1 TITLE: 2 The 2016 Bowman Lecture 3 Conjunctival curses: scarring conjunctivitis 30 years on 4 5 6 November 2016 6 Author: John K Dart 1,2 7 8 1. Ocular Biology and Therapeutics, UCL Institute of Ophthalmology, London, EC1V 9EL, United Kingdom 9 National Institute of Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS 10 Foundation Trust and the UCL Institute of Ophthalmology, London, EC1V 2PD, United Kingdom 11 12 **Conflict of Interest** 13 I am involved with the development of ALDH inhibition for scarring under the Patent PCT/GB2015/051292 held 14 by UCL Business plc. 15 16 **Running head:** Cicatrising (scarring) conjunctivitis 17 18 Keywords: cicatrising conjunctivitis, scarring conjunctivitis, mucous membrane pemphigoid, diagnosis, 19 pathogenesis, treatment, history 20 21 **Correspondence address:** 22 Professor John KG Dart 23 162 City Rd, 24 London, 25 EC1V 2PD, 26 **United Kingdom** 27 Telephone: 020 7566 2320 28 Fax: 020 7566 2109 29 Mobile: 07970 419309 30 Email: j.dart@ucl.ac.uk 31 32 Content 33 Text: 9141 words 34 Figures: 12 35 Tables: 4 36 References: 165 37 38 Supplementary data: 5 Appendices and 1 Table 39 Appendix 1 Observations on Sir William Bowman's life 40 Appendix 2 History of cicatrising conjunctivitis 41 Appendix 3 Protocol for investigation and diagnosis of patients with Cicatrising Conjunctivitis 42 Appendix 4 Systemic immunosuppression record and toxicity screening chart 43 Appendix 5 Intravenous immunoglobulin treatment protocol 44 Table 1 Direct immunofluorescence results in ocular only mucous membrane pemphigoid (MMP)

45

46

Abstract

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

66

67

68

69

This review is in two sections. The first section summarises thirty-five conditions, both common and infrequent, causing cicatrising conjunctivitis. Guidelines for making a diagnosis are given together with the use of diagnostic tests, including direct and indirect immunofluorescence, and their interpretation. The second section evaluates our knowledge of ocular mucous membrane pemphigoid, which is the commonest cause of cicatrizing conjunctivitis in most developed countries. The clinical characteristics, demographics and clinical signs of the disease are described. This is followed by a review and re-evaluation of the pathogenesis of conjunctival inflammation in mucous membrane pemphigoid (MMP), resulting in a revised hypothesis of the autoimmune mechanisms causing inflammation in ocular MMP. The relationship between inflammation and scarring in MMP conjunctiva is described. Recent research, describing the role of aldehyde dehydrogenase (ALDH) and retinoic acid (RA) in both the initiation and perpetuation of profibrotic activity in MMP conjunctival fibroblasts is summarised and the potential for antifibrotic therapy, using ALDH inhibition, is discussed. The importance of the management of the ocular surface in MMP is briefly summarised. This is followed with the rationale for the use of systemic immunomodulatory therapy, currently the standard of care for patients with active ocular MMP. The evidence for the use of these drugs is summarised and guidelines given for their use. Lastly the areas for research and innovation in the next decade are reviewed including the need for better diagnostics, markers of disease activity, and the potential for biological and topical therapies for both inflammation and scarring.

65

Keywords

conjunctival cicatrisation, conjunctival scarring, mucous membrane pemphigoid, autoimmunity, classification, diagnosis, pathogenesis, treatment

70

Introduction

It is a great privilege to have been asked to give this lecture and I am very grateful to the Scientific Committee of the College for inviting me to undertake it. I am going to discuss the results of 25 years of studies both collaborating with, and supported by, a large number of colleagues, including scientists and clinicians. I will use our studies in the context of the available evidence base to describe the causes and diagnosis of cicatrising conjunctivitis (CC) and follow this with a review of mucous membrane pemphigoid (MMP), which is the most common cause in developed countries. I hope that what I have to say about a group of diseases that is challenging for both patients and clinicians, will interest, entertain and challenge you as much as it has me.

All previous Bowman Lecturers have felt that their predecessors have been hard acts to follow and I am no different. However, all of us have worked in the shadow of Sir William Bowman. The portrait in Figure 1 has rarely been seen. It was painted by George Watts in about 1865 when Bowman was at the height of his powers. Watts was a friend of Bowman's and the leading portraitist of the day. Like many others who have given this lecture I became fascinated by Bowman's life and achievements. I wanted to know what it was that made him one of the foremost scientists of his day for his work on histology, bringing him Fellowship of the Royal Society at the age of 25, and then going on to become one of the founders of Ophthalmology as a scientific discipline in the UK. I have drawn on the biographies by his contemporary Power¹, and by James² for the background, but also on other sources to make some connections that have not, to my knowledge, been made before. These have been summarised in Supplementary Appendix 1.

In the early 19th century the classification of conjunctivitis was almost unrecognizable from what we know today. However, by the end of the 19th century trachoma, trauma, ocular rosacea, adenovirus, allergic eye disease, lupus and pemphigus (at that time a term including the all the pemphigoid diseases) had been recognized. A brief history of milestones in the recognition of the diseases causing CC is summarized in Supplementary Appendix 2 and Supplementary Table 1.

Current causes, epidemiology and classification of cicatrising conjunctivitis

Table 1 lists causes of CC from 2 studies. Thorne JE 2004³ provides the largest institutional case series of CC cases attending an MMP clinic, either to have the cause of CC diagnosed, or to have ocular involvement by MMP excluded from those patients with an established diagnosis of extraocular MMP. But, causes in single clinics are skewed by case selection and referral bias. For this reason, the epidemiology of CC is best described

by the study from Radford C 2012⁴ in the UK which lists the causes of cicatrizing conjunctivitis from the only national incidence study to have been reported. This study may have underestimated the incidence of CC by 15%, but the figures for MMP are in line with those reported for that disease from French and German estimates of 1.13.per million in France and 0.87 per million in Germany⁴.

Table 2 lists 30 causes of CC with references and notes about the associated diseases and the rarity of the conditions. Although not complete, it includes those reported causes for which there is reasonable evidence of causation. These diseases have been divided into three categories in Table 2, each of which relate to current treatment protocols.

Blinding scarring disorders include mucous membrane pemphigoid (MMP), and a rare subset of cases of drug induced scarring, and Stevens-Johnson syndrome/Toxic epidermal necrolysis (SJS/TEN), that behave like MMP and develop both inflammation and progressive scarring.

Ocular surface neoplasia (OSN) is an uncommon cause of inflammation and scarring which may mimic MMP although it is usually, but not always, unilateral unlike MMP which is usually, but not always, bilateral. The oncology protocols required for treatment will be delayed by misdiagnosis as CC.

Other conjunctival scarring diseases are those that have frequently been termed "pseudopemphigoid". The reason for this distinction, and for the term pseudopemphigoid, has been summarized in the study from Thorne JE 2004 comparing MMP and with pseudopemphigoid³ where pseudopemphigoid is used as the term for non MMP causes of CC. "Other ocular surface diseases can cause cicatrizing conjunctivitis and symblepharon formation that mimic MMP. These diseases may be grouped under the term "pseudopemphigoid". Although there are uncommon diseases associated with pseudopemphigoid that might require systemic immunosuppressive drug therapy (e.g., lichen planus or paraneoplastic pemphigus), most of the causes of pseudopemphigoid do not require systemic immunosuppressive drug therapy. Because long-term, systemic immune-suppressive drug therapy has potentially life-threatening side effects, differentiating ocular MMP from pseudopemphigoid is essential for the proper treatment of patients with cicatrizing conjunctivitis".

However, the use of "pseudopemphigoid" as a catch-all term for any cause of CC that shares the clinical features of MMP, but which demands a different therapeutic approach, is too non-specific to be helpful for several reasons. It groups diseases with very different aetiologies, some of which may be associated with severe inflammation, unresponsive to topical therapy, that require immunosuppressive treatment. These include severe atopic keratoconjunctivitis (AKC)⁵, graft versus host disease (GVHD)⁶, sarcoid⁷, ectrodactyly-ectodermal dysplasia-cleft syndrome EEC syndrome and ectodermal dysplasia (ED)⁸, even though the associated scarring rarely leads to pathology. Additionally, some of these conditions, with an established diagnosis, such as AKC and lupus, may also have concomitant MMP, which must be excluded. For these reasons the term pseudopemphigoid, as a specific term covering a disparate group of diseases, can be confusing and is best avoided.

Making the diagnosis

The diagnostic problem is shown by the similarity of the clinical appearances of the diseases illustrated in Figure 2. This has resulted in the difficulty we have in distinguishing many of the causes in Table 2 from ocular MMP and making a firm diagnosis, particularly for eye diseases without systemic associations or with dual pathology such as SJS/TEN, and for CC triggered by eye drops. Figure 3 summarises these dilemmas. Despite the similarities in the clinical characteristics some features of the clinical examination can help, as can a carefully taken history, and the appropriate investigations providing their limitations are understood.

Clinical examination, given the similarity of the clinical phenotype, is principally useful for establishing whether the disease is unilateral, and therefore potentially caused by *ocular surface neoplasia*, and to identify signs associated with a few of the other causes. Ocular MMP is rarely unilateral with only 7/115 (6%) of cases in one of our series³⁶. Other clinical findings that contribute are: severe aqueous tear deficiency (rare in early ocular MMP) caused by both *Sjögren's syndrome* and *severe non-Sjögren's dry eye*, for which a low Schirmer's 1 test (without anaesthesia) is indicative although not diagnostic because of limited sensitivity³⁷. For *ocular rosacea* the scarring is associated with meibomitis and a papillary and follicular conjunctivitis with or without the following: pseudopterygium, peripheral corneal vascularisation and usually the dermatological features of acne rosacea³⁸.

<u>History</u>: The other disorders listed in Table 2 have a clear history of an associated ocular condition or systemic disease with the exceptions listed below. Direct questions about the eye diseases, risks associated with scarring, and systemic diseases that are associated with CC (listed in Table 2) will identify whether the patient has an ocular or systemic disease in association with CC. This information will not always be volunteered by patients or referring physicians. The diseases which develop CC, without a history of an associated systemic disease, or which present in the eye before disease develop elsewhere are:

- Ocular MMP (conjunctival disease without involvement of other sites) has comprised 32-48% of 132
 cases in two UK studies including both cases with and without identified autoantibodies but meeting
 clinical criteria^{4,39}.
- Paraneoplastic pemphigoid and pemphigus (PNP) may rarely present in the conjunctiva at the same time as a neoplasm, and before other signs of malignancy²⁷.
- Pemphigus vulgaris (PV) may present in the conjunctiva before other signs: one case series of this
 presentation has been described³⁰.
- Sarcoid may present in the conjunctiva and be associated with severe scarring⁷. Conjunctival biopsy, even in the absence of conjunctival signs, can be diagnostic in 30% of patients with systemic sarcoidosis⁴⁰.

The rate of progression of scarring can usually be established by taking a careful history of the onset of symptoms of inflammation and scarring. The scarring may have been noticed by a healthcare professional or as a result of the onset of trichiasis. Estimating the rate of progression is important when deciding on the need for therapy, given the diagnostic delay for patients with ocular MMP (mean of 2.5 years and a range up to 10 years)⁴.

181 <u>Patients with multiple causes of CC</u>: as described in Table 2 patients with SJS/TEN and drug induced CC may

rarely develop disease that is indistinguishable from ocular MMP. We also have one unreported case of MMP

who later developed localised conjunctival OSSN. Other causes of CC including trachoma and atopic

keratoconjunctivitis may also develop MMP and this can be expected to occur occasionally for any of the other

causes. It follows that any patient with rapid progression of scarring and inflammation should have

investigations to exclude MMP and OSSN.

When to investigate?

Unilateral cases (to exclude OSN), patients without a clear history of an associated ocular or systemic disease presenting in the eye (such as occurs in ocular MMP, PNP, PV and Sarcoid), and those with an ocular or systemic disease, but with inflammation and scarring not responding to topical therapy. The latter is most common in SJS/TEN, and in topical drug associated CC, for whom there is often reluctance to discontinue topical therapy for advanced glaucoma. In the latter group severe inflammation and scarring may be associated with most non preserved glaucoma medications, as well as due to preservatives⁴¹: the inflammation will often rapidly resolve on withdrawal of the drops although this may take 2-6 weeks to improve and longer to resolve¹⁰. Oral acetazolamide will often be tolerated by this group of patients, at least for 1-2 weeks to confirm the diagnosis of toxicity/allergy to topical medications. If oral acetazolamide is not tolerated, topical unpreserved iopidine, to which few of these patients have been exposed, can be used as an alternative. We have had to carry out glaucoma tube surgery on some patients having these reactions.

Which investigations to use?

Our protocol for the investigation of CC cases is included in Supplementary Appendix 3.

- Blood tests are used in our department for the following:
- a. Indirect immunofluorescence and individual autoantibody detection to identify specific autoantibodies
 associated with MMP and PV.
 - Indirect immunofluorescence: Indirect immunofluorescence (IIF) microscopy on salt-split skin is used to identify the presence of serum autoantibodies in pemphigoid diseases. The structure of the basement membrane, its constituent proteins and the identification of these by immunofluorescence tests is illustrated in Figures 4 and 5⁹. However, IIF is non-specific for individual antibodies which cannot be identified from this test. The technique is clearly described by Hintner⁴².
 - Autoantibody detection: autoantibodies to BP180, laminin 332 BP230, α6β4 integrin, laminin 311, type VII collagen, Laminin γ1 (p200 protein) are associated with MMP and those to the intraepithelial proteins desmoglein 1 and 3 are associated with pemphigus vulgaris⁴³ which may occasionally present in the eye. ELISAs for antibodies to BP180, BP230, Collagen VII, Desmoglein 1 and Desmoglein 3 are

available from immunodermatology services and some laboratories offer immunoblotting to detect the remaining autoantibodies .

Although often negative in MMP, positive indirect immunofluorescence, or autoantibody detection, showing circulating anti-basement membrane zone antibodies provides additional evidence for an underlying autoimmune pathology⁴⁴ and may occasionally be positive when DIF is negative³.

- Autoantibody screening, and inflammatory markers to identify patients at risk of subclinical autoimmune disease which is present in over 30% patients with MMP (compared to less than 10% in a control population)⁴⁵.
- c. Baseline screening for patients who might need immunosuppressive therapy: full blood count, blood film, glucose 6 phosphate dehydrogenase (G6PD) level (dapsone is contraindicated if this is elevated, which is common in Mediterranean races), thiopurine methyltransferase (TPMT) levels relevant to metabolism of azathioprine, urea and electrolytes, creatinine, liver function tests, glucose and infection markers including quantiferon gold (for TB), hepatitis and HIV.

- <u>Biopsies</u> are taken for routine histopathology and direct immunofluorescence studies. Bulbar conjunctival biopsies are safe to take in patients with probable or proven MMP: of 344 cases having bulbar conjunctival biopsy no adverse effects were reported⁴⁶⁻⁴⁸. However, Foster reported that fornix conjunctival biopsies may be associated with an exacerbation of scarring in 3 cases, following which he abandoned this site for diagnostic biopsy⁴⁶. The technique for taking biopsies has recently been described⁴⁹.
- a. *Routine histopathology* with fixation in 10% formol saline is used to identify atopic keratoconjunctivitis⁵⁰, sarcoid⁴⁰ and is essential to exclude OSN, particularly in unilateral CC.
- b. Direct immunofluorescence (DIF) from perilesional tissue is required to confirm the diagnosis in the immunobullous diseases^{42,51}. Figure 5 illustrates the differences between the DIF findings for the intraepithelial "pemphigus" diseases and the subepithelial "pemphigoid" diseases, first defined by Lever in 1953⁵². Lichen planus, an inflammatory disease affecting skin and sometimes the oral and ocular mucosa, is also shown. DIF is used to differentiate these conditions and is described in Figure 5.

Interpretation of DIF results

- Causes of cicatrising conjunctivitis which may be DIF positive from the conjunctiva and skin, or other mucosal
 sites, include:
- MMP (including mucosal dominated EB. Linear IgA disease and anti-laminin 332 pemphigoid), some

 patients with SJS/TEN, some with drug induced scarring, all of which show IgG or IgA and complement on

 DIF at the epithelial basement membrane (shown in Figures 4 and 5), should be treated as MMP cases.

 Note that for the diagnosis of ocular MMP a conjunctival biopsy is NOT required for the diagnosis if there

 has been a positive biopsy from any other site (skin, buccal, genital, nasopharyngeal mucosa), providing

 the ocular findings are typical of MMP⁴⁴. The DIF findings in BP are the same as for MMP so that the

 diseases must be differentiated on clinical grounds.
- Pemphigus vulgaris may rarely present in the conjunctiva as conjunctivitis in addition to conjunctival scarring, and conjunctival DIF has shown the characteristic epithelial intercellular fluorescence to IgG and complement³⁰ shown in Figure 5, that is typically found in the skin at the dermoepidermal junction (basement membrane zone) in PV⁴².
- Lichen planus has a characteristic "ragged" fibrin basement membrane deposition as shown in Figure 5⁴⁴.
- Other diseases associated with CC, and which may have positive conjunctival DIF are:

258

263

- The ectodermal dysplasias with a pattern identical to that of MMP in the conjunctiva⁸.
- Lupus shows granular deposition of IgG, IgA, IgM and complement at the dermal basement membrane in
 many cases⁴² and also in the conjunctiva in CC cases associated with lupus⁵³.
- 264 Conjunctival scarring associated disease which may be DIF positive from skin, but not from the conjunctiva:
- Conjunctival DIF has not been demonstrated in paraneoplastic pemphigus, which may also be associated
 with conjunctival scarring, and show a similar pattern to that of PV, in addition to a pemphigoid like
 pattern at the dermal epidermal junction.
- Dermatitis herpetiformis having a typical granular pattern at the basement membrane zone⁴².
- Porphyria cutanea tarda also shows characteristic immunoglobulin deposition at the dermoepidermal
 junction but one study of associated conjunctival scarring showed repeatedly negative DIF results³⁵.

Negative immunofluorescence findings in ocular MMP

There is a strong recommendation, from an influential consensus document⁴⁴, that MMP can only be diagnosed when both clinical criteria and direct immunopathology criteria have been fulfilled. The clinical criteria are those distinguishing MMP from other diseases involving mucous membranes. Those that are listed in the document, and which cause CC, are lichen planus, SJS/TEN, PV, PNP, BP, and drug induced CC, to which the additional disorders summarized in Table 2 must be added. The direct immunopathology criteria that are recommended, as mandatory for diagnosis, are either direct immunofluorescence (DIF) microscopy, which is widely available, or immunohistochemistry which is not. Both positive IIF or specific autoantibody detection are widely accepted as alternative evidence when DIF findings are negative.

These criteria are usually positive in MMP that involves tissues other than the eye. However negative DIF results have long been recognized as presenting a diagnostic problem in patients with strong clinical evidence of ocular only MMP (in whom the conjunctiva is the only site of involvement). In three studies describing this group of 49 patients^{3,47,54} 25/49 (51%) were DIF+ of whom 13 (26%) required more than one biopsy to demonstrate this (Supplementary Table 2). Other problems with the use of DIF as a mandatory finding for the diagnosis of MMP are that the results can be initially positive, then subsequently negative when patients are in remission⁴⁷. Identical biopsy findings are found in bullous pemphigoid, which has to be distinguished by the clinical findings, and identical findings are also reported in patients some patients with SJS/TEN¹¹, drug induced progressive scarring⁵⁵, ectodermal dysplasia⁸ and ulcerative colitis⁵⁴.

