

Therapeutic Considerations: Symptoms, Cells and Mediators

Susan Lightman, PhD, FRCP, FRCOphth, Professor of Clinical Ophthalmology, Moorfields Eye Hospital, London, UK.

Summary

Immuno-histopathological studies of conjunctival tissue biopsied from patients with non-sight-threatening allergic conjunctivitis or with sight-threatening allergic keratoconjunctivitis should lead to more effective management of these eye conditions, based on the specific cellular involvement. The major difference between these two categories of eye disease was the occurrence of T-lymphocytes, which were absent in the former but prominent in the sight-threatening disorders. Seasonal and perennial allergic conjunctivitis both showed a heavy mast cell increase, due to infiltration of mucosal type mast cells, and allergen challenge studies linked mast cell histamine release to the early phase reaction occurring within 20 minutes. A second histamine peak at six hours after challenge might implicate basophils (or refractory mast cells) and was accompanied by a rise in eosinophil cationic protein. In sight-threatening, chronic allergic keratoconjunctivitis the responses were clearly directed by T-cells, themselves the primary effector cell in atopic keratoconjunctivitis, whereas vernal keratoconjunctivitis displayed a T-cell driven eosinophilia, with increased expression of the adhesion molecules involved in tissue invasion by these cells. Appropriate therapies for each different category of conjunctivitis should be based on the specific immunopathology, and directed at the activated cell types that are primarily responsible for the disease process.

Introduction

In considering the most effective therapies for allergic eye diseases, these can be classed as either non-sight-threatening or sight-threatening. Work undertaken at Moorfields (and in collaboration with Stephen Holgate in Southampton) on non-sight-threatening disorders arose from findings in the lung which had led to changes in clinical practice in the treatment of asthma patients. Examination of the

cells and mediators involved in the pathology of seasonal (SAC) and perennial (PAC) allergic conjunctivitis showed fundamental differences from allergic asthma, but nevertheless gave clear indications of the likely therapeutic potential of the treatments available. A similar detailed investigation into immunopathology of the sight-threatening disorders, vernal (VKC) and atopic (AKC) keratoconjunctivitis, has opened the path to an improved, immunotherapeutic approach to treatment, directed by the types of cells involved rather than the overt signs and symptoms of disease.

Non-sight-threatening allergic eye disease

Seasonal and perennial allergic conjunctivitis

The typical ocular pathology of patients with relatively severe seasonal allergic conjunctivitis shows increased numbers of mast cells in the conjunctiva and, particularly, moving into the epithelium, where they are not normally found¹. Eosinophils and neutrophils also increase but, unlike the findings in the allergic asthmatic lung which led to a change in management of asthma², there are no T-cells to be found, and no fibrous tissue.

Mast cells can be divided into different sub-types and it is the connective tissue type which normally predominates in the conjunctiva. By staining 1 µm thick glycol-methacrylate serial sections, it is possible to work out exactly what type of proteases are present in these mast cells. One perplexing finding has been resolved by this study. It was known that *in vitro* connective tissue type cells are relatively resistant to sodium cromoglycate, whereas clinically, cromoglycate is very useful for the management of SAC. This study demonstrated an influx of mucosal mast cells into the conjunctiva in SAC and it is these that are sensitive to cromoglycate *in vivo*. When the effects of ocular allergen challenge were examined, the *total* number of mast cells remained the same but their distribution was different: tryptase, common to all mast cells, was detected but without the chymase characteristic of connective tissue mast cells, showing a predominance of mucosal type cells. PAC was very similar in many ways to SAC but interestingly these patients had no epithelial mast cells.

Patients known to be sensitive to various allergens, particularly to mixed grass pollen in the majority of cases, but in some to house dust mite, were challenged with these antigens into the conjunctival sac. To determine whether this was a reliable model of allergic conjunctivitis, patients were challenged out of season, giving undiluted allergen into the lower fornix of one eye and a control solution into the other eye. Predictably, there was an early phase and a later stage reaction, during which serial tear samples were taken, followed by a conjunctival biopsy at six hours. Two histamine peaks were found – one at 20 minutes and one six hours after challenge – but only one tryptase peak occurred, during the early phase, whilst the only significant elevation of eosinophil cationic protein (ECP) was seen at six hours. These findings agree with reported events following nasal allergen challenge in patients with allergic rhinitis, which linked the early phase reaction to mast cell-derived histamine, whilst eosinophils and non-mast cell (basophil) histamine release were associated with the later stage reaction^{3, 4}. However, it is not yet clear in the ocular late phase reaction if the presence of histamine without mast cell tryptase indicates basophil involvement, or merely that the mast cells are refractory and need time to recover.

