp Med 1976;143:1299–1307.

16   Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH: Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor beta. J Exp Med 1987;165:251–256.

17   Albrink WS, Wallace AC: Aqueous humor as a tissue culture nutrient. Proc Soc Exp Biol Med 1951;77:754–758.

18   Hauviller V: Gonioscopic findings in trabeculectomies in young children. J Pediatr Ophthalmol Strabismus. 1989;26:133–135.

19   Ehrlich HP, Rajaratnam JBM: Cell locomotion forces versus cell contraction forces for collagen lattice contraction: An in vitro model of wound healing. Tissue Cell 1990;22:407–417.

20   Bell E, Ivarsson B, Merrill C: Production of a tissue-like structure by contraction of collagen lattice by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci USA 1979;76: 1274–1278.

21   Ehrlich HP, Wyler DJ: Fibroblast contraction of collagen lattices in vitro: Inhibition by chronic inflammatory cell mediators. J Cell Physiol 1983;116:345–351.

22   Pitaru S, Soldinger M, Madgar D, Metzger Z: Bacterial endotoxin inhibits migration, attachment, and orientation of human gingival fibroblasts in vitro and delays collagen gel contraction. J Dent Res 1987;66:1449–1455.

23   Guidry C: Fibroblast contraction of collagen gels requires activation of protein kinase C. J Cell Physiol 1993;155:358–367.

24   Hunt RC, Pakalnis VA, Choudhury P, Black EP: Cytokines and serum cause alpha2beta1 integrin-mediated contraction of collagen gels by cultures retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 1994;35:955–963.

25   Wilson SE, Schultz GS, Chegini N, Weng J, He Y: Epidermal growth factor, transforming growth factor alpha, transforming growth factor beta, acidic fibroblast growth factor, basic fibroblast growth factor and interleukin-1 proteins in the cornea. Exp Eye Res 1994;59:63–72.

26   Nishiyama T, Tominaga N, Nakajima K, Hayashi T: Quantitative evaluation of the factors affecting the process of fibroblast-mediated collagen gel contraction by separating the process into three phases. Coll Relat Res 1988;8:259–273.

27   Grinnell F: Mini-review on the cellular mechanisms of disease: Fibroblasts, myofibroblasts and wound contraction. J Cell Biol 1994;124:401–404.

4

28 Thomas DW, O'Neill ID, Harding KG, Shepherd JP: Cutaneous wound healing: A current perspective. J Oral Maxillofac Surg 1995;53:443–447.

29 Peacock E: Wound Repair. Philadelphia, Saunders, 1984, pp 102–140.

30 Woessner JFJ: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991;5:2145–2154.

31 Matrisian LM: The matrix-degrading metalloproteinases. Bioessays 1992;14:455–463.

32 Wahl SM: Glucocorticoids and wound healing; in Scleimer RP, Claman HN (eds): Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. New York, Academic Press, 1989, pp 280–302.

33 Roth SM, Starita RJ, Spaeth GL, Steinmann WC, Paryzees EM: The effects of postoperative corticosteroids on trabeculectomy: Long-term follow-up. Invest Ophthalmol Vis Sci 1988;29(suppl):367.

34 Migdal C, Hitchings R: The developing bleb: Effect of topical antiprostaglandins on the outcome of glaucoma fistulizing surgery. Br J Ophthalmol 1983;67:655–660.

35 Elder MJ, Lightman S, Dart JK: Role of cyclophosphamide and high dose steroid in ocular cicatricial pemphigoid. Br J Ophthalmol 1995;79:264–266.

36 Fern A, Jay JL, Young H, Mackie R: Dapsone therapy for the acute inflammatory phase of ocular pemphigoid. Br J Ophthalmol 1992;76:332–335.

37 Snyder RW, Lambrou FH, Williams GA: Intraocular fibrinolysis with recombinant human tissue plasminogen activator. Experimental treatment in a rabbit model. Arch Ophthalmol 1987;105:1277–1280.

38 Shah M, Foreman DM, Ferguson MWJ: Control of scarring in adult wounds by neutralising antibody to transforming growth factor beta. Lancet 1992;339:213–214.

39 Shah M, Foreman DM, Ferguson MWJ: Neutralising antibody to TGF-beta1,2 reduces cutaneous scarring in adult rodents. J Cell Sci 1994;107:1137–1157.

40 Shah M, Foreman DM, Ferguson MWJ: Neutralisation if TGF-beta1 and TGF-beta2 or exogenous addition of TGF-beta3 to cutaneous rat wounds reduces scarring. J Cell Sci 1995;108:985–1002.

41 Dennis PA, Rifkin DB: Cellular activation of latent transforming growth factor b requires binding to the cation-independent mannose-6-phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci USA 1991;88:580–584.

42 Wu-Pong S, Weiss TL, Hunt CA: Antisense c-*myc* oligonucleotide cellular uptake and activity. Antisense Res Dev 1994;4:155–163.