Immunopathology, as described in the Consensus document, refers to the autoantibody detection tests (DIF, IIF or other means of autoantibody detection) that are recommended for diagnosis in these immunobullous disorders. However, the presence of autoantibodies does not reflect the dominance of the cell mediated autoreactive response, to mucosal basement membrane epitopes, which is probably important in many cases of ocular MMP (see sections b. and c. in the review of the pathogenesis of ocular MMP below). In effect a positive DIF result, as recommended for diagnosis, is a biomarker for an autoantibody driven autoimmune pathology and not the cellular autoimmune response. Unfortunately, DIF has not proved to be either sensitive in ocular MMP, nor specific as a marker for ocular MMP. Although a positive DIF result is useful, and can distinguish MMP from lichen planus, lupus erythematosus and PV and PNP, which have characteristic immune-pathological features of their own, a negative result does not exclude ocular MMP. Currently patients are being seen at Moorfields who could benefit from treatment for MMP, but who have been untreated

because of uncertainty about the diagnosis due to their having negative autoantibody results. This has resulted in delayed therapy and deterioration of their scarring and inflammation. For these reasons we have proposed new criteria for the diagnosis of ocular MMP that are summarised below.

Recommended diagnostic criteria for ocular MMP.

We have proposed 3 sets of criteria for the diagnosis of ocular MMP^{4,56,57}:

- a. Patients with positive conjunctival DIF or positive DIF from another site meet currently agreed criteria 44.
- Patients with negative DIF from any site and positive indirect immunofluorescence can be diagnosed as having MMP.
- c. Patients with negative immunopathology can be diagnosed with ocMMP providing that they have a typical phenotype of progressive conjunctival scarring, and that other diseases that may cause this phenotype have been excluded. When ocular cases are reported, the detailed immunopathology findings should be recorded so that the diagnosis can be interpreted in light of future modifications to diagnostic criteria.

When to refer?

Patients with scarring & inflammation if there are no facilities for the specialist investigations or for delivering immunomodulatory therapies, for rarely described diseases, when the diagnosis is uncertain and when there is a poor response to therapy. In these circumstances a specialist centre with expertise in CC can provide the resources both to clarify the diagnosis and establish a treatment plan⁴. Guidelines for referral are given in Figure 6.

Review of mucous membrane pemphigoid with ocular involvement

This is the commonest disease causing cicatrising conjunctivitis in the UK, accounting for 60% of CC cases, and is the prototypic autoimmune mucosal scarring disease. For the latter reasons MMP has been the focus of much of the research into scarring eye diseases in developed countries and ocular involvement by MMP has threefold more citations on PubMed than any other cause of CC apart from trachoma. MMP has been the focus of most of my work in CC and is the topic of the rest of this review.

Terminology

The terminology for the immunobullous diseases is confusing, given the changes in nomenclature over the past 250 years, and more recently. All these bullous diseases were categorized as pemphigus, until Lever's seminal paper in which he differentiated the intraepithelial pemphigus diseases, from the subepithelial pemphigoid (meaning "resembling pemphigus") diseases. This separation of these diseases was based on his histological studies demonstrating the tissue that was the focus of the inflammatory response. Since then the immunobullous diseases have been classified into these two groups, which are described in Figure 5.

The terminology for MMP has also changed. Initially Lever's term for this was Benign Mucous Membrane Pemphigoid (BMMP) because, unlike pemphigus vulgaris, which had a very high mortality before the introduction of steroids, it was rarely fatal. In the 1980's the term BMMP was replaced by the term cicatricial pemphigoid (CP) to reflect the associated scarring. CP and MMP have been used interchangeably at different times, and the studies describing the sites of involvement have prefaced these terms with the name of site, most often ocular CP (OCP) and ocular MMP (OMMP). Cicatricial pemphigoid is a term that is now used to describe a rare disease, having the same immunopathology as MMP, but which is limited to the head and neck and is also called localised CP or Brunsting Perry cicatricial pemphigoid samples. The term OCP is still in occasional use although, following the 2002 Consensus document the term MMP has been recommended for any patient, with any site of involvement (because multiple sites are often involved). However, this recommendation is impractical for specialist publications, describing the diagnosis and management of the different mucosal sites, and the descriptive terms ocular MMP (instead of OCP) and oral MMP are now being used for studies focused on diseases at these sites.

The other principal terminological difficulty is the altering nomenclature of the basement membrane immunoreactant proteins: examples are BP180, also known as Bullous Pemphigoid Antigen II and classified as Collagen type XVII, BP230 is also known as Bullous Pemphigoid Antigen I, laminin 332, formerly called laminin 5 or epiligrin, and p200 protein which is now designated as laminin y1.

Clinical characteristics

a. <u>Sites of involvement</u>: Mucous membrane pemphigoid involves all the orificial mucosal sites (oral, ocular, nasopharyngeal, genital and anal) as well as, less often, tracheal and oesophageal. The skin may also be involved. Figure 7 shows examples of disease at many of the sites involved by MMP. All mucosal sites may become severely inflamed however scarring is rare in the oral mucosa whereas scarring is mandatory for

the diagnosis in the conjunctiva. Oral MMP, when restricted to the oral mucosa, has a relatively benign course in many patients, unlike ocular MMP. The conjunctiva is involved occurs in 70% of all MMP cases resulting in bilateral blindness in 20% of cases and severe sight loss in 30% of eyes^{36,39,59}. In a cross sectional study (unpublished) between 2009-12 of 73 MMP patients with conjunctival involvement seen at Moorfields, 27% of patients had ocular only MMP, 26% ocular and oral, and a further 26% ocular, oral and nasopharyngeal, with the remainder having a mixture of other sites involved.

Demographics: In the UK national incidence survey which included 50 MMP patients meeting clinical criteria for the disease the median age was 71 years (range 20-90), there was a preponderance of males with a M:F ratio of 29:21 (1.38:1), with 32/50 (64%) having extraocular MMP (as opposed to ocular only MMP) of whom 8 (16%) had another autoimmune disease and 26/40 (65%) were taking immunosuppressive therapy at a 12 month follow up⁴. These finding are similar to those in an institutional case series of 50 patients with a median age of 67 years (range 32-91), M:F ratio of 23:27 (0.85:1), extraocular MMP in 26/50 (52%) and 38/50 (76%) requiring immunosuppressive therapy after their initial assessment at the specialist centres³⁹. There is no good data on racial predisposition although MMP is probably less common in Indian Asians⁶⁰ and the Chinese^{61,62} than in Caucasians. The disease is reported in Japan.

Clinical signs of disease progression

Early diagnosis and initiation of appropriate treatment are essential to prevent the sight-threatening complications of the approximately 75% of ocular MMP patients with rapidly progressive disease²⁶. Clinical signs are illustrated in Figure 8, ocular MMP typically presents with a red eye and persistent conjunctivitis that has not responded to topical therapy, or with cicatricial entropion and trichiasis, that may have failed surgical repair. About 30% of patients develop severe inflammation with limbitis leading to surface failure if uncontrolled of patients present with acute conjunctivitis and limbitis leading to rapidly progressive scarring and surface failure if uncontrolled²⁶. Similarly, persistent epithelial defect has a poor prognosis occurring in about 20% of patients. The remaining patients present with subacute or low grade chronic inflammation and slowly progressive scarring. The earliest clinical sign in patients with subacute disease is often medial canthal scarring, with loss of the plica and caruncle. Medial canthal scarring is usually an early sign of MMP and is not as frequent in conjunctival scarring due to other causes. Linear scarring in the sulcus subtarsalis (marginal

sulcus) of the upper tarsus is sometimes present early in the disease. Other signs, in order of progression, are subepithelial reticular fibrosis, infiltration of the tarsal and bulbar ^{63,64}conjunctiva, shortening of the fornices, symblepharon and cicatricial entropion, followed by ankyloblepharon and then, subsequent to scarring of the lacrimal ductules which usually occurs late in the disease, a totally dry "skin like" eye. Figure 9 describes the events leading to morbidity and blindness in ocular MMP.

Pathogenesis

a. *Predisposing factors*. As described in the section on the demographics of MMP above the disease is probably more common in Caucasians than in Indian and Chinese Asians, although it may occur in any racial group. Also, other autoimmune diseases are more common in MMP patients. Many patients have a genetic predisposition to MMP, expressing the HLA-DQB*0301 gene⁶⁵. For the majority of patients there are no identifiable precipitating factors. However, in subsets of patients, including cases of ocular MMP following SJS/TEN^{11,66} and topical glaucoma treatment^{55,67}, it is possible that damage to the conjunctival basement membrane precipitates the disease by exposing basement membrane epitopes triggering a pathological autoimmune response to neoantigens. The latter mechanism is an alternative to the development of loss of tolerance to basement membrane antigens that is described below and thought to be the underlying mechanisms in most cases.

Loss of tolerance, autoantibodies and autoreactive T cells and mechanisms of disease activation and remission at different sites in MMP. As in other autoimmune disorders disease probably develops as a result of loss of tolerance. In the case of the autoimmune subepithelial bullous dermatoses, of which MMP is one, loss of tolerance is to epithelial basement membrane proteins. This has been shown to result in circulating autoreactive T cells in 2 cases⁶⁸ and in the generation of autoantibodies to a number of basement membrane proteins, most commonly BP 180, described in Figure 4. The pathogenic potential of antibodies has been demonstrated: anti-laminin 332 induces blistering in a mouse model and anti- α 6 β 4 integrin induces separation of the dermoepidermal junction in organ culture of human skin, 9 . In serum, antibody levels have been correlated with disease activity in MMP⁶⁹. As a result of these findings it has been proposed that autoantibodies must be demonstrated for the diagnosis of MMP⁴⁴ as for the other pemphigoid diseases⁹.

On the other hand, not all patients with MMP have demonstrable circulating antibodies. In ocular MMP only 50% of patients have demonstrable autoantibodies (see section on negative immunofluorescence findings in ocular MMP, above). In addition, few MMP patients suffer from involvement of all potential sites of involvement: of 112 patients with MMP in a cross sectional study in London (unpublished) 2/112 patients had 5 involved sites, and 6/112 had 4 involved sites, from a potential maximum of seven sites (ocular, oral, skin, nasopharyngeal, laryngeal, genital, perianal). The mechanisms that protect individual sites from involvement in a systemic disease, caused by circulating antibodies to proteins common to the basement membranes of all the target tissues, may involve factors local both to those tissues and to the local cell mediated inflammatory response. The latter is a feature of these diseases^{9,70} in which autoreactive T cells are central both to the autoantibody response, and to the cellular autoimmune response⁶⁸. Combined cellular and antibody mediated responses are common in autoimmune diseases, all of which require autoreactive T cells. Although some autoimmune diseases are dominated by the pathogenic effects of one effector pathway, either autoantibodies or effector T cells, both pathways are commonly involved. Examples are Graves' disease which is an autoantibody dominated disease, mediated by autoantibodies to thyroid stimulating hormone. At the other end of the spectrum, psoriasis is the result of an autoreactive T cell dominated response to skin associated antigens. However, most autoimmune diseases, exemplified by systemic lupus erythematosus and rheumatoid arthritis, result from the effects of both autoantibody and autoreactive T cell mediated inflammation in the target tissues⁷¹. In bullous pemphigoid there is ample evidence for combined autoimmune cellular and antibody mediated effector pathways⁹, as there is for mucous membrane pemphigoid⁷².

417

418

419

420

421

422

423

424

425

426

427

428

429

430

431

432

433

434

435

436

437

438

439

440

441

442

443

444

445

Autoimmune reactivity is normal in healthy individuals who may express both autoantibodies and autoreactive T cells without developing an effector response that results in disease⁷¹. In the last decade naturally occurring autoantibodies have been shown to have homeostatic functions in the clearance of oxidatively damaged body waste, and in the modulation of immune cell functions⁷³. *Autoantibodies in normal MMP controls*: several studies have examined the prevalence of autoantibodies to BP180 and BP230, the basement membrane proteins commonly precipitating an autoreactive response in pemphigoid diseases and show that these are probably present in about 10-15% of healthy age matched individuals. However, the prevalence of these antibodies has varied in relation to the sensitivity and specificity of the assay used and the control group. The lowest prevalence has been than 1% in a large cohort of blood

donors of unknown ages⁷⁴, other publications have given values of 7.5%⁷⁵, 13%, 16% and 26% for ELISA and or immunoblots⁷⁶ although higher values have been reported⁷⁷. Results for IIF have generally shown <5% positivity^{75,76} although this was 19% (n=32) in another study⁷⁸. *Autoreactive T cells* recognising the basement membrane protein BP 180, are also present in healthy individuals expressing the HLA-DQB*0301 gene (although not in those who did not)⁷⁹. These autoreactive T cells are thought to be prevented from developing a pathological response by the activity of both regulatory T cells (Treg)⁸⁰ and regulatory B cells (Breg)⁸¹. The role of these regulatory cells has been evaluated in only two studies in MMP: Treg were shown to be present in higher numbers in MMP lesional tissue compared to the skin of normal controls⁷² whereas a study of peripheral blood in patients, with a variety of pemphigus and pemphigoid diseases, showed reduced numbers of cells with Breg characteristics, compared to controls, in pemphigus only and not in pemphigoid⁸².

In summary a substantial proportion of normal individuals have antibodies to both BP180 and BP230, some also having autoreactive T cells to BP180. There is evidence that Treg are present in MMP lesional tissue. We can hypothesise that differences in the balance between autoreactive T cells and Tregs in the different lesional MMP tissues account for the development and resolution of the disease in different target tissues in any one individual affected by MMP. If this occurs disease activation and remission in MMP is likely to be occurring at a local lesional level in the tissues, and independent of circulating autoantibody production and local antibody deposition. If this hypothesis is correct then the balance, between autoantibody and autoreactive cellular effector mechanisms, may differ both for different sites, and at different stages of disease chronicity, accounting for the lack of evidence of circulating antibodies in some cases of MMP, particularly in those with ocular MMP.

However, this is an area which demands further study, and the control mechanisms are likely to be more complex than this. Given that anti-CD20 B cell depletion therapy (such as the anti-CD20 monoclonal, rituximab) is effective in many cases of T cell mediated diseases such as type I diabetes and rheumatoid arthritis, as well as MMP, the role of B cells in regulating T cell responses has been examined⁸³ and evidence suggests that, in some circumstances, B cell antigen presentation to autoreactive T cells may be necessary to develop an autoreactive T cell response. In addition, unstimulated B cells may also promote the development of Treg cells⁸³.

Summary of the pathogenesis of inflammation and scarring in ocular MMP.

This is described in Figure 10, for which the evidence is summarised both in Table 3 "Effectors and cytokines identified in MMP conjunctiva" and in the hypothesis synopsised above, that the inflammatory response is both the result of a variable balance between epithelial basement membrane autoreactive T cell mediated inflammation, in the lesional tissues, and also results from circulating autoantibodies to basement membrane proteins. Although the effects of inflammation and scarring are closely related it is easier to dissect out the mechanisms for each separately:

482

483

484

485

486

487

488

489

490

491

492

493

494

495

496

497

498

499

500

501

502

503

475

476

477

478

479

480

481

Inflammation: current descriptions ascribe the inflammatory response in MMP as arising from loss of tolerance to basement membrane proteins resulting in autoreactive T cells interacting with autoreactive B cells in the regional lymph nodes. This results in the generation of plasma cells, producing IgG or IgA circulating antibodies. Antibody may also be locally produced given that plasma cells are found in the lesional tissue, whereas the evidence for the presence of B cells in lesional tissue is mixed^{84,85}. Loss of tolerance is more common in individuals expressing HLA-DQB*0301 which may promote (restrict) the activation of T cells that are autoreactive to basement membrane proteins⁷⁹. Following this IgG and/or IgA antibody binds to the basement membrane in the conjunctival mucosa, resulting in complement fixation and the development of an acute inflammatory response leading to an influx of neutrophils, macrophages, dendritic cells and both cytotoxic (Tc) and helper (Th) T cells and plasma cells^{84,85}. MHC class II protein (required to present peptides to the immune system) are highly expressed in MMP conjunctiva⁸⁵. Mast cells and eosinophils, capable of producing pro-fibrotic cytokines are also found in acute disease⁸⁶. Transforming growth factor beta (TGF β) is overexpressed in acute disease compared to controls but not in chronic disease and, although the profibrotic factors, platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) are present they are not overexpressed, suggesting that in chronic disease fibroblast activity remains functionally and morphologically abnormal after the withdrawal of the influence of growth factors^{85,87}. The B7-2 costimulatory molecule required for T cell proliferation in the presence of IL-2 is also overexpressed and can be expected to lead to increased T cell expansion⁸⁴. Of the cytokines, IL-2, IFNγ and TNFα (Th1 signature cytokines) are all present although not overexpressed compared to controls^{85,87}. However, serum TNF α , an important pro-inflammatory mediator, has been shown to be elevated in MMP compared to controls and, in a more recent study, to be overexpressed in inflamed compared to treated

MMP conjunctiva⁸⁸. Evidence for the activity of Th2 cells in ocular MMP comes from the finding of elevated levels of IL-13 in inflamed MMP conjunctiva⁸⁹ and IL-5 in serum⁸⁶, although the latter is also produced by mast cells.

b. *Scarring*: Profibrotic mediators have been investigated in several studies including TGFβ, PDGF, FGF and IL13 as described above. Collagen type I and III are increased in ocular MMP stromal tissue. Cultured *in vitro*MMP conjunctival fibroblasts respond to TGFβ by the induction of heat shock protein 47 (HSP47), which is
thought to influence procollagen synthesis and fibrosis resulting in collagen Type I production⁹⁰.

Macrophage accumulation in the conjunctiva is probably an important event in the pathogenesis of MMP
conjunctival scarring stimulated by macrophage derived cytokines and growth factors, including IL-4,
PDGF, and TGFβ. Increased levels of macrophage inhibition factor (MIF)⁹¹ and macrophage colony
stimulating factor (m-CSF)⁹², have been demonstrated in both *in vitro* cultured MMP fibroblasts and in
whole conjunctiva and associated with increased numbers of macrophages. Connective tissue growth
factor (CTGF), a downstream profibrotic mediator of TGF-β1, is overexpressed in both ocular MMP whole
conjunctiva and in MMP cultured fibroblasts⁹³. These studies demonstrate that the effector cells, cytokines
and growth factors necessary for fibrosis are present in the MMP conjunctiva. However, the mechanisms
that relate this inflammatory milieu to the production of the extracellular matrix (ECM) by fibroblasts, that
results in scarring, have not been identified ⁹⁴⁻⁹⁶. However, our recent studies outlined below have
identified one control mechanism.