Conjunctival biopsies were carried out by everting the upper lid and with a 3 mm trephine under local anaesthetic. The findings were very similar to those seen in SAC patients in-season, with increased neutrophils, mast cells (particularly the mucosal type), eosinophils and macrophages. Again, no change was seen in the T-cells, and although increased adhesion molecules were detected there was no change in VCAM-1 – which may just reflect the fact that this was only six hours and not 24 hours after allergen challenge⁵. No 24-hour data are available as it is difficult to re-biopsy the patients, but the lack of any significant increase in T-cells at six hours after ocular allergen challenge is an extremely important difference from findings in the asthmatic lung. Also, there were no signs of collagen deposition, such as occurs in the lung, and in general the conjunctiva appears to have a much greater similarity to the skin than to the airways mucosa.

Applying this knowledge to the treatment of SAC and PAC, there are a variety of drugs that can be used to good effect. Among mast cell stabilisers, nedocromil sodium has the advantage over sodium cromoglycate in its effectiveness against both subtypes of mast cell to be found in the allergic eye^{6, 7}. Lodoxamide is another mast cell stabiliser which is useful in treating allergic eye diseases^{8, 9} and new generation antihistamines include levocabastine for topical use in the eye¹⁰. Topical steroids can be used to dampen down inflammation generally, but the major problem with using them in disorders such as

SAC and PAC is that they have potentially sight-threatening consequences which the diseases themselves do not. Nedocromil sodium has a variety of advantages: it does stabilise both mast cell subsets, but in addition it reduces leukotriene secretion by activated eosinophils and it inhibits eosinophil and neutrophil chemotaxis – all of which are features of the early allergic response¹¹ (Fig. 1). One of its major advantages is a twice-daily dosage, because trying to remember to take anything four times a day is often impractical. The fact that nedocromil sodium also has effects on both eosinophil migration and adhesion molecules would suggest that it has a major role to play in the future¹².

Sight-threatening allergic eye disease

Vernal and atopic keratoconjunctivitis

The sight-threatening allergic eye diseases are more difficult to manage, yet if not treated adequately the early signs may have blinding sequelae. In severe VKC the problem lies in the development of corneal scars, initially with epithelial defects, which then go on to form macro-erosions. Even worse is AKC, a life-long condition for which the range of drugs available to date is really not satisfactory. Understanding the immunopathology of these diseases may lead to better immuno-therapeutic approaches to their treatment in the future.

The involvement of T-lymphocytes in these disorders was obvious when tissue samples were stained for the cell surface markers CD3 and CD4, showing many T-cells to be present. It was clear too that activated T-cells, having increased expression of CD25 (interleukin IL-2 receptor), were predominant in VKC and AKC, whereas there were very few to be found in normal ocular tissue. The presence of macrophages, staining for MAC-1, was again statistically elevated in VKC, AKC, and also GPC (giant papillary conjunctivitis), which shares many of the immunopathological features of VKC. This combination of CD4⁺ (helper) T-cells, in an activated state as evidenced by increased IL-2 receptor expression, and macrophages showing MHC class-II expression, sets the scene for a T-cell mediated response.

One of the major features of chronic allergic eye disease is cicatrization, and whatever collagen antibody may be used for staining, that type of collagen (3, 4, 5, etc.) will be found in the diseased conjunctiva, whilst proline hydroxylase activity reveals the involvement of activated fibroblasts. The end result is that the patient with VKC may develop tarsal scarring despite the inflammation being quiescent.

Widespread expression of ICAM-1, VCAM-1 and E-selectin in the conjunctiva was found in all allergic eye diseases. To investigate the lymphokines po-