43 Beardsley T: Making antisense. Sci Am 1992;266:107–108.

44 Fakler B, Herlitze S, Amthor B, Zenner H, Ruppersberg J: Short antisense oligonucleotide-mediated inhibition is strongly dependent on oligo length and concentration but almost independent of location of the target sequence. J Biol Chem 1994;269:16187–16194.

45 Wilson C, Szostak JW: In vitro evolution of a self-alkylating ribozyme. Nature 1995;374:777–782.

46 Bartel DP, Szostak JW: Isolation of new ribozymes from a large pool of random sequences. Science 1993;261:1411–1418.

47 Moore MJ: Exploration by lamp light. Nature 1995;374:766–767.

48 Altman S: RNA enzyme-directed gene therapy. Proc Natl Acad Sci USA 1993;90:10898–10900.

49 Dorai T, Kobayashi H, Holland JF, Ohnuma T: Modulation of platelet-derived growth factor-beta mRNA expression and cell growth in a human mesothelioma cell line by a hammerhead ribozyme. Mol Pharmacol 1994;46:437–444.

50 Khaw PT, Occleston NL, Larkin G, Shad H, Schultz G, Grierson I, Grant MB: The effects of growth factors on human ocular fibroblast proliferation, migration and collagen production. Invest Ophthalmol Vis Sci 1994;35(suppl):1898.

51 Metcalfe RA, Weetman AP: Stimulation of extraocular muscle fibroblasts by cytokines and hypoxia: Possible role in thyroid-associated ophthalmology. Clin Endocrinol 1994;40:67–72.

52 Jampel HD, Leong KW, Koya P, Quigley HA: The use of hydrophobic drugs incorporated into polyanhydrides in experimental glaucoma surgery. Invest Ophthalmol Vis Sci 1990;31(suppl):2.

53 Blumenkranz MS, Ophir A, Claflin AJ, Hajek AS: Flourouracil for the treatment of massive periretinal proliferation. Am J Ophthalmol 1982;94:458–467.

54 Blumenkranz MS, Hartzer MK, Hajek AS: Selection of therapeutic agents for intraocular proliferative disease. Arch Ophthalmol 1987;105:396–399.

4

55   The Fluorouracil Filtering Surgery Study Group: Fluorouracil filtering surgery study one-year follow-up. Am J Ophthalmol 1989;108:625–635.

56   Lee DA, Shapourifar-Tehrani D, Kitada S: The effect of 5-fluorouracil and cytarabine on human fibroblasts from Tenon's capsule. Invest Ophthalmol Vis Sci 1990;31:1848–1855.

57   Litin BS, Herschler J: Silver staining of human aqueous humour proteins resolved by gel electrophoresis. Graefes Arch Clin Exp Ophthalmol 1984;221:290–292.

58   Schmidt JA, Mizel SB, Cohen D, Green I: Interleukin-1, a potential regulator of fibroblast proliferation. J Immunol 1982;128:2177.

59   Lee DA, Shapourifar-Tehrani S, Stephenson TR, Kitada S: The effects of the fluorinated pyrimidines FUR, FUdR, FUMP, and FdUMP on human tenon's fibroblasts. Invest Ophthalmol Vis Sci 1991; 32:2599–2609.

60   Gillies M, Su T, Sarossy M, Hollows F: Interferon-alpha 2b inhibits proliferation of human Tenon's capsule fibroblasts. Graefes Arch Clin Exp Ophthalmol 1993;231:118–121.

61   Maity A, McKenna WG, Muschel RJ: The molecular basis for cell cycle delays following ionizing radiation: A review. Radiat Ther Oncol 1994;31:1–13.

62   Blumenkranz MS, Hartzer MK, Hajek AS: Selection of therapeutic agents for intraocular proliferative disease. II. Differing antiproliferative activity of the fluoropyrimidines. Arch Ophthalmol 1987; 105:396–399.

63   Senderoff RI, Weber PA, Smith DR, Sokoloski TD: Evaluation of antiproliferative agents using a cell culture model. Invest Ophthalmol Vis Sci 1990;31:2572–2578.

64   Skuta GL, Assil K, Parrish RKI, Folberg R, Weinreb RN: Filtering surgery in owl monkeys treated with the antimetabolite 5-fluorouridine 5′-monophosphate entrapped in multivesicular liposomes (letter). Am J Ophthalmol 1987;103:714–715.

65   Kay JS, Litin BS, Jones MA, Fryczkowski AW, Chvapil M, Herschler J: Delivery of antifibroblast agents as adjuncts to filtration surgery. II. Delivery of 5-fluorouracil and bleomycin in a collagen implant: Pilot study in the rabbit. Ophthalmic Surg 1986;17:796–801.

66   Sachdev S, Zou X, Higginbotham E: The effect of 5-fluorouracil impregnated collagen shield implants in filtration surgery. Invest Ophthalmol Vis Sci 1990;31(suppl):3.