We have previously confirmed that OMMP fibroblasts maintain a profibrotic phenotype *in vitro* and have hypothesised that progressive fibrosis may be precipitated by the inflammation associated with ocular MMP, and then persist in eyes having clinical control of inflammation because of the continuing activity of persistently profibrotic fibroblasts ⁹⁷. In recently published studies ⁹⁸ we have identified that the aldehyde dehydrogenase (ALDH)/retinoic acid (RA) metabolic pathway regulates this profibrotic activity in ocular MMP conjunctival fibroblasts *in vitro*. We have shown that ALDH is overexpressed in ocular MMP conjunctiva at the gene and protein level, compared to controls, and that ALDH inhibition with disulfiram abolished the profibrotic phenotype in MMP conjunctiva, resulting in the adoption of a normal control phenotype. Conversely *in vitro* fibroblasts from normal controls adopt a profibrotic phenotype when treated with RA, the metabolic product of ALDH. These findings provide evidence for ALDH/RA

autoregulation in ocular MMP fibroblasts as a mechanism underlying progressive conjunctival scarring seen in this disease and the potential for ALDH inhibition with disulfiram as a therapy for fibrosis. These findings were further confirmed in a mouse model of ovalbumin induced severe conjunctival inflammation that was developed for allergic eye disease studies in which we have shown that conjunctival scarring develops concurrent with inflammation. ALDH inhibition in this model, using topical disulfiram, was effective in preventing scarring in vivo, and also restored in vitro in mouse conjunctival fibroblasts to a normal phenotype, as in ocular MMP. Furthermore, another paper published with this study, has shown that in the same mouse model conjunctival scarring is initiated by the key role of dendritic cells, through paracrine production of ALDH/RA effecting conjunctival fibroblasts⁹⁹. Given our hypothesis that the scarring in ocular MMP is the result of the inflammatory response in MMP, rather than due to the autoimmune pathogenesis per se, we believe this mouse model provides a surrogate for studying immunemediated conjunctival scarring. Disulfiram is a drug already licensed for alcohol abuse control. These studies suggest that it could be repurposed for the topical treatment of conjunctival scarring in ocular MMP and provide justification for a randomized controlled trial of disulfiram therapy in this disease. Other mucosal scarring diseases are potential targets for further study using these techniques. Conjunctival diseases that may share these fibrotic mechanisms are Stevens-Johnson syndrome, atopic keratoconjunctivitis and trachoma.

Although ALDH inhibition is effective in these models we do not understand the molecular mechanisms underlying these profibrotic effects of ALDH/RA. It is possible that these profibrotic effects are mediated by similar mechanisms to those described in liver fibrosis 8,9 , by the induction of the TGF β 1 gene, and/or activation of latent TGF β 1, for example by increased plasminogen activator levels. Active TGF β drives critical changes in fibroblast metabolism, activation and ECM production in concert with proinflammatory cytokines and growth factors that affect fibroblast activity 10 . Alternatively, ALDH/RA might cause its effects through altered cellular energy metabolism via activation of TGF β or as a consequence of the modulation of the transcription of metabolic genes 11 . The ALDH/RA metabolic pathway and these potential molecular mechanisms are described in Figure 10.

559

560

561

533

534

535

536

537

538

539

540

541

542

543

544

545

546

547

548

549

550

551

552

553

554

555

556

557

558

Treatment summary

a. Treatment outcomes for ocular MMP before 1980

These were poor until the 1980's when immunomodulation techniques were introduced for their management for the first time. In the first series of cases reported by Morris in 1889 the outcomes were summarised: 28 cases (12F, 13M) onset from infancy to 76 years. "Disease began in the skin in 16, other mucosae in 4, and in the eye in 8. Twelve cases had conjunctival blistering, and others a pseudomembrane, entropion, progressive conjunctival shrinkage, cloudy cornea, thickened bulbar conjunctiva and xerophthalmia. Generally, vision is lost apart from perception of light although corneal perforation and destruction of the globe has been reported. One case had been described with spontaneous remission. Entropion surgery and application of lotions for the inflammation are palliative, there is no treatment for the progressive scarring. The pathology is obscure. It is a very rare disease" 104. In Swanzy's 1895 textbook he stated "Treatment is helpless in respect of arresting the progress of disease, or of restoring sight when lots in consequence of it. The most one can do is to relieve the distressing symptoms by emollients to the conjunctiva, and by the use of closely fitting goggles, to protect from wind, dust and sun. Internally arsenic is indicated 105." By 1951 Sorsby, in Systemic Ophthalmology stated of ocular pemphigus "Treatment is uniformly unsuccessful" 106. The first textbook I owned when I started ophthalmology in 1978 was Parsons' Diseases of the Eye 16th Edition in which it was stated that in the treatment of Benign Mucous Membrane Pemphigoid "Local treatment is unavailing as also, indeed, is general treatment" 107. The use of dapsone for the management of pemphigus and pemphigoid was introduced in the 1970's, but not for ocular MMP until 1982¹⁰⁸. Systemic steroids for the control of acute disease in ocular MMP was described by Mondino in 1979¹⁰⁹. The first use of immunosuppressive therapy, with azathioprine and cyclophosphamide, was described by Foster for 2 cases in 1980¹¹⁰, followed by a larger series using these drugs in 1982¹¹¹. Subsequent publications have proliferated and are described below.

582583

584

585

586

587

562

563

564

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

580

581

b. <u>Current treatment</u>

Successful management of disease demands the integration of the following:

- (1) Control of surface disease
 - i. Blepharitis
- 588 ii. Dry eye
- 589 iii. Corneal punctate epitheliopathy
- 590 iv. Keratinisation

591	٧.	Trichiasis, entropion and lagophthalmos

- vi. Persistent epithelial defects
- 593 vii. Corneal perforation
- 594 viii. latrogenic toxicity
- 595 (2) Control of immune mediated inflammation with systemic immunomodulation
- 596 (3) Control of fibrosis
 - (4) Prophylaxis of corneal ulceration and exposure
- 598 (5) Improving vision in patients with corneal blindness

The ocular surface disease management, and a synopsis of management with systemic immunomodulatory therapy, and visual rehabilitation, has been discussed in detail in two reviews from our group^{63,64} and a further recent review has summarized the use of immunomodulatory therapy in detail⁵⁷. In this publication I have restricted the description of treatments to a summary of the role of immunomodulatory techniques and the evidence for their use in ocular MMP, with guidelines for their use.

Use of systemic immunomodulation to control immune mediated inflammation

a. Lack of effect of topical immunosuppressive therapy in ocular MMP. Topical steroid treatment is ineffective in controlling progressive ocular MMP, offering only variable symptomatic relief^{59,109}. Its adverse effects of cataract and glaucoma generally outweigh the benefits. Subconjunctival steroids may be temporarily effective, but relapses occur when the injections are stopped¹⁰⁹ and prolonged use also leads to cataract and glaucoma. Anecdotally, topical steroid will relieve discomfort and inflammation in some patients with mild/moderate disease activity but has not reduced the activity of severe inflammatory disease or the progression of scarring. Topical ciclosporin has been used in only 4 reported cases of whom 2 had some response¹¹²; we have little experience with this for ocular MMP and it is probable that the poor results reported for systemic therapy with ciclosporin may have inhibited further investigation of this modality. As a result, systemic immunomodulatory therapy is currently the standard of care for these MMP patients ^{46,113,114}.

b. Evidence for the effect of systemic immunosuppression on progression of disease. The primary goals of treatment of ocular MMP are to control inflammation and arrest fibrosis, in order to prevent progression of disease to more advanced stages and blindness. Most cicatrisation is occurs during active inflammation ¹⁰⁹, but despite control of inflammation in 70% -78% of patients with systemic immunosuppression, progressive fibrosis was still observed in 61/115 (53%)³⁶ and 23/54 (42%)³⁹ of patients. Without treatment, conjunctival scarring in ocular MMP progresses in 13/20 (64%) of patients over 10 to 53 months¹¹⁵. Progression is more frequent in the advanced stages of disease¹¹⁶. Use of immunosuppressive therapy has been shown to slow progression of disease in one case series 116 and control of inflammation has been shown to prevent progression in one RCT ⁴⁶. With current immunosuppressive regimens, progression of cicatrisation has still been observed in 10 to 53% of ocular MMP patients ¹¹⁶⁻¹¹⁸ and is more rapid in patients <60 years of age ¹¹⁹. A subset of MMP patients with ocular involvement have ongoing conjunctival fibrosis without overt clinical signs of inflammation¹²⁰. However, despite the absence of clinical signs of inflammation, there may still be significant cellular infiltrate on histological evaluation ("white inflammation")^{87,100,121}. Further systemic immunosuppression with potential systemic toxicity may not necessarily be helpful in these cases, for whom more specific local therapy targeting the cellular infiltrate or fibrogenic process would be ideal. There is evidence for both ongoing residual subclinical inflammation 89,122 and transformed profibrotic fibroblasts 97 as the putative drivers of scarring which progresses, despite apparent clinical control of inflammation with systemic immunosuppression. No current medical therapy is able to reverse the cicatrisation or ocular surface problems once they have developed.

620

621

622

623

624

625

626

627

628

629

630

631

632

633

634

635

636

637

638

639

640

641

642

643

644

645

646

647

648

c. *Criteria for initiating immunosuppressive therapy.* About 25% of OcMMP patients do not require immunosuppression¹¹⁷ as they have few symptoms, limited scarring, mild or no inflammation and slow progression, or are in remission. In end-stage "burned out" disease, immunosuppression is also unnecessary as eyes are usually comfortable, albeit blind, and treatment only slows scarring without significantly reversing it. In these patient groups, if conjunctival incision surgery is planned, such as that required for cataract extraction when the fornices are very short, or before fornix reconstruction, then immunosuppression should be started beforehand, to prevent an exacerbation of postoperative inflammation and scarring which may be severe, and will result in a poor surgical result and disease

progression. For cataract surgery through a clear corneal incision, without conjunctival surgery ¹²³ or fornix incision for subtenon's anaesthesia, and for lid surgery without a conjunctival incision, it is not necessary in my experience, to introduce preoperative immunosuppressive therapy.

d. *Identifying inflammation due to underlying disease rather than to the secondary effects of a poor ocular surface.* Identification of inflammation that is primarily due to the disease process in MMP, rather than to inflammation secondary to the effects of ocular surface disease or topical drug therapy, is essential before initiating immunomodulatory therapy. This is rarely a problem in those patients presenting with more severe disease. However, it can be difficult in those with less severe inflammation. It is necessary to differentiate inflammation due to the associated surface disease (dry eye, blepharoconjunctivitis, and lash abrasion), from immune mediated inflammation. Clinically this can be done by evaluating the degree of bulbar conjunctival inflammation under the upper lid, which is free of the worst effects of surface disease. However sectoral inflammation, although not common, does occur and may affect any quadrant of the bulbar conjunctiva as shown in Figure 11. Topical drop toxicity/allergy, typically resulting from glaucoma medications or preservatives may cause diffuse inflammation and be impossible to distinguish from that causes by MMP unless the topical therapy is discontinued: it takes 1-2 weeks for this to start to improve after withdrawing glaucoma medication, and any other topical drops, apart from non-preserved saline (see Section 3).

e. Evidence for the efficacy of different immunosuppressive regimens in controlling inflammation and guidelines for its delivery. Evidence for the effect of current immunosuppressive therapy in ocular MMP is summarised in Table 4 and comes from cohort studies^{124,125}, interventional and retrospective case series^{63,108,116,126-134} and two randomized trials⁴⁶. These have indicated a role for dapsone, sulfasalazine or sulphapyridine for mild to moderate inflammation, azathioprine, mycophenolate or methotrexate for moderate inflammation, or for disease not responding to sulphonamide therapy, and cyclophosphamide with a short course of prednisolone, for severe inflammation. Combinations of sulfas and myelosuppressive drugs (methotrexate, azathioprine, mycophenolate, and cyclophosphamide) with or without prednisolone can be effective. Notes on the use of these drugs, as "step up and step down therapy", including combination therapy as favoured by UK specialists, are in Table 4 and Figure 12.

Supplementary Appendix 4 includes a synopsis of immunosuppression management guidelines for use in clinic.

continue immunomodulation, if well tolerated, for at least 12 months. Following this, the dose is reduced and can be stopped, if the patient wishes, providing they understand that it will need to be recommenced if disease activity recurs. Life-long follow-up is necessary, because disease recurs in up to 1/3 of patients¹²⁹. Our series of 115 patients showed remission without therapy of at least 6 months in 20 (17%) of patients with a relapse in 4/20 (20%). In our experiences long term remissions are uncommon using conventional drug therapies. However, Thorne et al. using high dose cyclophosphamide and prednisolone, achieved better remission rates, of at least 3 months without therapy, in 40/44 (91%) within 2 years of initiating therapy, followed by an 8/40 (20%) relapse rate. However, we think the risks of adverse effects outweigh the benefits as they require cumulative cyclophosphamide doses which probably exceed safe levels for the development of malignancy (see Table 4, rows 14 and 19).

The last thirty years and horizon scanning for the next decade

In the last 30 years there have been great advances in the understanding of the pathogenesis and therapy of three of the commonest causes of conjunctival scarring: trachoma¹⁵⁹, mucous membrane pemphigoid⁶⁴ and atopic keratoconjunctivitis⁵. These include the introduction of mass azithromycin delivery for trachoma control and the understanding that the scarring response in trachoma is only indirectly related to the precipitating infection resulting from the secondary profibrotic effects of infection. The unravelling of the pathogenesis of atopic keratoconjunctivitis and the randomised controlled trials, demonstrating the effectiveness of topical ciclosporin in its management. For ocular mucous membrane pemphigoid, the introduction of effective therapy with systemic myelosuppressives, currently the standard of care, had only just been introduced 30 years ago before which a majority of patients progressed to bilateral blindness. There was minimal understanding of the pathogenesis of MMP at that time and what we now know is feeding into the application of new therapies for inflammation. The latter include the use of monoclonal CD20 mediated B cell elimination, and the potential development of anti-scarring therapies. There are 4 areas in particular where advances can be expected to contribute substantially to improved outcomes for patients: improved diagnostic tests, the identification of

biomarkers of disease activity, the further development of biological drugs for therapy, and the development of topical therapies for both inflammation and scarring.

a. Better diagnostics

The limitations of the current reliance on the detection of autoantibodies for disease identification, particularly for ocular MMP, but also for MMP affecting other sites, have been outlined above. We have some preliminary data using short peptides of BP180 that appear to be more sensitive than currently available substrates for the identification of autoantibodies in ocular MMP. An alternative approach has been used for the investigation of trachoma phenotypes using genetic biomarkers, that are up and down regulated during the inflammation, scarring and resolution ^{160,161}. A similar approach in MMP, and other causes of CC, may be expected to provide an alternative route to autoantibody detection, for both diagnosis and for the assessment of disease activity.

b. Biomarkers of disease activity

More objective biomarkers than clinical slit-lamp examination are needed to identify for both inflammatory and scarring activity, to help identify the effects of therapy and guide their initiation and withdrawal. Some progress has been made using neutrophils and their products for this 100,101. Expression of genes that are up and down regulated in inflammation and scarring may also be worth investigating for this purpose.

c. Biological drugs for MMP

Conventional non biological immunomodulatory therapy with cyclophosphamide is effective antiinflammatory therapy from between 80% to 92% of cases in the larger series. However, the CD20
monoclonal Rituximab has been increasingly used as rescue therapy for patients with severe ocular
MMP who have failed cyclophosphamide therapy and with combined success rates of 80% in this
group. Although efforts have been made to carry out a randomised trial of this drug in ocular MMP
the cost of therapy has prohibited this, and manufacturers have been unwilling to support research
into an orphan indication. However, there are numerous CD20 monoclonal biosimilars in development
and it is probable that these drugs will become available for prospective case series, and randomised

controlled trials. The latter are needed to establish the effect of this drug in a number of areas. These include the effect in early ocular MMP, as opposed to unresponsive later stage disease. The length of remission, evidence for the induction of tolerance to autoantigens, and the identification of responders and non-responders needs to be investigated, as has been done in pemphigus¹⁶². The optimum treatment regimen needs to be established, and the side effect profile compared to that of the other drugs used in this, relatively elderly, patient group having MMP.

742

743

744

745

746

747

748

749

750

751

752

753

754

755

756

757

758

759

760

761

736

737

738

739

740

741

d. Topical therapies for inflammation and scarring

There is an unmet need for effective topical therapies for inflammation and scarring to eliminate the morbidity associated with the side effects of systemic therapies. There are potential new topical therapies for both scarring and inflammation in CC. We have outlined the evidence for a promising new therapy, using ALDH inhibition, which has the potential to control scarring with topical therapy. ALDH inhibition is also in development elsewhere as an anti-inflammatory therapy for allergic eye disease and anterior uveitis. Rapidly effective anti-scarring therapy for CC is critical in preventing morbidity, given the speed of scarring during severe inflammatory episodes which take 2-4 months to resolve with current anti-inflammatory therapies in ocular MMP, and which are also very rapid in diseases like Stevens-Johnson syndrome. The latter is another additional target disease for topical anti-scarring therapy which will be effective during acute phase inflammation, when most of the scarring occurs. Perfenidone is a new class of immunosuppressant with anti-inflammatory and antifibrotic effects. Its mechanisms of action are not fully established. In different model systems it has been shown to abrogate TGF-β1-stimulated collagen synthesis by inhibiting the upregulation of HSP47 and Col1 RNA, it blocks the proliferative effects of PDGF; reduces fibroblast proliferation and downregulates the proinflammatory cytokines, TNFα, IFNγ, IL-1B and IL-6¹⁶³. Topical application has been studied in human scleroderma¹⁶⁴ and in glaucoma scarring models¹⁶⁵. Lastly, the topical use of anti-inflammatory monoclonal antibodies, such as the anti TNF α drugs which could be given subconjunctivally, should be evaluated.

Although a lot has been done in the last 30 years, the tools for understanding the pathogenesis of these diseases have expanded exponentially, and the potential for new therapies has seldom been greater, for patients cursed with these diseases.

Acknowledgements

Manuscript. For the following illustrations: Dr Jane Setterfield for Figure 5 mucosal lichen planus, and Figure 7 D, B, E-F. Professor Mike Gleeson for Figure 7 C, Professor Enno Schmidt for pemphigus vulgaris in Figure 5. Dr John Mee, of the St John's Institute of Dermatology Immunofluorescence Laboratory, for the DIF images of pemphigus, pemphigoid and the IIF image in Figure 5. For review of the pathogenesis section of the manuscript: Professor Enno Schmidt, Professor Detlef Zillikens, Dr Virginia Calder and Dr Danny Saban. For their review of the entire manuscript Dr Saaeha Rauz and Mr Ewan Craig.

Collaborators for these studies: This work is the result of collaboration with a lot of individuals: Dr J. Setterfield and Professor M. Gleeson of Guys and St Thomas's Hospital and King's College London, Professor V. Lund and Mr G. Sandhu of the UCL Royal National ENT Hospital, Ms. S. Rauz and Mr G. Williams of the Institute of Inflammation and Ageing, University of Birmingham, Dr E. Schmidt, Professor D. Zillikens of the Dept of Dermatology, Luebeck, Germany Dr Chuan-Hui Kuo and Dr K. Morohoshi of the Dept. of Ophthalmology, Division of Allergy and Immunology, Cincinnati Children's Hospital, USA, Mr R. Keeler, Archivist at the Royal College of Ophthalmologists, Professor J. Daniels, Dr V. Calder and Dr S. Ahadome of the UCL Institute of Ophthalmology, Professor D. Abraham, Dr J. Norman and Mr S. Rayurapeddi of the Research Department of Inflammation, Division of Medicine, UCL Royal Free, Dr W. Bernauer, Mr M. Elder, Dr V. Saw, Dr C. Bunce, Sister M. Mason, and Dr C. Radford of Moorfields Eye Hospital NHS Foundation Trust, Dr D. Minassian of Epivision, UK and Mrs A Dart. Also, many other members of Staff at Moorfields Eye Hospital Foundation Trust and the UCL Institute of Ophthalmology.