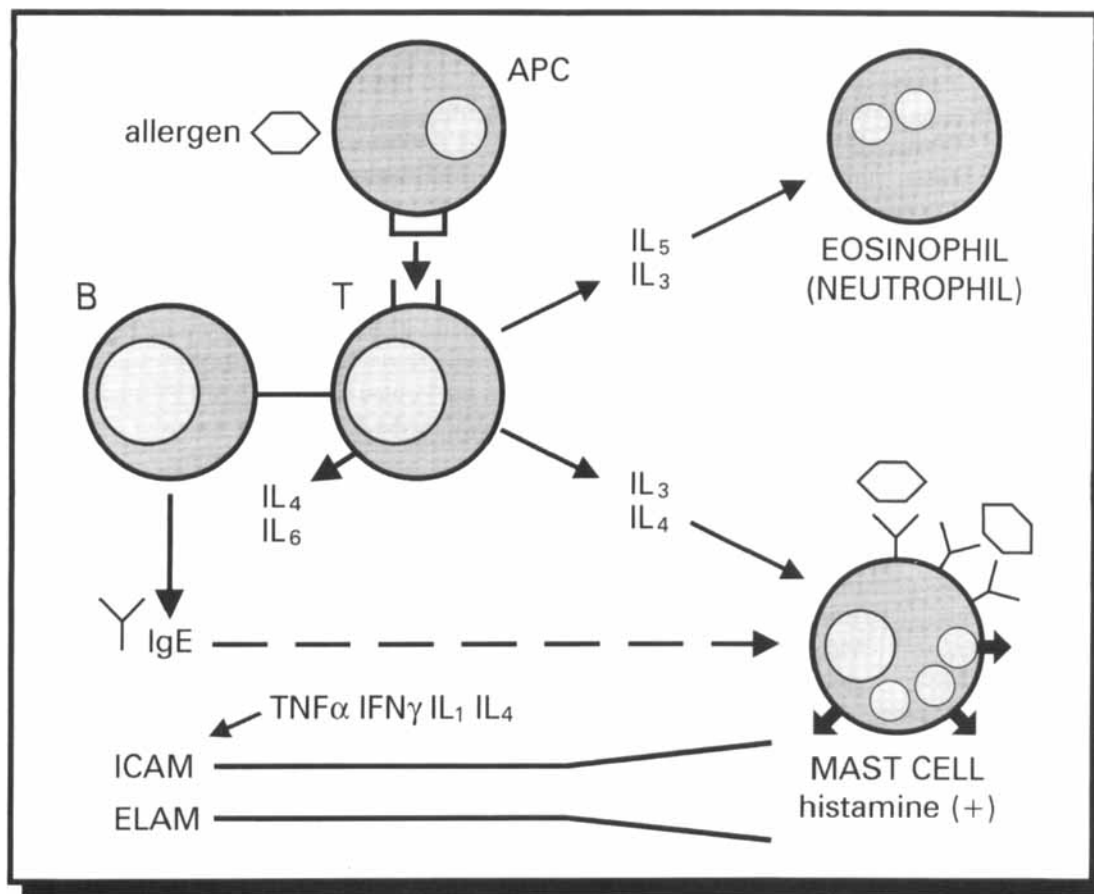


Fig. 1. Schematic representation of the possible events taking place during allergic ocular reactions.

tentially secreted by the activated T-cells, *in situ* hybridisation histochemistry with ribo-probes specific for human lymphokine (messenger) mRNA was used. Large amounts of IL-3 mRNA and IL-4 mRNA, and even greater amounts of IL-5 mRNA, were present in both VKC and AKC, whereas IL-2 mRNA production was associated only with AKC and was not a feature of VKC.

Potential treatments for VKC include a variety of different therapeutic approaches. Typically, most of these patients remain on sodium cromoglycate during the inactive stage, with topical steroids playing a major role during exacerbations. Cyclosporin eye drops have also shown benefits¹³. Cyclosporin not only has a suppressive effect on T-cells but also on eosinophils, which should make it a useful drug for VKC. Major basic protein (MBP) secreted by the activated eosinophils has a direct effect on the corneal epithelium and may be one cause of the corneal problems that are seen; in addition, it causes mast cell degranulation and can potentially perpetuate the inflammatory cycle. One theoretical problem to bear in mind is that cyclosporin has been shown to upregulate (transforming growth factor) TGF-β mRNA¹⁴ and, in disorders which have a scarring

tendency anyway, it is important not to control the inflammation at the cost of increasing scarring: that may be too high a price to pay. The mast cell stabilising drugs sodium cromoglycate¹⁵, nedocromil sodium¹¹ and lodoxamide¹⁶ can also inhibit activated eosinophils, and the two more recent compounds have shown better clinical activity than sodium cromoglycate in patients with VKC^{17, 18}, perhaps due to greater activity against eosinophils and mast cells.

The cellular mechanisms involved in allergic eye disorders in general are summarised in Fig. 1. Sight-threatening, chronic allergic keratoconjunctivitis may involve two different types of mechanism. In AKC, the T-cell is almost certainly the primary effector cell, and ongoing studies will provide more information about this. In VKC, the T-cells may drive the eosinophils, which then cause the major pathological changes. Taking into account all the other cell types that are present, these are the factors that should direct the approach to therapy for the future. In collaboration with Stephen Holgate from Southampton, these two groups hope to find better ways of managing the patients with these sight-threatening disorders.