67   Lee DA, Flores RA, Anderson PJ, Leong KW, Teekheoscoree C, de Koder AW, Hestzmark E: Glaucoma filtration surgery in rabbits using bioeradicable polymers and 5-fluorouracil. Ophthalmology 1987;94:1523–1530.

68   Jampel HD, Leong KW, Dunkelburger GR, Quigley HA: Glaucoma filtering surgery in monkeys using 5-fluorouridine in polyanhydride discs. Arch Ophthalmol 1990;108:430–435.

69   Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–159.

70   Chen C, Huang H, Bair JS, Lee C: Trabeculectomy with simultaneous topical application of mitomycin-c in refractory glaucoma. J Ocul Pharmacol 1990;6:175–182.

71   Chen CW: Enhanced intraocular pressure controlling effectiveness of trabeculectomy by local application of mitomycin c. Trans Asia Pacif Acad Ophthalmol 1983;9:172–177.

72   Khaw PT, Sherwood MB, MacKay SLD, Rossi MJ, Schultz GS: Five-minute treatments with fluorouracil, floxuridine, and mitomycin have long-term effects on human Tenon's capsule fibroblasts. Arch Ophthalmol 1992;110:1150–1154.

73   Khaw PT, Ward S, Grierson I, Rice NSC: The effects of beta-radiation on proliferating human Tenon's capsule fibroblasts. Br J Ophthalmol 1991;75:580–583.

74   Khaw PT, Ward S, Porter A, Grierson I, Hitchings RA, Rice NSC: The long-term effects of 5-fluorouracil and sodium butyrate on human Tenon's fibroblasts. Invest Ophthalmol Vis Sci 1992; 33:2043–2052.

75   Doyle JW, Sherwood MB, Khaw PT, McGorray S, Smith MF: Intraoperative 5-fluorouracil for filtration surgery in the rabbit. Invest Ophthalmol Vis Sci 1993;34:3313–3319.

76   Khaw PT, Doyle JW, Sherwood MB, Smith FM, McGorray S: Effects of intraoperative 5-fluorouracil or mitomycin C on glaucoma filtration surgery in the rabbit. Ophthalmology 1993;100:367–372.

77   Khaw PT, Doyle JW, Sherwood MB, Grierson I, Schultz GS, McGorray S: Prolonged localized tissue effects from 5-minute exposures to fluorouracil and mitomycin C. Arch Ophthalmol 1993; 111:263–267.

4

78   Khaw PT, Sherwood MB, Doyle JW, Smith MF, Grierson I, McGorray S, Schultz GS: Intraoperative and postoperative treatment with 5-fluorouracil and mitomycin-C: Long-term effects in vivo on subconjunctival and scleral fibroblasts. Int Ophthalmol 1992;16:381–385.
79   Siegel RC: Collagen cross-linking effect of *D*-penicillamine on cross-linking in vivo. J Biol Chem 1977;252:254.
80   McGuigan LJB, Cook DJ, Yablonski ME: Dexamethasone, *D*-penicillamine, and glaucoma filtering surgery in rabbits. Invest Ophthalmol Vis Sci 1986;27:1755.
81   McGuigan LJB, Mason RP, Sanchez R, Quigley HA: *D*-penicillamine and beta-aminopropionitrile effects on experimental filtering surgery. Invest Ophthalmol Vis Sci 1987;28:1625–1629.
82   Moorhead LC, Stewart RH, Kimbrough PL, Smith J: Use of beta-aminopropionitrile following glaucoma filtering surgery. Invest Ophthalmol Vis Sci 1990;31(suppl):3.
83   D'Amore PA: Mechanisms of endothelial growth control. Am J Respir Cell Mol Biol 1992;6:1–8.
84   Favard C, Moukadiri H, Dorey C, Praloran V, Plouet J: Purification and biological properties of vasculotropin a new angiogenic cytokine. Biol Cell 1991;73:1–6.
85   Clarke MSF, West DC: The identification on proliferation and tumor-induced proteins in human endothelial cells: A possible target for tumour therapy. Electrophoresis 1991;12:500–508.
86   Furlong RA: The biology of hepatocyte growth factor/scatter factor (review). Bioessays 1992;14:613.
87   Miller MH, Rice NS: Trabeculectomy combined with beta irradiation for congenital glaucoma. Br J Ophthalmol 1991;75:584–590.
88   Khaw PT, Rice NSC, Baez KA: The congenital glaucomas; in El Sayyad F (ed): The Refractory Glaucomas. Tokyo, Igaku-Shoin, 1995, pp 1–21.
89   Cooper JS: Postoperative irradiation of pterygia: Ten more years of experience. Radiology 1978;28:753–756.
90   Aswad MI, Baum J: Optimal time for postoperative irradiation of pterygia. Ophthalmology 1987;94:1450–1451.
91   MacKenzie FD, Hirst LW, Kynaston B, Bain C: Recurrence rate and complications after beta irradiation for pterygia. Ophthalmology 1991;98:1776–1781.

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