Grant funding: The studies that are reported here, from me and my collaborators, have been generously funded by the following businesses, charities and individuals: UCL Business, Fight for Sight, NIHR Biomedical Research Centre for Ophthalmology, Action Medical Research, Moorfields League of Friends, Moorfields Special Trustees (now Moorfields Eye Charities), Anonymous Donors for this research through Moorfields Eye Charities and Guide Dogs for the Blind.

795 References

- 796 1. Power, H. Prefatory Memoir. in The Collected Papers of Sir W Bowman Bart. FRS, Vol. 1 (eds. J, B.-S. & JW,
- H.) 13 (Harrison and Sons, St Martin's Lane, London, 1892).
- 798 2. James, R.R. BRITISH MASTERS OF OPHTHALMOLOGY SERIES: 16.-SIR WILLIAM BOWMAN, BART., F.R.S.,
- 799 1816-1892. *Br J Ophthalmol* **9**, nil2-494 (1925).
- 800 3. Thorne, J.E., Anhalt, G.J. & Jabs, D.A. Mucous membrane pemphigoid and pseudopemphigoid.
- 801 *Ophthalmology* **111**, 45-52 (2004).
- 4. Radford, C.F., Rauz, S., Williams, G.P., Saw, V.P. & Dart, J.K. Incidence, presenting features, and diagnosis of
- cicatrising conjunctivitis in the United Kingdom. Eye (Lond) 26, 1199-1208 (2012).
- 804 5. Guglielmetti, S., Dart, J.K. & Calder, V. Atopic keratoconjunctivitis and atopic dermatitis. *Current opinion in*
- 805 *allergy and clinical immunology* **10**, 478-485 (2010).
- 806 6. Shikari, H., Antin, J.H. & Dana, R. Ocular graft-versus-host disease: a review. Survey of ophthalmology 58,
- 807 233-251 (2013).
- 808 7. O'Donnell, J.J., Jr., Karakus, S., Doroslovacki, P. & Akpek, E.K. Sarcoidosis Presenting with Cicatrizing
- 809 Conjunctivitis. Optometry and vision science: official publication of the American Academy of Optometry
- **92**, e173-175 (2015).
- 811 8. Saw, V.P., Dart, J.K., Sitaru, C. & Zillikens, D. Cicatrising conjunctivitis with anti-basement membrane
- autoantibodies in ectodermal dysplasia. *Br J Ophthalmol* **92**, 1403-1410 (2008).
- 9. Schmidt, E. & Zillikens, D. Pemphigoid diseases. *Lancet* **381**, 320-332 (2013).
- 814 10. Dart, J. Corneal toxicity: the epithelium and stroma in iatrogenic and factitious disease. Eye (Lond) 17, 886-
- 815 892 (2003).
- 11. De Rojas, M.V., Dart, J.K. & Saw, V.P. The natural history of Stevens Johnson syndrome: patterns of chronic
- 817 ocular disease and the role of systemic immunosuppressive therapy. Br J Ophthalmol 91, 1048-1053
- 818 (2007).
- 819 12. Loeffler, K. & Perlman, J. Diffuse intraepithelial sebaceous carcinoma of the conjunctiva. *Br J Ophthalmol*
- **820 81**, 168 (1997).
- 13. Foster, C.S. & Calonge, M. Atopic Keratoconjunctivitis. *Ophthalmology* **97**, 992-1000 (1990).
- 14. Taylor, H.R., Burton, M.J., Haddad, D., West, S. & Wright, H. Trachoma. *Lancet* **384**, 2142-2152 (2014).

- 823 15. Rajak, S.N., et al. The clinical phenotype of trachomatous trichiasis in Ethiopia: not all trichiasis is due to
- 824 entropion. *Invest Ophthalmol Vis Sci* **52**, 7974-7980 (2011).
- 16. Chintakuntlawar, A.V. & Chodosh, J. Cellular and tissue architecture of conjunctival membranes in
- 826 epidemic keratoconjunctivitis. Ocular immunology and inflammation 18, 341-345 (2010).
- 827 17. Hammer, L.H., Perry, H.D., Donnenfeld, E.D. & Rahn, E.K. Symblepharon formation in epidemic
- 828 keratoconjunctivitis. *Cornea* **9**, 338-340 (1990).
- 18. Tullo, A.B. & Higgins, P.G. An outbreak of adenovirus keratoconjunctivitis in bristol. *Br J Ophthalmol* **63**,
- 830 621-626 (1979).
- 831 19. Wright, P. Cicatrizing conjunctivitis. *Transactions of the ophthalmological societies of the United Kingdom*
- **105 (Pt 1)**, 1-17 (1986).
- 20. Mendez, C.A. & Singh, A.D. Radiation therapy: anterior segment complications. *Developments in*
- 834 *ophthalmology* **52**, 102-113 (2013).
- 835 21. Creamer, D., et al. U.K. guidelines for the management of Stevens-Johnson syndrome/toxic epidermal
- necrolysis in adults 2016. *Br J Dermatol* **174**, 1194-1227 (2016).
- 837 22. Mitsuya, J., et al. Metastatic ovarian carcinoma-associated subepidermal blistering disease with
- 838 autoantibodies to both the p200 dermal antigen and the gamma 2 subunit of laminin 5 showing unusual
- 839 clinical features. *Br J Dermatol* **158**, 1354-1357 (2008).
- 840 23. Solomon, L.W., Helm, T.N., Stevens, C., Neiders, M.E. & Kumar, V. Clinical and immunopathologic findings
- in oral lichen planus pemphigoides. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **103**, 808-813 (2007).
- 842 24. Henry, S. Recognizing presentations of pemphigoid gestationis: a case study. Case reports in obstetrics and
- 843 *gynecology* **2014**, 415163 (2014).
- Venning, V.A., Frith, P.A., Bron, A.J., Millard, P.R. & Wojnarowska, F. Mucosal involvement in bullous and
- cicatricial pemphigoid. A clinical and immunopathological study. Br J Dermatol 118, 7-15 (1988).
- 26. Elder, M.J., Bernauer, W., Leonard, J. & Dart, J.K. Progression of disease in ocular cicatricial pemphigoid. *Br*
- 847 *J Ophthalmol* **80**, 292-296 (1996).
- 27. Ahuero, A.E., Jakobiec, F.A., Bhat, P., Ciralsky, J.B. & Papaliodis, G.N. Paraneoplastic conjunctival
- cicatrization: two different pathogenic types. *Ophthalmology* **117**, 659-664 (2010).
- 28. Daoud, Y.J., Foster, C.S. & Razzaque Ahmed, A. Eyelid Skin Involvement in Pemphigus Foliaceus. *Ocular*
- 851 *immunology and inflammation* **13**, 389-394 (2009).

- 852 29. Broussard, K.C., Leung, T.G., Moradi, A., Thorne, J.E. & Fine, J.D. Autoimmune bullous diseases with skin
- 853 and eye involvement: Cicatricial pemphigoid, pemphigus vulgaris, and pemphigus paraneoplastica. Clinics
- 854 in dermatology **34**, 205-213 (2016).
- 855 30. Chirinos-Saldana, P., et al. Cicatricial changes in ocular pemphigus. Eye (Lond) 28, 459-465 (2014).
- 31. Karamursel Akpek, E., Merchant, A., Pinar, V. & Foster, C.S. Ocular Rosacea. Ophthalmology 104, 1863-
- 857 1867 (1997).
- 858 32. Gorouhi, F., Davari, P. & Fazel, N. Cutaneous and mucosal lichen planus: a comprehensive review of clinical
- subtypes, risk factors, diagnosis, and prognosis. ScientificWorldJournal 2014, 742826 (2014).
- 860 33. Thorne, J.E., et al. Lichen planus and cicatrizing conjunctivitis: characterization of five cases. Am J
- 861 *Ophthalmol* **136**, 239-243 (2003).
- 34. Burge, S.M., Frith, P.A., Juniper, R.P. & Wojnarowska, F. Mucosal involvement in systemic and chronic
- cutaneous lupus erythematosus. *Br J Dermatol* **121**, 727-741 (1989).
- 35. Park, A.J., Webster, G.F., Penne, R.B. & Raber, I.M. Porphyria cutanea tarda presenting as cicatricial
- 865 conjunctivitis. *Am J Ophthalmol* **134**, 619-621 (2002).
- 866 36. Saw, V.P., et al. Immunosuppressive Therapy for Ocular Mucous Membrane Pemphigoid Strategies and
- 867 Outcomes. *Ophthalmology* **115**, 253-261 (2008).
- 868 37. Sullivan, B.D., et al. Clinical utility of objective tests for dry eye disease: variability over time and
- implications for clinical trials and disease management. *Cornea* **31**, 1000-1008 (2012).
- 870 38. Alvarenga, L.S. & Mannis, M.J. Ocular Rosacea. *The Ocular Surface* 3, 41-58 (2005).
- 871 39. Williams, G.P., Radford, C., Nightingale, P., Dart, J.K. & Rauz, S. Evaluation of early and late presentation of
- patients with ocular mucous membrane pemphigoid to two major tertiary referral hospitals in the United
- 873 Kingdom. *Eye (Lond)* **25**, 1207-1218 (2011).
- 874 40. Spaide, R.F. & Ward, D.L. Conjunctival biopsy in the diagnosis of sarcoidosis. *Br J Ophthalmol* 74, 469-471
- 875 (1990).
- 876 41. Servat, J.J. & Bernardino, C.R. Effects of common topical antiglaucoma medications on the ocular surface,
- 877 eyelids and periorbital tissue. *Drugs & aging* **28**, 267-282 (2011).
- 42. Pohla-Gubo, G. & Hintner, H. Direct and indirect immunofluorescence for the diagnosis of bullous
- autoimmune diseases. *Dermatologic clinics* **29**, 365-372, vii (2011).

- 43. Baum, S., Sakka, N., Artsi, O., Trau, H. & Barzilai, A. Diagnosis and classification of autoimmune blistering
- diseases. *Autoimmunity reviews* **13**, 482-489 (2014).
- 44. Chan, L.S., et al. The first international consensus on mucous membrane pemphigoid: definition, diagnostic
- criteria, pathogenic factors, medical treatment, and prognostic indicators. Arch. Dermatol. 138, 370-379
- 884 (2002).
- 885 45. Nayar, M., Wojnarowska, F., Venning, V. & Taylor, C.J. Association of autoimmunity and cicatricial
- pemphigoid: is there an immunogenetic basis? J Am Acad Dermatol 25, 1011-1015 (1991).
- 46. Foster, C.S. Cicatricial pemphigoid. *Trans.Am.Ophthalmol.Soc.* **84**, 527-663 (1986).
- 47. Bernauer, W., Elder, M.J., Leonard, J.N., Wright, P. & Dart, J.K. The value of biopsies in the evaluation of
- chronic progressive conjunctival cicatrisation. *Graefes Arch.Clin.Exp.Ophthalmol.* **232**, 533-537 (1994).
- 48. Power, W.J., Neves, R.A., Rodriguez, A., Dutt, J.E. & Foster, C.S. Increasing the diagnostic yield of
- conjunctival biopsy in patients with suspected ocular cicatricial pemphigoid. *Ophthalmology* **102**, 1158-
- 892 1163 (1995).
- 893 49. Grau, A.E., Setterfield, J. & Saw, V.P. How to do conjunctival and buccal biopsies to investigate cicatrising
- conjunctivitis: improving the diagnosis of ocular mucous membrane pemphigoid. Br J Ophthalmol 97, 530-
- 895 531 (2013).
- 896 50. Power, W.J., Tugal-Tutkun, I. & Foster, C.S. Long-term follow-up of patients with atopic
- keratoconjunctivitis. *Ophthalmology* **105**, 637-642 (1998).
- 898 51. Magro, C.M., Roberts-Barnes, J. & Crowson, A.N. Direct immunofluorescence testing in the diagnosis of
- 899 immunobullous disease, collagen vascular disease, and vascular injury syndromes. Dermatologic clinics 30,
- 900 763-798, viii (2012).
- 901 52. Lever, W.F. PEMPHIGUS. *Medicine* **32**, 1-123 (1953).
- 902 53. Thorne, J.E., Jabs, D.A., Nikolskaia, O., Anhalt, G. & Nousari, H.C. Discoid lupus erythematosus and
- 903 cicatrizing conjunctivitis: clinicopathologic study of two cases. Ocular immunology and inflammation 10,
- 904 287-292 (2009).
- 905 54. Leonard, J.N., et al. Immunofluorescent studies in ocular cicatricial pemphigoid. Br.J.Dermatol. 118, 209-
- 906 217 (1988).
- 907 55. Butt, Z., Kaufman, D., McNab, A. & McKelvie, P. Drug-induced ocular cicatricial pemphigoid: a series of
- 908 clinico-pathological reports. *Eye* **12 (Pt 2)**, 285-290 (1998).

- 909 56. Tauber, J. Ocular cicatricial pemphigoid. *Ophthalmology* **115**, 1639-1640; author reply 1640-1631 (2008).
- 910 57. Dart, J.K. & Saw, V.P. Immune Modulation in Ocular Mucous Membrane Pemphigoid. in *Immune*
- 911 Modulation and Anti-Inflammatory Therapy in Ocular Disorders IOIS Guidelines (eds. Pleyer, U., Alió, J.,
- 912 Barisani-Asenbauer, T., Le Hoang, P. & Rao, N.A.) 19-37 (Springer Berlin Heidelberg, 2014).
- 913 58. Leenutaphong, V., von Kries, R. & Plewig, G. Localized cicatricial pemphigoid (Brunsting-Perry): electron
- 914 microscopic study. *J Am Acad Dermatol* **21**, 1089-1093 (1989).
- 915 59. Hardy, K.M., Perry, H.O., Pingree, G.C. & Kirby, T.J., Jr. Benign mucous membrane pemphigoid.
- 916 *Arch.Dermatol.* **104**, 467-475 (1971).
- 917 60. De, D., et al. Clinical, demographic and immunopathological spectrum of subepidermal autoimmune
- 918 bullous diseases at a tertiary center: A 1-year audit. Indian journal of dermatology, venereology and
- 919 *leprology* **82**, 358 (2016).
- 920 61. Jin, P., Shao, C. & Ye, G. Chronic bullous dermatoses in China. Int J Dermatol 32, 89-92 (1993).
- 921 62. Wong, S.N. & Chua, S.H. Spectrum of subepidermal immunobullous disorders seen at the National Skin
- 922 Centre, Singapore: a 2-year review. *Br J Dermatol* **147**, 476-480 (2002).
- 923 63. Saw, V.P.J., Dart, J.K.G. & Reinhard T, L.F. Management of ocular mucous membrane pemphigoid. in
- 924 Cornea and External Eye Disease (eds. Kriegelstein, G.K. & Weinreb, R.N.) 154-175 (Springer, Berlin
- 925 Heidelberg, 2008).
- 926 64. Saw, V.P. & Dart, J.K. Ocular mucous membrane pemphigoid: diagnosis and management strategies. *Ocul*
- 927 *Surf* **6**, 128-142 (2008).
- 928 65. Setterfield, J., et al. Mucous membrane pemphigoid: HLA-DQB1*0301 is associated with all clinical sites of
- 929 involvement and may be linked to antibasement membrane IgG production. Br.J.Dermatol. 145, 406-414
- 930 (2001).
- 931 66. Chan, L.S., Soong, H.K., Foster, C.S., Hammerberg, C. & Cooper, K.D. Ocular cicatricial pemphigoid occurring
- 932 as a seguela of Stevens-Johnson syndrome. *JAMA* **266**, 1543-1546 (1991).
- 933 67. Tauber, J., Melamed, S. & Foster, C.S. Glaucoma in patients with ocular cicatricial pemphigoid.
- 934 *Ophthalmology* **96**, 33-37 (1989).
- 935 68. Black, A.P., et al. Rapid effector function of circulating NC16A-specific T cells in individuals with mucous
- 936 membrane pemphigoid. *Br J Dermatol* **151**, 1160-1164 (2004).

- 937 69. Setterfield, J., et al. Cicatricial pemphigoid: serial titres of circulating IgG and IgA antibasement membrane
- 938 antibodies correlate with disease activity. Br.J.Dermatol. 140, 645-650 (1999).
- 939 70. Hertl, M. Humoral and cellular autoimmunity in autoimmune bullous skin disorders. *International archives*
- 940 *of allergy and immunology* **122**, 91-100 (2000).
- 941 71. Murphy, K. & Weaver, C. Janeway's Immunobiology. 652-669 (Garland Science, New York and Oxford,
- 942 2016).
- 943 72. Torchia, D., Caproni, M., Volpi, W. & Fabbri, P. Naturally occurring regulatory T cells in mucous membrane
- 944 pemphigoid lesions. Acta dermatovenerologica Alpina, Pannonica, et Adriatica 18, 3-6 (2009).
- 945 73. Lutz, H.U. & Miescher, S. Natural antibodies in health and disease: an overview of the first international
- 946 workshop on natural antibodies in health and disease. *Autoimmunity reviews* **7**, 405-409 (2008).
- 947 74. Prussmann, W., et al. Prevalence of pemphigus and pemphigoid autoantibodies in the general population.
- 948 Orphanet journal of rare diseases 10, 63 (2015).
- 949 75. Wieland, C.N., et al. Anti-bullous pemphigoid 180 and 230 antibodies in a sample of unaffected subjects.
- 950 *Arch Dermatol* **146**, 21-25 (2010).
- 951 76. van Beek, N., et al. Serological diagnosis of autoimmune bullous skin diseases: prospective comparison of
- 952 the BIOCHIP mosaic-based indirect immunofluorescence technique with the conventional multi-step single
- 953 test strategy. *Orphanet journal of rare diseases* **7**, 49 (2012).
- 954 77. Desai, N., Allen, J., Ali, I., Venning, V. & Wojnarowska, F. Autoantibodies to basement membrane proteins
- 955 BP180 and BP230 are commonly detected in normal subjects by immunoblotting. Australas J Dermatol 49,
- 956 137-141 (2008).
- 957 78. Hachisuka, H., Kurose, K., Karashima, T., Mori, O. & Maeyama, Y. Serum from normal elderly individuals
- contains anti-basement membrane zone antibodies. Arch Dermatol 132, 1201-1205 (1996).
- 959 79. Budinger, L., et al. Identification and characterization of autoreactive T cell responses to bullous
- pemphigoid antigen 2 in patients and healthy controls. J Clin Invest 102, 2082-2089 (1998).
- 961 80. Thorlacius-Ussing, G., Sorensen, J.F., Wandall, H.H. & Pedersen, A.E. Auto-reactive T cells revised.
- 962 Overestimation based on methodology? *Journal of immunological methods* **420**, 56-59 (2015).
- 963 81. Rosser, E.C. & Mauri, C. Regulatory B cells: origin, phenotype, and function. *Immunity* 42, 607-612 (2015).
- 82. Kabuto, M., Fujimoto, N., Takahashi, T. & Tanaka, T. Decreased level of IL-10-producing B cells in patients
- with pemphigus but not with pemphigoid. *Br J Dermatol* (2016).