References

1. BADDELEY SM, BRADDING P, HEUSSER C, BACON A, ROCHE W. Immunolocalisation of IL-4 in normal and allergic conjunctivitis. *Clin Exp Allergy* 1994; 24: 980 (abstract).
2. AZZAWI M, BRADLEY B, JEFFERY PK, FREW AJ, WARDLAW AJ, KNOWLES G, ASSOUEFI B, COLLINS JV, DURHAM S, KAY AB. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990; 142: 1407-1413.
3. NACLERIO RM, PROUD D, TOGIAS AG, ADKINSON NF, MEYERS DA, KAGEY-SOBOTKA A, PLAUT M, NORMAN PS, LICHTENSTEIN LM. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985; 313: 65-70.
4. KLEMENTSSON H, ANDERSSON M, BAUMGARTEN CR, VENGE P, PIPKORN U. Changes in non-specific nasal reactivity and eosinophil influx and activation after allergen challenge. *Clin Exp Allergy* 1990; 20: 539-547.
5. HOLGATE ST. Asthma: past, present and future. *Eur Respir J* 1993; 6: 1507-1520.
6. PEARCE FL, AL-LAITH M, BOSMAN L, BROSTOFF J, CUNIFFE TM, FLINT KC, HUDSPITH BN, JAFFAR ZH, JOHNSON NMCI, KASSESSINOFF TA, LAU HYA, LEE PY, LEUNG KBP, LIU WL, TAINSH KR. Effects of sodium cromoglycate and nedocromil sodium on histamine secretion from mast cells from various locations. *Drugs* 1989; 37(suppl.1): 37-43.
7. VAN BIJSTERVELD OP, MOONS L, VERDONCK M, KEMPE-NEERS HP. Nedocromil sodium treats symptoms of perennial allergic conjunctivitis not fully controlled by sodium cromoglycate. A double-masked placebo controlled group comparative study. *Ocular Immunol Inflamm* 1994; 2: 177-186.
8. YANNI JM, WEIMER LK, GLASER RL, LANG LS, ROBERTSON SM, SPELLMAN JM. Effect of lodoxamide on in vitro and in vivo conjunctival immediate hypersensitivity responses in rats. *Int Arch Allergy Immunol* 1993; 101: 102-106.
9. FAHY GT, EASTY DL, COLLUM LMT, BENEDICT-SMITH A, HILLERY M, PARSONS DG. Randomised double-masked trial of lodoxamide and sodium cromoglycate in allergic eye disease. *Eur J Ophthalmol* 1992; 2: 144-149.
10. DECHANT KL, GOA KL. LEVOCABASTINE. A review of its pharmacological properties and therapeutic potential as a topical antihistamine in allergic rhinitis and conjunctivitis. *Drugs* 1991; 41: 202-224.
11. MOQBEL R, CROMWELL O, WALSH GM, WARDLAW AJ, KURLAK L, KAY AB. The effects of nedocromil sodium (Tilade) on activation of human eosinophils, neutrophils and histamine release from mast cells. *Allergy* 1988; 43: 268-276.
12. TRIGG C, MANOLITSAS N, MCAULEY A, NORTON A, WANG J, DAVIES RJ. A pilot comparative study of the effects of inhaled nedocromil sodium and albuterol on bronchial biopsies in asthma. *Am Rev Respir Dis* 1993; 147: A522.
13. BEN-EZRA D, PE'ER J, BRODSKY M, COHEN E. Cyclosporin A for the treatment of severe vernal keratoconjunctivitis. *Am J Ophthalmol* 1986; 101: 278-281.
14. LI B, SEHAJPAL PK, KHANNA A, VLASSARA H, CERAMI A, STENZEL KH, SUTHANTHIRAN M. Differential regulation of transforming growth factor beta and interleukin 2 genes in human T cells: demonstration by usage of novel competitor DNA constructs in the quantitative polymerase chain reaction. *J Exp Med* 1991; 174: 1259-1262.
15. KAY AB, WALSH GM, MOQBEL R, MACDONALD AJ, NAGAKURA T, CARROLL MP, RICHESON HB. Disodium cromoglycate inhibits activation of human inflammatory cells in vitro. *J Allergy Clin Immunol* 1987; 80: 1-8.
16. SANTOS CI, HUANG AJ, ABELSON MB, FOSTER CS, FRIEDLAENDER M, MCCULLEY JP. Efficacy of lodoxamide 0.1% ophthalmic solution in resolving corneal epitheliopathy associated with vernal keratoconjunctivitis. *Am J Ophthalmol* 1994; 117: 488-497.
17. CALDWELL DR, VERIN P, HARTWICH YOUNG R, MEYER SM, DRAKE M. Efficacy and safety of lodoxamide 0.1% vs cromolyn sodium 4% in patients with vernal keratoconjunctivitis. *Am J Ophthalmol* 1992; 113: 632-637.
18. EL HENNAWI M. A double-blind placebo controlled group comparative study of ophthalmic sodium cromoglycate and nedocromil sodium in the treatment of vernal keratoconjunctivitis. *Br J Ophthalmol* 1994; 78: 365-369.