- 966 83. Marino, E. & Grey, S.T. B cells as effectors and regulators of autoimmunity. *Autoimmunity* **45**, 377-387
- 967 (2012).
- 968 84. Tesavibul, N., et al. Costimulatory molecules in ocular cicatricial pemphigoid. *Invest Ophthalmol.Vis.Sci.* 39,
- 969 982-988 (1998).
- 970 85. Bernauer, W., Wright, P., Dart, J.K., Leonard, J.N. & Lightman, S. The Conjunctiva in Acute and Chronic
- 971 Mucous Membrane Pemphigoid. *Ophthalmology* **100**, 339-346 (1993).
- 972 86. Letko, E., Bhol, K., Colon, J., Foster, C.S. & Ahmed, A.R. Biology of interleukin-5 in ocular cicatricial
- pemphigoid. Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur
- 974 klinische und experimentelle Ophthalmologie **240**, 565-569 (2002).
- 975 87. Elder, M.J., Dart, J.K. & Lightman, S. Conjunctival fibrosis in ocular cicatricial pemphigoid--the role of
- 976 cytokines. *Exp.Eye Res.* **65**, 165-176 (1997).
- 977 88. Saw, V.P., et al. Tumor necrosis factor-alpha in ocular mucous membrane pemphigoid and its effect on
- 978 conjunctival fibroblasts. *Invest Ophthalmol Vis Sci* **50**, 5310-5317 (2009).
- 979 89. Saw, V.P., et al. Conjunctival interleukin-13 expression in mucous membrane pemphigoid and functional
- 980 effects of interleukin-13 on conjunctival fibroblasts in vitro. Am J Pathol 175, 2406-2415 (2009).
- 98.1 90. Razzaque, M.S., Foster, C.S. & Ahmed, A.R. Role of collagen-binding heat shock protein 47 and
- 982 transforming growth factor-beta1 in conjunctival scarring in ocular cicatricial pemphigoid. *Invest*
- 983 *Ophthalmol.Vis.Sci.* **44**, 1616-1621 (2003).
- 984 91. Razzaque, M.S., Foster, C.S. & Ahmed, A.R. Role of Macrophage Migration Inhibitory Factor in Conjunctival
- 985 Pathology in Ocular Cicatricial Pemphigoid. Investigative Opthalmology & Visual Science 45, 1174 (2004).
- 986 92. Razzaque, M.S., Foster, C.S. & Ahmed, A.R. Role of enhanced expression of m-CSF in conjunctiva affected
- 987 by cicatricial pemphigoid. *Invest Ophthalmol. Vis. Sci.* **43**, 2977-2983 (2002).
- 988 93. Razzaque, M.S., Foster, C.S. & Ahmed, A.R. Role of Connective Tissue Growth Factor in the Pathogenesis of
- 989 Conjunctival Scarring in Ocular Cicatricial Pemphigoid. Investigative Opthalmology & Visual Science 44,
- 990 1998 (2003).
- 991 94. Kasperkiewicz, M., Zillikens, D. & Schmidt, E. Pemphigoid diseases: pathogenesis, diagnosis, and treatment.
- 992 *Autoimmunity* **45**, 55-70 (2012).
- 95. Kourosh, A.S. & Yancey, K.B. Pathogenesis of mucous membrane pemphigoid. *Dermatologic clinics* 29, 479-
- 994 484, x (2011).

- 96. Kirzhner, M. & Jakobiec, F.A. Ocular cicatricial pemphigoid: a review of clinical features, immunopathology,
- 996 differential diagnosis, and current management. Seminars in ophthalmology 26, 270-277 (2011).
- 997 97. Saw, V.P., et al. Profibrotic phenotype of conjunctival fibroblasts from mucous membrane pemphigoid. Am
- 998 *J Pathol* **178**, 187-197 (2011).
- 98. Ahadome, S.D., et al. Aldehyde dehydrogenase inhibition blocks mucosal fibrosis in human and mouse
- ocular scarring. *JCI Insight* **1**, e87001 (2016).
- 1001 99. Ahadome, S.D., et al. Classical dendritic cells mediate fibrosis directly via the retinoic acid pathway in
- severe eye allergy. JCI Insight 1(2016).
- 1003 100. Williams, G.P., et al. Conjunctival Neutrophils Predict Progressive Scarring in Ocular Mucous Membrane
- 1004 Pemphigoid. *Invest Ophthalmol Vis Sci* **57**, 5457-5469 (2016).
- 1005 101. Arafat, S.N., et al. Neutrophil collagenase, gelatinase, and myeloperoxidase in tears of patients with
- stevens-johnson syndrome and ocular cicatricial pemphigoid. Ophthalmology 121, 79-87 (2014).
- 1007 102.Bernauer, W., Wright, P., Dart, J.K., Leonard, J.N. & Lightman, S. Cytokines in the conjunctiva of acute and
- 1008 chronic mucous membrane pemphigoid: an immunohistochemical analysis. *Graefes*
- 1009 *Arch.Clin.Exp.Ophthalmol.* **231**, 563-570 (1993).
- 1010 103.Lee, S.J., Li, Z., Sherman, B. & Foster, C.S. Serum levels of tumor necrosis factor-alpha and interleukin-6 in
- ocular cicatricial pemphigoid. *Invest Ophthalmol.Vis.Sci.* **34**, 3522-3525 (1993).
- 1012 104.Morris M, R.H. Pemphigus of the skin and mucous membranes of the mouth associated with "essentil
- shrinking" and pemphigus of the conjunctivae". Br J Dermatol 1, 175-181 (1889).
- 1014 105.Swanzy HR. A Handbook of the Diseases of the Eye and their Treatment, (HK Lewis, London, 1895).
- 1015 106. Sorsby, A. Systemic Ophthalmology, (Butterworth and Mosby, London and St Louis, 1951).
- 1016 107. Miller, S. *Parson's Diseases Of The Eye*, (Churchill Livingstone, Edinburgh, London, New York, 1978).
- 1017 108.Rogers, R.S., III, Seehafer, J.R. & Perry, H.O. Treatment of cicatricial (benign mucous membrane)
- pemphigoid with dapsone. *J.Am.Acad.Dermatol.* **6**, 215-223 (1982).
- 1019 109. Mondino, B.J., Brown, S.I., Lempert, S. & Jenkins, M.S. The acute manifestations of ocular cicatricial
- pemphigoid: diagnosis and treatment. *Ophthalmology* **86**, 543-555 (1979).
- 1021 110. Foster, C.S. Immunosuppressive Therapy for External Ocular Inflammatory Disease. Ophthalmology 87,
- 1022 140-150 (1980).

- 1023 111. Stephen Foster, C., Wilson, L.A. & Ekins, M.B. Immunosuppressive Therapy for Progressive Ocular
- 1024 Cicatncial Pemphigoid. Ophthalmology 89, 340-353 (1982).
- 1025 112. Holland, E.J., et al. Topical cyclosporin A in the treatment of anterior segment inflammatory disease.
- 1026 *Cornea* **12**, 413-419 (1993).
- 1027 113. Sacher, C. & Hunzelmann, N. Cicatricial pemphigoid (mucous membrane pemphigoid): current and
- emerging therapeutic approaches. *Am.J Clin.Dermatol.* **6**, 93-103 (2005).
- 1029 114. Chang, J.H. & McCluskey, P.J. Ocular cicatricial pemphigoid: manifestations and management. *Current*
- 1030 allergy and asthma reports **5**, 333-338 (2005).
- 1031 115. Mondino, B.J. & Brown, S.I. Ocular cicatricial pemphigoid. *Ophthalmology* 88, 95-100 (1981).
- 1032 116.Mondino, B.J. & Brown, S.I. Immunosuppressive therapy in ocular cicatricial pemphigoid. Am.J.Ophthalmol
- **96**, 453-459 (1983).
- 1034 117. Elder, M.J., Bernauer, W., Leonard, J. & Dart, J.K. Progression of disease in ocular cicatricial pemphigoid.
- 1035 *Br.J.Ophthalmol.* **80**, 292-296 (1996).
- 1036 118. Miserocchi, E., Baltatzis, S., Roque, M.R., Ahmed, A.R. & Foster, C.S. The effect of treatment and its related
- side effects in patients with severe ocular cicatricial pemphigoid. Ophthalmology 109, 111-118 (2002).
- 1038 119.Rauz, S., Maddison, P.G. & Dart, J.K. Evaluation of mucous membrane pemphigoid with ocular involvement
- in young patients. *Ophthalmology* **112**, 1268-1274 (2005).
- 1040 120. Elder, M.J. The role of cytokines in chronic progressive conjunctival cicatrisation. Dev. Ophthalmol. 28, 159-
- 1041 175 (1997).
- 1042 121.Bernauer, W., Wright, P., Dart, J.K., Leonard, J.N. & Lightman, S. The conjunctiva in acute and chronic
- mucous membrane pemphigoid. An immunohistochemical analysis. Ophthalmology 100, 339-346 (1993).
- 1044 122.Saw, V.P., et al. Tumor necrosis factor-alpha in ocular mucous membrane pemphigoid and its effect on
- conjunctival fibroblasts. *Invest Ophthalmol Vis Sci* **50**, 5310-5317 (2009).
- 1046 123. Geerling, G. & Dart, J.K. Management and outcome of cataract surgery in ocular cicatricial pemphigoid.
- 1047 *Graefes Arch Clin.Exp.Ophthalmol* **238**, 112-118 (2000).
- 1048 124.Foster, C.S., Wilson, L.A. & Ekins, M.B. Immunosuppressive therapy for progressive ocular cicatricial
- 1049 pemphigoid. *Ophthalmology* **89**, 340-353 (1982).
- 1050 125. Mondino, B.J. Cicatricial pemphigoid and erythema multiforme. *Ophthalmology* **97**, 939-952 (1990).

- 1051 126.Doan, S., et al. Treatment of ocular cicatricial pemphigoid with sulfasalazine. Ophthalmology 108, 1565-
- 1052 1568 (2001).
- 1053 127. Elder, M.J., Leonard, J. & Dart, J.K. Sulphapyridine--a new agent for the treatment of ocular cicatricial
- 1054 pemphigoid. *Br.J.Ophthalmol* **80**, 549-552 (1996).
- 1055 128. Elder, M.J., Lightman, S. & Dart, J.K. Role of cyclophosphamide and high dose steroid in ocular cicatricial
- 1056 pemphigoid. *Br.J.Ophthalmol* **79**, 264-266 (1995).
- 1057 129. Foster, C.S., Neumann, R. & Tauber, J. Long-term results of systemic chemotherapy for ocular cicatricial
- 1058 pemphigoid. *Doc.Ophthalmol.* **82**, 223-229 (1992).
- 1059 130.Letko, E., Ahmed, A.R. & Foster, C.S. Treatment of ocular cicatricial pemphigoid with tacrolimus (FK 506).
- 1060 *Graefes Arch. Clin. Exp. Ophthalmol.* **239**, 441-444 (2001).
- 1061 131.McCluskey, P., Chang, J.H., Singh, R. & Wakefield, D. Methotrexate therapy for ocular cicatricial
- pemphigoid. *Ophthalmology* **111**, 796-801 (2004).
- 1063 132. Neumann, R., Tauber, J. & Foster, C.S. Remission and recurrence after withdrawal of therapy for ocular
- cicatricial pemphigoid. *Ophthalmology* **98**, 858-862 (1991).
- 1065 133.Tauber, J., Sainz, D.L.M. & Foster, C.S. Systemic chemotherapy for ocular cicatricial pemphigoid. *Cornea* 10,
- 1066 185-195 (1991).
- 1067 134.Thorne, J.E., Woreta, F.A., Jabs, D.A. & Anhalt, G.J. Treatment of ocular mucous membrane pemphigoid
- with immunosuppressive drug therapy. *Ophthalmology* **115**, 2146-2152 e2141 (2008).
- 1069 135. Reiche, L., Wojnarowska, F. & Mallon, E. Combination therapy with nicotinamide and tetracyclines for
- 1070 cicatricial pemphigoid: further support for its efficacy. Clin. Exp. Dermatol. 23, 254-257 (1998).
- 1071 136. Knight, A., Askling, J., Granath, F., Sparen, P. & Ekbom, A. Urinary bladder cancer in Wegener's
- granulomatosis: risks and relation to cyclophosphamide. *Ann Rheum Dis* **63**, 1307-1311 (2004).
- 1073 137.Bernatsky, S., Ramsey-Goldman, R. & Clarke, A.E. Malignancies and cyclophosphamide exposure in
- 1074 Wegener's granulomatosis. *J Rheumatol* **35**, 11-13 (2008).
- 138. Durrani, K., Papaliodis, G.N. & Foster, C.S. Pulse IV cyclophosphamide in ocular inflammatory disease:
- efficacy and short-term safety. *Ophthalmology* **111**, 960-965 (2004).
- 1077 139. Harper, L., et al. Pulse versus daily oral cyclophosphamide for induction of remission in ANCA-associated
- vasculitis: long-term follow-up. *Ann Rheum Dis* **71**, 955-960 (2012).

1079 140.Zurdel, J., et al. [Early clinical results with mycophenolate mofetil in immunosuppressive therapy of ocular 1080 pemphigoid]. Klin. Monatsbl. Augenheilkd. 218, 222-228 (2001). 1081 141.Letko, E., et al. A nonrandomized comparison of the clinical outcome of ocular involvement in patients 1082 with mucous membrane (cicatricial) pemphigoid between conventional immunosuppressive and 1083 intravenous immunoglobulin therapies. Clin.Immunol. 111, 303-310 (2004). 1084 142. Sami, N., et al. Intravenous immunoglobulin therapy in patients with ocular-cicatricial pemphigoid: a long-1085 term follow-up. Ophthalmology 111, 1380-1382 (2004). 1086 143. John, H., Whallett, A. & Quinlan, M. Successful biologic treatment of ocular mucous membrane 1087 pemphigoid with anti-TNF-alpha. Eye (Lond) 21, 1434-1435 (2007). 1088 144. Prey, S., et al. Treatment of ocular cicatricial pemphigoid with the tumour necrosis factor alpha antagonist 1089 etanercept. Acta Derm Venereol 87, 74-75 (2007). 1090 145. Sacher, C., et al. Treatment of recalcitrant cicatricial pemphigoid with the tumor necrosis factor alpha 1091 antagonist etanercept. J Am. Acad. Dermatol. 46, 113-115 (2002). 1092 146. Canizares, M.J., Smith, D.I., Conners, M.S., Maverick, K.J. & Heffernan, M.P. Successful treatment of 1093 mucous membrane pemphigoid with etanercept in 3 patients. Arch Dermatol 142, 1457-1461 (2006). 1094 147. Heffernan, M.P. & Bentley, D.D. Successful treatment of mucous membrane pemphigoid with infliximab. 1095 Arch Dermatol. 142, 1268-1270 (2006). 1096 148.Le Roux-Villet, C., et al. Rituximab for Patients With Refractory Mucous Membrane Pemphigoid. Arch 1097 Dermatol (2011). 1098 149. Taverna, J.A., Lerner, A., Bhawan, J. & Demierre, M.F. Successful adjuvant treatment of recalcitrant 1099 mucous membrane pemphigoid with anti-CD20 antibody rituximab. J Drugs Dermatol 6, 731-732 (2007). 1100 150.Ross, A.H., Jaycock, P., Cook, S.D., Dick, A.D. & Tole, D.M. The use of rituximab in refractory mucous 1101 membrane pemphigoid with severe ocular involvement. Br J Ophthalmol 93, 421-422, 548 (2009). 1102 151. Schumann, T., Schmidt, E., Booken, N., Goerdt, S. & Goebeler, M. Successful treatment of mucous 1103 membrane pemphigoid with the anti-CD-20 antibody rituximab. Acta Derm Venereol 89, 101-102 (2009). 1104 152.Lourari, S., et al. Bullous and mucous membrane pemphigoid show a mixed response to rituximab: 1105 experience in seven patients. J Eur Acad Dermatol Venereol (2010). 1106 153. Doan, S., Roux-Villet, C., Prost-Squarcioni, C., Caux, F. & Hoang-Xuan, T. Rituximab in Severe Ocular 1107 Cicatricial Pemphigoid. ARVO Meeting Abstracts 47, 594 (2006).

1108	154. Rubsam, A., Stefaniak, R., Worm, M. & Pieyer, U. Rituximab preserves vision in ocular mucous membrane
1109	pemphigoid. Expert opinion on biological therapy 15, 927-933 (2015).
1110	155. Maley, A., et al. Rituximab combined with conventional therapy versus conventional therapy alone for the
1111	treatment of mucous membrane pemphigoid (MMP). J Am Acad Dermatol 74, 835-840 (2016).
1112	156. Foster, C.S., Chang, P.Y. & Ahmed, A.R. Combination of rituximab and intravenous immunoglobulin for
1113	recalcitrant ocular cicatricial pemphigoid: a preliminary report. Ophthalmology 117, 861-869 (2010).
1114	157.Papaliodis, G.N., Chu, D. & Foster, C.S. Treatment of ocular inflammatory disorders with daclizumab.
1115	Ophthalmology 110 , 786-789 (2003).
1116	158. Sobolewska, B., Deuter, C. & Zierhut, M. Current medical treatment of ocular mucous membrane
1117	pemphigoid. Ocul Surf 11, 259-266 (2013).
1118	159.Taylor, H.R. Doyne Lecture: trachoma, is it history? Eye (Lond) 23, 2007-2022 (2009).
1119	160. Holland, M.J., et al. Pathway-focused arrays reveal increased matrix metalloproteinase-7 (matrilysin)
1120	transcription in trachomatous trichiasis. Invest Ophthalmol Vis Sci 51, 3893-3902 (2010).
1121	161.Burton, M.J., Bailey, R.L., Jeffries, D., Mabey, D.C. & Holland, M.J. Cytokine and fibrogenic gene expression
1122	in the conjunctivas of subjects from a Gambian community where trachoma is endemic. Infection and
1123	immunity 72 , 7352-7356 (2004).
1124	162. Colliou, N., et al. Long-term remissions of severe pemphigus after rituximab therapy are associated with
1125	prolonged failure of desmoglein B cell response. Science translational medicine 5, 175ra130 (2013).
1126	163. Selvaggio, A.S. & Noble, P.W. Pirfenidone Initiates a New Era in the Treatment of Idiopathic Pulmonary
1127	Fibrosis. <i>Annu Rev Med</i> 67 , 487-495 (2016).
1128	164. Rodriguez-Castellanos, M., Tlacuilo-Parra, A., Sanchez-Enriquez, S., Velez-Gomez, E. & Guevara-Gutierrez,
1129	E. Pirfenidone gel in patients with localized scleroderma: a phase II study. Arthritis research & therapy 16,
1130	510 (2015).
1131	165.Zhong, H., Sun, G., Lin, X., Wu, K. & Yu, M. Evaluation of pirfenidone as a new postoperative antiscarring
1132	agent in experimental glaucoma surgery. Invest Ophthalmol Vis Sci 52, 3136-3142 (2011).
L133	

1	Legends for Figures
2	Figure 1
3	Portrait of Sir William Bowman by George Watts painted about 1865.
4	I am grateful to Rachel Clarkson, Sir William's great-great-granddaughter, for providing this image.
5	
6	Figure 2
7	Illustrations of some of the causes of cicatrising conjunctivitis
8	A. Mucous membrane pemphigoid, B. Trachoma, C. Adenovirus, D. Sebaceous carcinoma, E. Ocular surface
9	squamous neoplasia, F. Ectrodactyly Ectodermal Dysplasia Cleft Lip/Palate G. Glaucoma drops, H. Atopic
10	keratoconjunctivitis, I. Stevens-Johnson syndrome, J. Ocular rosacea, K. Lichen Planus, L. Sarcoid.
11	
12	Figure 3
13	The diagnostic problem in cicatrising conjunctivitis
14	This Figure illustrates the classification of CC used in Table 2 with notes on the prevalence, which causes are
15	often overlooked, which are usually known but which may co-exist with mucous membrane pemphigoid and
16	causes associated with systemic diseases that may have their first manifestations in the eye.
17	
18	Figure 4
19	The epithelial basement membrane structure and immunofluorescent tests
20	The cartoon describes the basement membrane (dermoepidermal junction) structure and its constituent
21	proteins. The proteins that have been shown to be target antigens in MMP are listed. The position of the
22	basement membrane (BM) is shown on a direct immunofluorescence (DIF) specimen which also shows positive
23	immunofluorescence to IgG in the BM zone. An example of indirect immunofluorescence (IIF) on salt split skin
24	is also shown demonstrating positive fluorescence to the floor (see Figure 5). The cartoon is reproduced with
25	permission from Figure 1 Schmidt, E. & Zillikens, D. Pemphigoid diseases. Lancet 381, 320-332 (2013).
26	
27	Figure 5
28	Autoimmune bullous dermatoses and lichen planus

Direct immunofluorescence (DIF) and indirect immunofluorescence

30	The illustrations in the top left panel shows pemphigus vulgaris (PV) with DIF on perilesional skin showing
31	intraepithelial antibody binding. In the top right panel the skin lesions in bullous pemphigoid are shown for
32	comparison with those in PV together with an example of a positive DIF in conjunctiva with antibody binding at
33	the basement membrane. The pemphigus and pemphigoid diseases are listed separating those that may be
34	associated with conjunctival scarring and those that are not. The panel on mucosal lichen planus shows the
35	typical appearance of a "shaggy" fibrin staining band at the dermoepidermal junction. The bottom panel shows
36	indirect immunofluorescence on human salt split skin from a patient with MMP.
37	
38	Figure 5
39	The spectrum of disease in mucous membrane pemphigoid (MMP)
40	A. Gingival inflammation and ulceration, B. Palatal inflammation and ulceration, C. Supraglottic inflammation
41	and scarring, D. Oesophageal stenosis, E. Skin ulceration and scarring, F. Foreskin scarring, G. Conjunctival
42	inflammation and scarring, H. Intraoperative photograph showing a normal subconjunctival space under
43	incised severely scarred and shortened conjunctiva demonstrating that subconjunctival tissue is unaffected.
44	
45	Figure 6
46	Criteria for referral
47	Criteria for referral of patients with cicatrising conjunctivitis to a specialist ocular surface disease service.
48	Adapted from Figure 5 in Williams, G.P et al. Eye (Lond) 25, 1207-1218 (2011).
49	
50	Figure 6
51	Ocular mucous membrane pemphigoid (MMP)
52	Clinical signs in ocular MMP
53	
54	Figure 7
55	Factors contributing to progression of disease in ocular mucous membrane pemphigoid (MMP)
56	
57	Figure 8

Summary of the pathogenesis of inflammation and scarring in ocular MMP

This illustrates the description in this section in the text, for which the evidence is summarised in Table 3. In brief a loss of tolerance to mucosal epithelial basement membrane proteins results in the development of pathogenic autoreactive T cells (aT) which help autoreactive B cells (aB) to proliferate in the regional lymph nodes, and differentiate into plasma cells. The latter produce circulating IgG and IgA autoantibodies to the mucosal basement membrane, which are detectable in the serum of some patients by indirect immunofluorescence (and other antibody specific assays). Plasma cells are also found in the conjunctival mucosal substantia propria where they may produce local antibody. In the mucosal epithelium direct immunofluorescence tests may show the presence of IgG and/or IgA fixed to the basement membrane where C3 (complement 3) is also identified in some patients. Activation of C3 at the basement membrane precipitates the complement cascade and acute inflammation at the basement membrane. This is the injury and inflammation phase of the disease causing an accumulation of inflammatory effector cells (neutrophils, dendritic cells, mast cells, eosinophils, macrophages and T cells) and the associated cytokines and growth factors IL-2, IL-5, IL-13 (interleukins-) IFNγ (interferon gamma, TNFα (tumour necrosis factor alpha), resulting in, often severe, inflammation and expansion of both Th1 and Th2 cells into a chronic inflammatory response.

An alternative effector pathway may be more important, in the absence of autoantibody, in some subsets of patients whereby autoreactive T cells to basement membrane components home in on the mucosa. Her they can create an inflammatory response, in the absence of antibody, through their cytokines and growth factors including IFN γ , TNF α , IL-4, IL-5 and IL-13.

This inflammatory response results in fibrosis through the effects of profibrotic mediators released by macrophages, T cells, mast cells and eosinophils on fibroblasts, including PDGF (platelet derived growth factor), IL-13, TGF β (transforming growth factor beta) and HSP47 (heat shock protein). Fibrosis also results from ALDH/RA (aldehyde dehydrogenase/retinoic acid) mediated paracrine effects of dendritic cells that activate a profibrotic phenotype in fibroblasts. During both active inflammation, and once inflammation has resolved, the activated MMP fibroblasts continue to scar as these remain profibrotic because of an ALDH/RA mediated autocrine effect. The latter probably results in RA dependent TGF β activation and or induction, further driving fibrosis.

Figure 9

Sectoral conjunctival inflammation in ocular mucous membrane pemphigoid (MMP)

The same eye of a patient showing the superior bulbar conjunctiva free of inflammation but a localised area of inflammation in the inferior bulbar conjunctiva.

Figure 10

Immunosuppression for ocular mucous membrane pemphigoid (MMP)

This illustrates "step up" and "step down" therapy to control conjunctival inflammation as described in the section on Evidence for the efficacy of different immunosuppressive regimens in controlling inflammation and guidelines for its delivery and in Table 4 Row 34, and Saw VP 2008³⁶.

- a. Drugs in different coloured boxes can be combined. The drugs in the dark red boxes (cyclophosphamide, mycophenolate, azathioprine and methotrexate) are all myelosuppressives: combining these results in unacceptable toxicity. Some authors have described the use of CD 20 inhibitors (such as Rituximab) with existing myelosuppressive therapy for unresponsive ocular MMP¹⁵³ but this may be unsafe and we follow the recommendations of restricting adjunctive drugs to sulfas¹⁴⁶ and stop the myelosuppressives immediately before the first CD20 monoclonal infusion. Prednisolone can generally be given for a short term effect with any of the other drugs listed but is usually needed in high doses, with its associated side effects.
- b. In general, all of these drugs, except high dose steroids take 2-4 months to take effect. This period is probably shorter for the CD 20 monoclonals than the other drugs. A full effect will often take longer and for this reason, unless inflammation is deteriorating, I do not usually alter therapy for 12 weeks.
- c. For mild/moderate ocular MMP use step up therapy. Start with a sulfa and, there is no effect, or toxicity, introduce mycophenolate. If the effect is limited but the drug is tolerated, add mycophenolate to the sulfa. Azathioprine (in grey typeface) is the second line drug when mycophenolate is not tolerated and methotrexate (in grey typeface) the third line drug in this situation because these are generally less well tolerated than mycophenolate and no more effective. If none of these drugs works switch to cyclophosphamide without using adjunctive steroid unless the patient is deteriorating. Failing these then a CD20 monoclonal is the next choice of drug and, if there is a lack of response to that 2 months after a second cycle of therapy, then start IV immunoglobulin therapy.

d. For severe MMP (severe conjunctival inflammation +/- limbitis +/- corneal ulceration) use step down therapy. Start oral Prednisolone 1 mg/kg WITH cyclophosphamide 1.0-1.5 mg/kg. Very severe cases will get a short term benefit from intravenous methylprednisolone 1 gram IV daily for 3 days before starting oral prednisolone with cyclophosphamide (unpublished data). If cyclophosphamide and prednisolone are not effective within 2-3 months, add a sulfa and then switch to a CD20 monoclonal. If this fails, as in the previous example, then start IVIG. Once control of inflammation has been established the patient can start stepdown therapy.

- i. For cyclophosphamide follow the toxicity guidelines in Table 4 row 19, I usually maintain therapy for up to 12 months in the hope that a longer period of therapy might induce a remission. Two months before discontinuing cyclophosphamide, introduce a sulfa, if tolerated. After discontinuing cyclophosphamide patients with full control of inflammation can discontinue sulfas after another 2-4 months therapy. For patients with partial control the sulfa is maintained and mycophenolate (azathioprine or methotrexate) added. Drugs can be discontinued as required dependent on disease control and the development of side effects.
- ii. After CD 20 monoclonals patients may be in remission and any sulfa therapy can be discontinued after 2-4 months therapy. In the event of a relapse a decision has to be made whether to control this with step up therapy or a further cycle of a CD 20 monoclonal.
- iii. After IVIG patients will usually still be in myelosuppressive agents which can be stepped down or stepped up as described above.



Figure 2

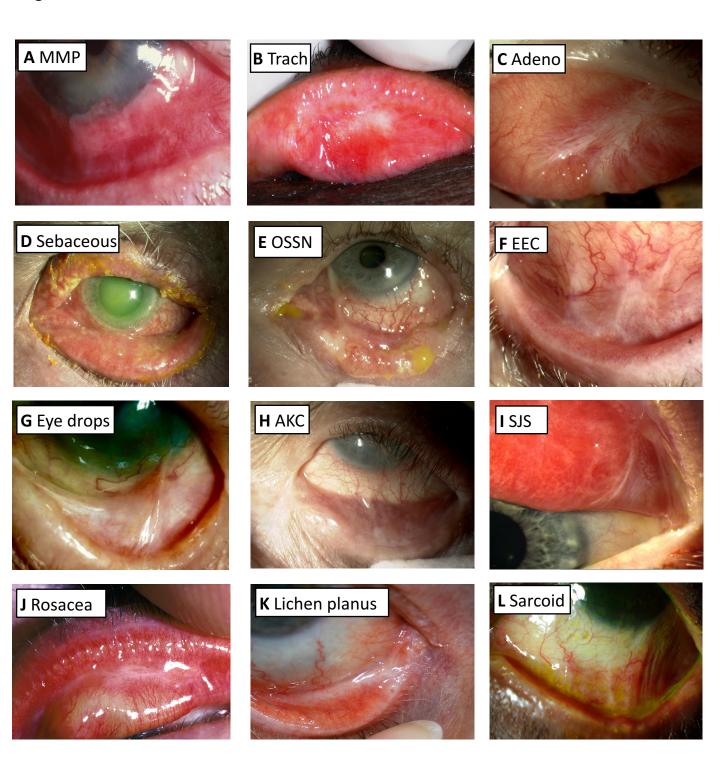


Figure 3

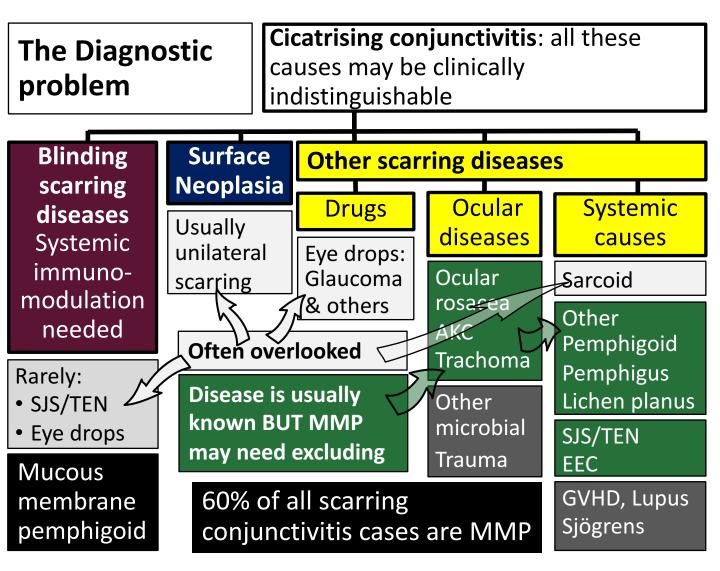
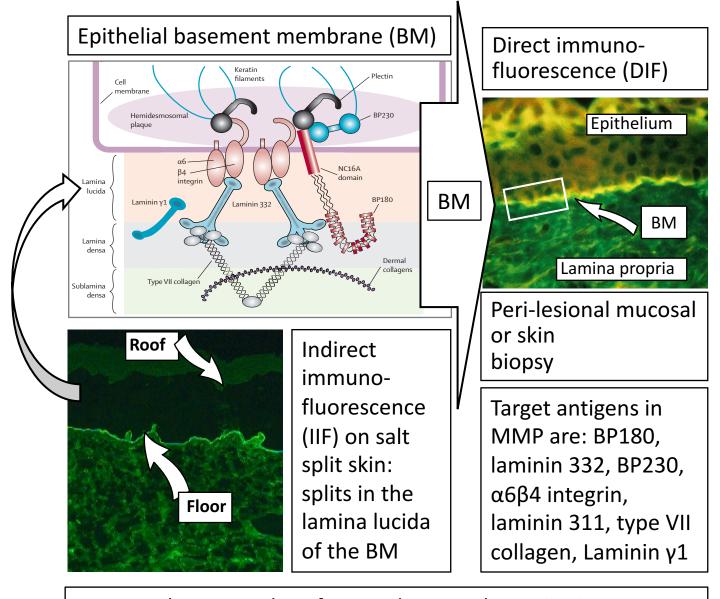


Figure 4

Epithelial basement membrane structure and immunofluorescence tests



Further examples of DIF and IIF are shown in Figure 5

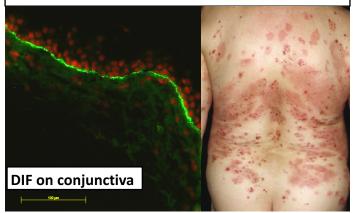
Autoimmune bullous dermatoses and lichen planus

Direct immunofluorescence (DIF) Indirect immunofluorescence (IIF)

Pemphigus is intraepithelial



Pemphigoid is subepithelial



Pemphigus diseases with ocular scarring

- Pemphigus vulgaris
- Paraneoplastic pemphigus

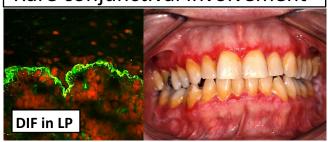
Pemphigus diseases with no ocular scarring

- Pemphigus foliaceous
- Pemphigus gestationis
- IgA pemphigus

Pemphigoid diseases with ocular scarring

- Mucous membrane pemphigoid now also includes mucosal dominated linear IgA, epidermolysis bullosa acquisita, anti-laminin 332 pemphigoid (previously laminin 5 and epiligrin)
- Bullous pemphigoid

Mucosal lichen planus (LP) Rare conjunctival involvement



Pemphigoid diseases with no ocular scarring

- Anti-p200/anti-laminin γ1 pemphigoid
- Lichen planus pemphigoides
- Pemphigus gestationis

Indirect immunofluorescence on human salt split skin

Detects antibodies to BP180, BP230, and $\alpha6\beta4$ integrin (on the "roof" of the split) and laminin 332, laminin $\gamma1$ and type VII collagen (on the "floor" of the split)

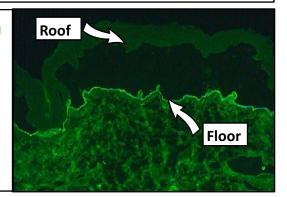


Figure 6

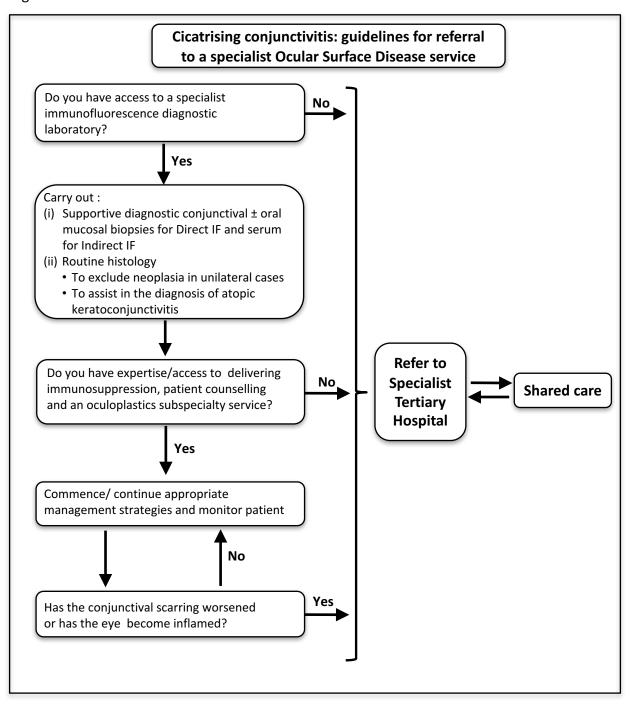


Figure 7

Spectrum of disease in mucous membrane pemphigoid

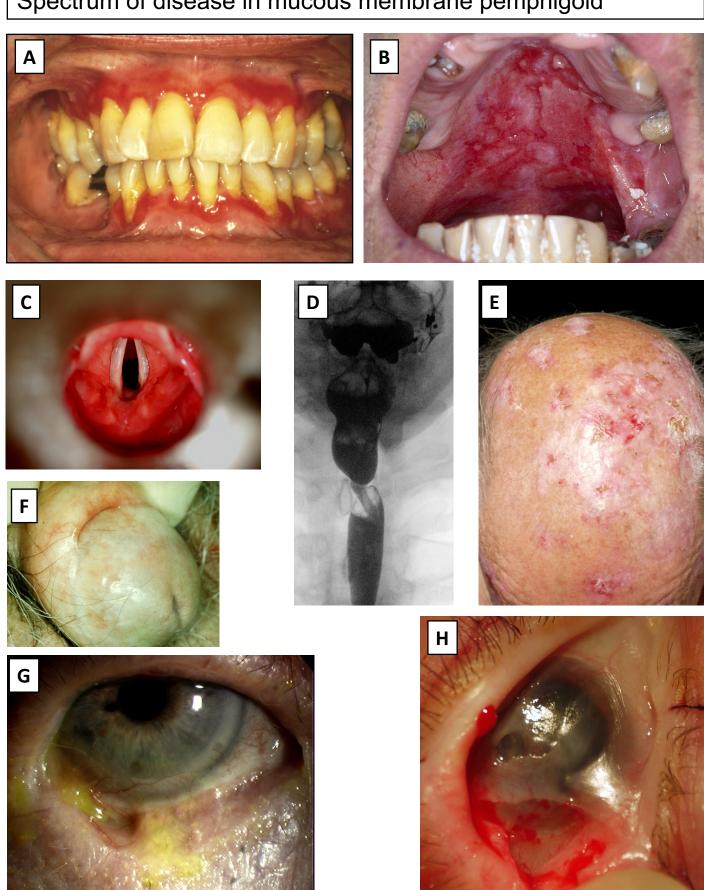


Figure 8

Ocular mucous membrane pemphigoid

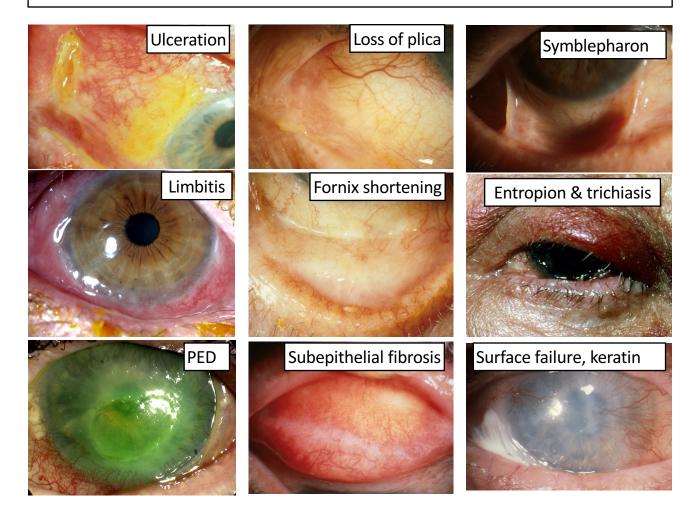


Figure 9

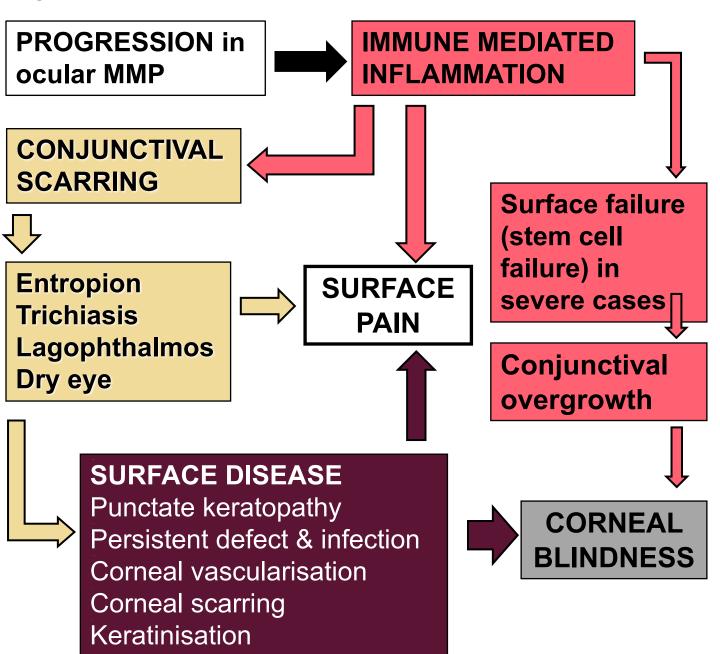


Figure 10

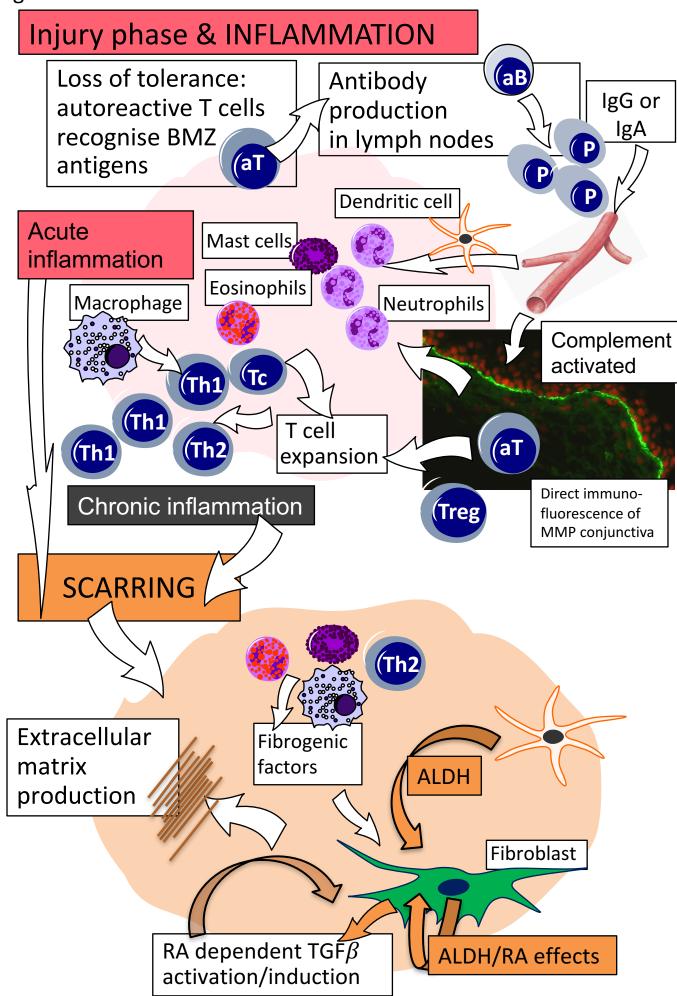
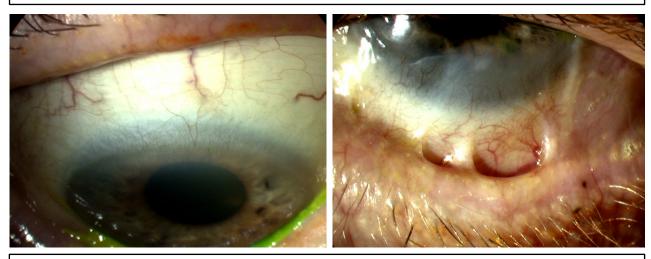


Figure 11

Sectoral conjunctival inflammation in ocular MMP



Superior and inferior bulbar conjunctiva showing persistent sectoral inflammation in partially controlled disease

Figure 12

Immunosuppression for ocular MMP Drugs in Oral prednisolone 1mg/kg (taper over different 12 weeks) for rapid effect colour **CD 20 monoclonals** groups can be IV immunoglobulin combined Cyclophosphamide Wait for 12 weeks Mycophenolate Mycophenolate before switching **Azathioprine Azathioprine Methotrexate Methotrexate** Dapsone or For mild Dapsone or Sulphapyridine **MMP** Sulphapyridine

Table 1Institutional and national surveys of cicatrising conjunctivitis

Institutional case series of cicatris	UK national survey of new cases of CC		
(CC) Thorne JE 2004 ³ . Includes 74 of	Radford C ⁴ n=82		
conjunctivitis caused by Mucous m			
pemphigoid (MMP) and 145 cause	d by other diseases		
	Number (%)	Number (%)	Incidence per
	74 cases of MMP	Total n=82	million
	and 145 others		
MMP	74 (33.7%)	50 (60.95%)	0.8
Other causes of CC	145 ^a		
Linear IgA bullous dermatosis ^b	None (N)	1 (1.25%)	
Topical glaucoma drug induced	(28.3%)	3 (3.65%)	
Rosacea blepharoconjunctivitis	(20%)	3 (3.65%)	
Atopic keratoconjunctivitis	(8.3%)	3 (3.65%)	
Sjögren's syndrome or KCS ^c	(7.6%)	1 (1.25%)	
Stevens Johnson syndrome	(6.3%)	16 (19.51%)	0.2
Graft versus host disease	(1.4%)	2 (2.43%)	
Lichen planus	N	2 (2.43%)	
Ocular surface neoplasia	N	1 (1.21%)	
Non-trachomatous infection	(5.5%)	N	
Trachoma	(2.8%)	N	
Sarcoidosis	(3.5%)	N	
Pemphigus vulgaris	(3.4%)	N	
Paraneoplastic pemphigus	(2.8%)	N	
Trauma or lid surgery	(2.8%)	N	
Ectodermal dysplasia	(2.1%)	N	
Chronic cutaneous lupus	(1.4%)	N	
Aplasia cutis congenital	(0.7%)	N	
Ectopic geographic tongue	(0.7%)	N	

^aPercentages of causes for the 145 cases of cicatrising conjunctivitis not caused by MMP. Percentages total above 100% because some patients had more than one diagnosis.

^bNow classified as MMP

^cKeratoconjunctivitis sicca

Table 2

Current classification of cicatrising conjunctivitis.

Causes are underlined. Some causes appear twice because they result in either progressive or non-progressive scarring disease.

The pemphigus and pemphigoid diseases NOT currently known to cause conjunctival scarring are also listed.

CLASSIFICATION	Description
BLINDING SCARRING DISORDERS	Progressive inflammatory and scarring diseases for which systemic immunomodulation is often needed for control of scarring & inflammation
Mucous membrane pemphigoid (MMP) ⁴	Currently 60% of all CC cases in the UK are caused by MMP which now includes the conditions mucosal dominated epidermolysis bullosa acquisita, linear IgA disease and anti-laminin 332 (formerly anti-epiligrin or anti-laminin 5) pemphigoid ⁹
Drug-induced progressive conjunctival cicatrisation ¹⁰	A rare complication of topical medication, usually for glaucoma. A small subset of patients with these diseases develop autoantibody-positive or negative progressive conjunctival indistinguishable from MMP
<u>Stevens-Johnson Syndrome</u> (SJS) & <u>Toxic Epidermal Necrolysis</u> (TEN) with progressive scarring ¹¹	Only a small subset of patients with these diseases develop autoantibody-positive or negative progressive conjunctival scarring similar to that in MMP which may continue from the acute episode or develop acutely years later
OCULAR SURFACE NEOPLASIA	
Ocular surface squamous carcinoma (OSSN) ⁴ and sebaceous cell carcinoma	Rare causes of conjunctival scarring and inflammation indistinguishable clinically from MMP, except that cases are usually unilateral
OTHER SCARRING CONJUNCTIVAL DISEASES Sometimes referred to as "Pseudopemphigoid" see discussion of Thorne JE 2004 ³ and in section on Current causes, epidemiology and classification of cicatrising conjunctivitis	
Ocular diseases with no systemic involvement	
Drug induced scarring ¹⁰	Usually due to topical preservatives or unpreserved glaucoma medication and, rarely, other drugs. Inflammation resolves after withdrawing the drops and scarring stabilizes
Atopic keratoconjunctivitis ^{3,5,13}	Usually a tarsal papillary reaction in addition to scarring although this may be minimal in severe longstanding cases
<u>Trachoma</u> ¹⁴	Tarsal scarring typical but symblepharon occurs ¹⁵
Adenoviral conjunctivitis 16-18	Scarring with entropion and symblepharon are occasionally present. These patients must have a history of an acute severe conjunctivitis to confirm the diagnosis
Conjunctival trauma: chemical, thermal ¹⁹ , surgical and radiation injury ²⁰	Diagnosis is clear from the history

Stevens-Johnson syndrome and Toxic Epidermal Necrolysis ²¹	Diagnosis clear from history. A small subset of patients develop progressive scarring disease see the section on Blinding Scarring Disorders above
Pemphigoid diseases (sub-epithelial immunobullous diseases) ⁹	Pemphigoid diseases with conjunctival scarring unreported or very rare: one case of anti-p200 with anti-laminin 332 pemphigoid ²² , lichen planus pemphigoides may involve oral but not ocular mucosa ²³ , pemphigoid gestationis rarely involves mucosa and conjunctival involvement is unreported to my knowledge ²⁴
Bullous pemphigoid ²⁵	Conjunctival scarring infrequent
Mucous membrane pemphigoid	See Blinding Scarring Disorders above. In about 20% of cases the disease progresses very slowly or is in remission and systemic immunosuppression is not required ²⁶
Paraneoplastic pemphigoid	Severe scarring may occur ²⁷
Pemphigus diseases (intra-epithelial immunobullous diseases)	Pemphigus diseases in which conjunctival disease is unreported: pemphigus gestationis, pemphigus foliaceous (may involve lid skin but not the conjunctiva) ²⁸ and IgA pemphigus
Pemphigus vulgaris ^{29,30}	Conjunctivitis occurs but scarring is rare. However, several cases have presented in the eye accompanied by severe scarring and corneal perforation ³⁰
Paraneoplastic pemphigus ^{27,29}	Conjunctivitis occurs. Severe scarring may occur but is uncommon ²⁷
Dermal diseases with variable ocular involvement	
Dermal diseases with variable ocular involvement Ocular rosacea ^{3,31}	Some scarring is common
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹	Some scarring is common Rarely causes scarring
Ocular rosacea ^{3,31}	-
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹	Rarely causes scarring
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹ Lichen planus ^{32,33}	Rarely causes scarring Rarely causes scarring
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹ Lichen planus ^{32,33} Systemic lupus and discoid lupus ³⁴	Rarely causes scarring Rarely causes scarring
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹ Lichen planus ^{32,33} Systemic lupus and discoid lupus ³⁴ Multisystem disorders	Rarely causes scarring Rarely causes scarring Rarely causes scarring The diagnosis has usually been made by the oncologist. Conjunctival scarring and
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹ Lichen planus ^{32,33} Systemic lupus and discoid lupus ³⁴ Multisystem disorders Graft versus Host Disease ⁶	Rarely causes scarring Rarely causes scarring Rarely causes scarring The diagnosis has usually been made by the oncologist. Conjunctival scarring and inflammation is common
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹ Lichen planus ^{32,33} Systemic lupus and discoid lupus ³⁴ Multisystem disorders Graft versus Host Disease ⁶ Sarcoid ^{3,7}	Rarely causes scarring Rarely causes scarring Rarely causes scarring The diagnosis has usually been made by the oncologist. Conjunctival scarring and inflammation is common Rare but may present in the eye as severe scarring conjunctivitis
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹ Lichen planus ^{32,33} Systemic lupus and discoid lupus ³⁴ Multisystem disorders Graft versus Host Disease ⁶ Sarcoid ^{3,7} Sjögren's syndrome and keratoconjunctivitis sicca ^{3,4} Miscellaneous congenital and acquired disease Ectodermal dysplasia (ED) diseases (includes ectrodactyly-ectodermal dysplasia-cleft syndrome (EEC) cases as well as ED ⁸	Rarely causes scarring Rarely causes scarring Rarely causes scarring The diagnosis has usually been made by the oncologist. Conjunctival scarring and inflammation is common Rare but may present in the eye as severe scarring conjunctivitis Scarring is rare Some patients develop circulating basement membrane antibodies or mucosal direct immunofluorescence: inflammation may be severe with scarring
Ocular rosacea ^{3,31} Dermatitis herpetiformis ¹⁹ Lichen planus ^{32,33} Systemic lupus and discoid lupus ³⁴ Multisystem disorders Graft versus Host Disease ⁶ Sarcoid ^{3,7} Sjögren's syndrome and keratoconjunctivitis sicca ^{3,4} Miscellaneous congenital and acquired disease Ectodermal dysplasia (ED) diseases (includes ectrodactyly-ectodermal	Rarely causes scarring Rarely causes scarring Rarely causes scarring The diagnosis has usually been made by the oncologist. Conjunctival scarring and inflammation is common Rare but may present in the eye as severe scarring conjunctivitis Scarring is rare Some patients develop circulating basement membrane antibodies or mucosal direct

Table 3Effectors and cytokines identified in MMP conjunctiva

GLOSSARY (alphabetical in the order of the abbreviations in the Table with additional notes on origin and function

- > B7-2: a costimulatory T cell ligand expressed on dendritic cells and macrophages.
- > CD40, CD80, CD154: costimulatory molecules on T cells.
- > FGF: a family of fibroblast growth factors, including basic FGF (bFGF) produced by monocytes and important in wound repair.
- \triangleright CTGF: connective tissue growth factor produced by fibroblasts and is a downstream mediator of TGF β 1 induced collagen synthesis, promotes fibroblasts proliferation & enhances matrix production.
- > HSP family: heat shock proteins produced by fibroblasts and involved in the maturation of collagen
- > ICAM: intercellular adhesion molecule expressed on macrophages and lymphocytes facilitating migration into inflamed tissues
- \triangleright IFN γ : interferon gamma, produced by activated T_H1 lymphocytes, epithelia and fibroblasts with multiple roles in inflammation.
- ➤ IL-1: interleukin 1 produced by macrophages, B cells lymphocytes, fibroblasts, and endothelium is a multifunctional cytokine critical in initiating and maintaining inflammation and immune responses.
- > IL-2: interleukin 2, produced by T cells, and responsible for T and B cells proliferation and activation.
- ➤ IL-4: interleukin 4, produced by mast cells, Th2 cells and fibroblasts affecting B cell, T cell and fibroblast functions with multiple profibrotic and inflammatory effects. Induces m-CSF, type I collagen & collagen-binding HSP47 production by conjunctival fibroblasts.
- > IL-5: interleukin 5, produced by mast cells, Th2 cells, eosinophils and monocytes and affects eosinophil chemotaxis, differentiation, activation and prolongs survival.
- > IL-6: interleukin 6, produced by macrophages, and stimulates B cells to differentiate into plasma cells
- > IL-13: interleukin 13, produced by Th2 cells, mast cells and basophils and is a potent stimulator of eosinophil, lymphocyte and macrophage rich inflammation and tissue fibrosis
- > m-CSF: macrophage colony stimulating factor produced by monocytes, fibroblasts and endothelial cells having effects on the proliferation, differentiation and activation of monocytes macrophages
- ➤ MIF: macrophage inhibition factor produced by T cells and macrophages inhibiting macrophage migration and stimulating macrophage activation.
- > MPO: myeloperoxidase. This enzyme is produced and stored by neutrophils
- > MMP (in italics to distinguish the abbreviation from that for mucous membrane pemphigoid) : matrix metalloproteinase family of enzymes including MMP-8 and MMP-9 that degrade extracellular matrix

- > PDGF: platelet derived growth factor produced by macrophages and fibroblasts amongst others.
- > Tc (Cytotoxic T cells) expressing CD8
- \succ TGF β family: transforming growth factor beta, produced by many cell types (B & T cells, monocytes, macrophages, fibroblasts). These are profibrotic mediators with immunosuppressive effects.
- \succ Th1: T helper subset 1 expressing CD4 secreting their signature cytokine interferon gamma (IFN γ) and also producing TNF α .
- > Th2: T helper subset 2 expressing CD4 and secreting the signature cytokine IL-4 and also producing IL-5 and IL-13
- > TNFα: tumour necrosis factor alpha, produced by macrophages, mast cells and lymphocytes. It has multiple inflammatory functions

Effector cells & proteins	Findings in MMP	Author
Circulating autoreactive T cells to BP180	2/10 patients with MMP demonstrated IFNγ production to BP180 NC16A in peripheral blood mononuclear cells suggesting that this segment of BP 180 is a T cell target antigen in MMP	Black AP 2004 ⁶⁸
Neutrophils, macrophages, dendritic cells, Th (CD4), Tc (CD8), plasma cells. (TGFβ FGF and PDGF)	Conjunctival biopsies from 20 patients with acute (n4) subacute (n8) and chronic (n8) MMP investigated showed an excess of neutrophils, macrophages and dendritic cells in acute disease. Increased T cells present in all subsets, with Tc:Th ratio higher except in acute disease when these were equal. Plasma cells increased, but not B cells or NK cells. PDGF, FGF and TFGß present in both diseased and controls. TFGß was overexpressed in acute disease. MHC class II highly expressed in diseased tissue (potential to present antigen to Th cells). The activated T lymphocytes were identified with anti-IL-2 showing these were Th1 T cells.	Bernauer W 1993 ⁸⁵
Neutrophils in the epithelium	Neutrophils are elevated in the MMP conjunctival epithelium shown by impression cytology and increased levels are associated with progressive scarring in both inflamed and clinically uninflamed eyes and may be a useful biomarker of progressive fibrosis and response to therapy	Williams G 2016 ¹⁰⁰
Neutrophil derived enzymes (MPO, MMP-8 and MMP- 9)	MPO is neutrophil derived, and neutrophils are also a source of <i>MMP's</i> which are elevated in the tears of MMP patients potentially accounting for their predisposition to corneal melting and also potentially of use as biomarkers of disease activity	Arafat S 2014 ¹⁰¹
Dendritic cells and	MMP epithelium and control did not differ. Substantia propria: predominantly	Tesavibul N

T cell co-	increased increased macrophages and T cells but also Langerhans & B cells. B7-2	1998 ⁸⁴
stimulatory	costimulatory ligand (usually expressed on dendritic cells and macrophages)	
molecules	overexpressed (but not the B7-1 ligand). B7-2 overexpression may be expected to be	
	associated with increased T cell proliferation via IL-2 production	
Eosinophils and	Serum levels of both IL-5 (produced by Th2 cells, monocytes and mast cells) and	Letko E 2002 ⁸⁶
mast cells with IL-5	eosinophils were increased in MMP. Mast cell and eosinophils were increased in the	
in acute disease	substantia propria in MMP. Mast cells released profibrogenic cytokines in pulmonary	
	and hepatic fibrosis and may be important in MMP scarring.	
Cytokine	Both diseased and normal control conjunctiva expressed IL-2, IFNγ (Th1 signature),	Bernauer W
expression IFN γ	TGF β , TNF α , PDGF and bFGF as well as a proliferating cell marker. No IL-4 was	1993 ¹⁰²
TGFeta, $TNFlpha$, $PDGF$	expressed (Th2 signature). TGF eta and proliferating cells were overexpressed in the acute	
	group and IL-2, bFGF and PDGF (fibrogenic cytokines) were more expressed in the	
	subacute disease group.	
TGFβ1& β3	TGF β 1& β 3 increased in the stroma in association with fibroblasts and macrophages in	Elder MJ 1997 ⁸⁷
increased	acute MMP (not chronic), PDGF and FGF undetectable	
HSP47, TGFβ and	Collagen I & III increased in OMMP conjunctival stromal tissue, and HSP47 and TGFβ	Razzaque MS
collagen I and III	increased in MMP fibroblasts. TGFß1 induced HSP47 and collagen I in OMMP	2003 ⁹⁰
expression in	fibroblasts. Increased expression of the latter may contribute to conjunctival scarring in	
conjunctiva and	OMMP.	
role of TGFβ 1		
induction of		
HSP47 and		
collagen I by		
fibroblasts		
Serum TNF $lpha$ and	Serum levels for TNF α , but not IL-6, were elevated in ocular MMP patients (n=35)	Lee SJ 1993 ¹⁰³
IL-6 in active MMP	compared to controls consistent with expectations for a systemic autoimmune disease.	
TNFα	TNF $lpha$ is overexpressed in inflamed OMMP conjunctiva compared to treated OMMP but	Saw VP 2009 ⁸⁸
	the latter still shows greater expression than control levels. TNF $lpha$ treatment of normal	
	fibroblasts stimulated increased migration, mmp-9 production, decreased timp-2 and	
	timp-4 and upregulated CD40 and ICAM. CD40 may interact with T lymphocytes	
		•

	stimulating proliferation and profibrogenic cytokine production.	
IL-13	IL-13 is overexpressed in ocular MMP conjunctiva (both inflamed and non-inflamed) compared to controls. IL-13 promotes pro-fibrotic effects on normal human conjunctival fibroblasts and also upregulates the expression of T cell costimulatory molecules (CD80, CD40 and CD154) on fibroblasts potentially promoting interaction with T cells to drive fibrosis.	Saw VP 2009. ⁸⁹
MIF protein and mRNA	Increased MIF was demonstrated by immunohistochemistry and qPCR in both MMP conjunctiva and fibroblasts. The expression of MIF was associated with increased macrophage numbers. MIF was also secreted by fibroblasts (shown by ELISA). IL-1, TNF and TGFβ1 induced MIF expression in fibroblasts.	Razzaque MS 2004 ⁹¹
CTGF in stroma and fibroblasts	CTGF is a downstream profibrotic mediator of TGF-β1 and was overexpressed in both ocular MMP whole conjunctiva and in cultured fibroblasts.	Razzaque MS 2003 ⁹³
m-CSF	Macrophage colony stimulating factor (m-CSF) was overexpressed in ocular MMP conjunctiva, macrophages, fibroblasts and epithelial cells. m-CSF correlated with macrophage numbers and some macrophages expressed a proliferation marker. Fibroblast m-CSF expression was increased by treatment with IL-1a or $TNF\alpha$.	Razzaque MS 2002 ⁹²

 Table 4

 Studies on the use of immunomodulatory drugs for ocular mucous membrane pemphigoid, notes on the evidence, and guidance for the use of the drugs

Row	Immunosuppressive agent Author, reference	% of patients or eyes responding to treatment	Notes on the evidence and guidance on the use of the drugs
1	Nicotinamide and tetracycline Reiche L 1998 ¹³⁵	63% (n=8) extraocular and 2 ocular MMP	Small case series. Rarely used for ocular disease. I have no experience of this therapy
2	Dapsone Saw VP 2008 ³⁶	77% 9 (n=114) ^a ocular MMP	Dapsone has common side effects, most often anaemia (95% of all patients to a variable extent), malaise and skin rashes. However, it's not an immunosuppressive drug, may be very effective, and
3	Rogers RS 1982 ¹⁰⁸	83% (n=24) extraocular and ocular MMP	can also be combined with immunosuppressive drugs for added effect. Although unfashionable I still use it for these reasons. Compared to cyclophosphamide and steroid it is not as effective for severe
4	Tauber J 1991 ¹³³	45% (n=69) ocular MMP	disease (see below)
5	Foster CS 1986 ⁴⁶	70% (n=20) ocular MMP	
6	Sulphapyridine Saw VP 2008 ³⁶	65% (n=55) ^a ocular MMP	Usually now given as sulfasalazine (in which sulphapyridine is an active agent) because sulphapyridine is no longer widely available. I use this as an alternative when dapsone causes
7	Elder MJ 1996 ¹²⁷	50% (n=20) ocular MMP	anaemia, or is not tolerated
8	Sulfasalazine Doan S 2001 ¹²⁶	45% (n=9) ocular MMP	
9	Azathioprine Saw VP 2008 ³⁶	71% (n=80) ^a ocular MMP	Azathioprine has similar effects to mycophenolate but side effects are more common such that mycophenolate has largely replaced azathioprine, except as a second line agent for patients not
10	Tauber J 1991 ¹³³	33% (n=11) ocular MMP	tolerating mycophenolate
11	Ciclosporin Neumann R 1991 ¹³² Foster CS 1992 ¹²⁹	2/22 patients (9%) ocular MMP	Calcineurin inhibitors have generally had poor success in ocular MMP and cannot be recommended on current evidence
12	Tacrolimus Letko E 2004 ¹³⁰	33% (n= 6) ocular MMP	

13	Oral corticosteroids Hardy KM 1971 ⁵⁹ Mondino BJ 1979 ¹⁰⁹ Foster CS 1986 ⁴⁶	65% (n=23) ^b ocular MMP	Doses above 0.5 mg/kg (about ≥ 40 mg) are usually needed to gain control of the disease and relapse is usual when doses are reduced. Side effects are unacceptable at the doses need as monotherapy Long term lower dose therapy has not been evaluated, but has not been needed in my patients having alternative steroid sparing immunosuppressive therapy. High doses (1-2mg/kg) are used for short term control in severe disease while the steroid sparing drug takes effect. I use a 3 months tapering course, usually in combination with cyclophosphamide
14	Cyclophosphamide and steroids Thorne JE 2008 ¹³⁴	91% (n=44) ocular MMP	This study used high dose (2mg/kg), prolonged (12-18 months) cyclophosphamide for most patients (68/78 or 87%). At 12 months 58/70 (83%) had complete success. However, serious complication rates, including malignancies (1 bladder cancers, 2 leukaemias and 6 other tumours), and 2 pneumonias, were relatively common and probably relate to a combination of the high doses used and the prolonged treatment period of 12-18 months which exceed safe doses (see Row 19)
15	Elder MJ 1995 ¹²⁸	15/19 eyes (79%) ocular MMP	2.0 mg/kg for up to 8 months
16	Foster CS 1996 ⁴⁶	100% (n=12) ocular MMP	2.0 mg/kg
17	Saw VP 2008 ³⁶	90% (n=73) ^a ocular MMP	1.0-1.5 mg /kg for up to 12 months
18	Tauber J 1991 ¹³³	92% (n=35) ocular MMP	2.0 mg/kg for a mean of 13 months (1-65 months)

19 Additional notes on cyclophosphamide use:

- <u>Cyclophosphamide monotherapy</u>. Cyclosphosphamide is typically used for patients with severe inflammation in ocular MMP, and is recommended by all authors as the drug of choice for this group of patients. It is usually combined with oral steroids for rapid disease control while the cyclophosphamide takes effect over 2-4 months. As a result, it is difficult to establish the effect of cyclophosphamide used as a single agent: in two of these studies 14/73³⁶ and 2/25¹³³ had cyclophosphamide without steroid. I use cyclophosphamide as single agent when patients have not responded adequately to other therapies, but do not have severe enough disease to risk oral prednisolone.
- Cyclophosphamide dose and malignancy. The dose of cyclophosphamide is given as between 1.0-2.0 mg/kg in the different studies and reviews summarized in rows 14-18. However, cyclophosphamide has a wide range of serious side effects listed in the studies below. In the last decade a maximum safe dose below which bladder cancer, and acute myeloid leukaemia are unlikely to occur, have been established in patients treated with cyclophosphamide for polyangiitis with granulomatosis. The probable cumulative safe doses for bladder cancer are 25gms, or 12 months treatment and up to 36 gms, to limit the risk of both bladder cancer and acute myeloid leukaemia 137.
- How to use cyclophosphamide. Cyclophosphamide tablets in the UK are available in 50mg or 100 mg and doses other than multiples of these can only be given by alternating therapy e.g. 50 mg one day alternating with 100 mg the next day to give a mean daily dose of 75 mg. I now use 1.0-1.5mg/kg³⁶ to restrict cumulative doses to the proposed safe limits. For most males I start with 100 mg per day for up to 12 months (a 36 gm cumulative dose for a 70kg person at 1.5 mg/kg). For women of 50kg I use 1 mg/kg for 12 months (an 18 gm cumulative dose over 12 months) but adjust the dose, and length of therapy, to get control of disease. Most patients will need 8 months of therapy to give them a period of one or two months of controlled inflammation and some will need more. This is less than the dose of 2.0mg/kg quoted in all the other studies, including our early publication¹²⁸, to optimize safety. After the cumulative safe dose is reached I usually switch to a sulfa, before stopping cyclophosphamide, and add an alternative myelosuppressive if needed after discontinuing cyclophosphamide.

	and may be as effec	tive ¹³⁸ . However, for polyangiit	route have been rarely reported in ocular MMP case series: it is probably safer than daily oral therapy is with granulomatosis a randomized trial of daily oral dosing versus intravenous cyclophosphamide ore effective in the long term ¹³⁹ . I have no experience of this modality of administration.
20	Methotrexate McCluskey P 2004 ¹³¹	83% (n=12) ocular MMP	This is the only study describing methotrexate in detail. Other large studies have not used the drug $\left(0/78\right)^{134}$ or only in small numbers $\left(4/115\right)^{36}$, or the effects is not clear from the publication 118. I have little experience with this drug and use it as a third line agent when patients cannot tolerate, or don't respond to, dapsone/suphasalazine, azathioprine, mycophenolate, and when cyclophosphamide cannot be used
21	Mycophenolate Saw VP 2008 ³⁶	82% (n=46) ^a ocular MMP	Mycophenolate is one of the best tolerated and safest immunosuppressive drugs to use and is my first line recommendation for moderately severe disease
22	Zurdel J 2001 ¹⁴⁰	9/10 eyes (90%) ocular MMP	
23	IVIG Letko E 2004 ¹⁴¹ Sami N 2004 ¹⁴²	89% patients (n=18) ^c ocular MMP	This is a very expensive therapy and a scarce resource, but is approved for use in mucous membrane pemphigoid in the UK and accessible through local IVIG committees. I use this for patients who have failed other therapies. Supplementary Appendix 5 is a protocol for use in ocular MMP with acknowledgement to Foster CS, who has provided the evidence for its use in ocular MMP
24	John H 2007 ¹⁴³ Prey S 2007 ¹⁴⁴ Sacher C 2002 ¹⁴⁵ Canizares MJ 2006 ¹⁴⁶ Heffernan MP ¹⁴⁷	100% (n=7) ^d ocular and extraocular MMP	These $TNF\alpha$ inhibitors have been little used in small case series. They probably deserve further study. I have no experience in their use for ocular MMP
25	Rituximab Le Roux-Villet C 2011 ¹⁴⁸ Taverna JA 2007 ¹⁴⁹ Ross AH 2009 ¹⁵⁰ Schumann T 2009 ¹⁵¹ Lourari S 2010 ^{152,153} Rubsam A 2015 ¹⁵⁴ Maley A 2016 ¹⁵⁵	80% (51/64) ^e ocular and extraocular MMP although in addition to the 13 reported failures 3 patients in one series only had partial control ¹⁵⁴ and another 5 did not respond after late relapse ¹⁵⁵ .	Rituximab in these studies has been most often given using the rheumatology protocol in which one cycle is a 1gram infusion repeated 14 days later. All these patients have failed conventional therapy first. Repeat cycles given after 4 months or later have been effective in most patients not responding to one cycle. The effect of the drug in early cases, the relapse rate, safety and efficacy of adjunctive immunosuppressives, and long term management protocols have not been established. However, this is currently the most widely used rescue therapy for patients having failure to control inflammation by conventional therapy. I use this in patients in whom cyclophosphamide has failed or is contraindicated
26	IVIG and Rituximab Foster CS 2010 ¹⁵⁶	100% (n=6) ocular MMP	Very high doses of Rituximab were used in this study. I have no experience of combined therapy however I have recommended IVIG in Rituximab failures if two cycles of Rituximab 4 months apart have been ineffective 2 months after a failure to respond to Rituximab; at this stage there should be some residual effect from Rituximab

	T		
27	Daclizumab	100% (n=1) ocular MMP	One case report only. I have no experience with this
	Papaliodis GN 2003 ¹⁵⁷		
28	Cyclophosphamide	(n=24) 12/12 responded to	Confirms use of a steroid sparing drug with prednisolone versus prednisolone alone. No progression
	and prednisolone vs.	cyclophosphamide and oral	of disease in cyclophosphamide group versus 7/12 progressing in prednisolone group.
	oral prednisolone	prednisolone versus 5/12 to	
	Foster CS 1986 ⁴⁶	oral prednisolone.	
29	Dapsone versus	(n=40) 14/20 responded to	Confirms the use of cyclophosphamide rather than dapsone for more severe disease.
	cyclophosphamide	dapsone versus 20/20 to	
	Foster CS 1986 ⁴⁶	cyclophosphamide	
30	Combination therapy with drugs other than prednisolone and cyclophosphamide		
31	Tauber J 1991 ¹³³	Tauber J 1991: 105 patients.	This influential paper was published over 2 decades ago and was the first to describe the effects of
31	Tauber 3 1331	Combination therapy was	treatment of ocular MMP with most non-biological immunomodulators used today, in a large cohort
		used in 24% of patients at	of patients. The breakdown of outcomes of different therapies and combinations are less clear.
		the end of follow up	However, combination therapy was used in 24% of patients and a recommendation is made for this
		including dapsone +/-	
			in the paper.
		prednisolone +/- a	
		myelosuppressive OR a	
		myelopsuppressive +	
	118	prednisolone.	
32	Miserocchi E 2002 ¹¹⁸	61 patients	22% requiring monotherapy, 21% requiring combinations of 3 or more drugs, and 10% 4 drugs
33	Saw VP 2008 ³⁶	388 treatment episodes in 115 patients: 198 single agent treatment episodes (51%) with success or partial success in 73%. 93	
	Williams GP	treatment episodes with a myelosuppressive (cyclophosphamide, azathioprine, mycophenolate, methotrexate) OR a sulfa (dapsone,	
	2011 ³⁹ The same	sulphapyridine) + prednisolone having 87% success or partial success. 47 treatment episodes with a myelosuppressive + a sulfa	
	approach described in	having 75% success or partial success. 31 treatment episodes with a myelosuppressive (cyclophosphamide, azathioprine,	
	a different cohort of 54	mycophenolate, methotrexate) + a sulfa (dapsone, sulphapyridine) + prednisolone having an 87% success or partial success rate. The	
	patients.	paper includes the data on the outcomes of all 115 patients as supplementary data.	
34	Additional notes on combination therapy		
	• Saw VP 2008 ³⁶ is the most comprehensive study on treatment methodology for conventional (non-biological immunomodulatory) therapies. The paper		
	includes outcomes of single agent therapy, and multiple agent combinations with rates of side effects. Multiple agent therapy resulted in improved		
	response rates compared to single therapy, without an identifiable increase in complication rates over those for monotherapy. A "step up and step down"		
	approach is described with the choice of initial therapy related to the severity of disease. Additional drugs are added to, as combination therapy, or		
	substituted as monotherapy, when necessary to gain disease control or to avoid side effects. Cyclophosphamide is reserved for the more severe cases, less		
	toxic drugs for others, and combinations of sulfas and myelosuppressives when disease control on monotherapy is inadequate. Prednisolone, used in short		
	courses, was added to any of the other drugs but most widely in combination with cyclophosphamide.		
	 Guidelines for cyclophosphamide therapy are given in Row 19. Dapsone or sulphapyridine was either added to myelosuppressives, when reducing therapy 		
	• duidennes for cyclophosphannide therapy are given in row 19. Dapsone of sulphapyridine was either added to myelosuppressives, when reducing therapy		

- (stepping down) after disease control, or supplemented with myelosuppressives and/or prednisolone, when disease control was inadequate (stepping up). This approach allows maintenance of therapy while additional new agents are introduced.
- This regimen is what is currently used in the Moorfields Eye Hospital Clinics. This approach has been adopted in UK specialist centres⁴ and is also what has been described also been described in the Foster publications summarized above. However, three review articles^{113,114,158} have not mentioned the use of combined therapy, excepting the use of prednisolone as adjunctive therapy, and have not discussed the value of step up and step down therapy, using drug combinations, to maintain therapy during treatment changes and to minimize risk, for ocular MMP. The approach used by Thorne JE et al. 2008 using cyclophosphamide as monotherapy after a course of adjunctive prednisolone is effective but probably less safe (see Rows 14,19)

^aresults for success (fully controlled inflammation) and qualified success (partial control of inflammation) of 388 individual treatment episodes in 115 patients

^bcombined results of three case series

^ccombined results of two case series

dcombined results of five case series

^ecombined result of 3 case series and 4 case reports