

Vernal keratoconjunctivitis

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REVIEW

Abstract

Vernal keratoconjunctivitis (VKC) is an allergic eye disease that especially affects young boys. The most common symptoms are itching, photophobia, burning, and tearing. The most common signs are giant papillae, superficial keratitis, and conjunctival hyperaemia.

Patients with VKC frequently have a family or medical history of atopic diseases, such as asthma, rhinitis, and eczema. However, VKC is not associated with a positive skin test or RAST in 42–47% of patients, confirming that it is not solely an IgE-mediated disease. On the basis of challenge studies as well as immunohistochemical and mediator studies, a Th2-driven mechanism with the involvement of mast cells, eosinophils, and lymphocytes has been suggested. Th2 lymphocytes are responsible for both hyperproduction of IgE (interleukin 4, IL-4) and for differentiation and activation of mast cells (IL-3) and eosinophils (IL-5). Other studies have demonstrated the involvement of neural factors such as substance P and NGF in the pathogenesis of VKC, and the overexpression of oestrogen and progesterone receptors in the conjunctiva of VKC patients has introduced the possible involvement of sex hormones. Thus, the pathogenesis of VKC is probably multifactorial, with the interaction of the immune, nervous, and endocrine systems.

The clinical management of VKC requires a swift diagnosis, correct therapy, and evaluation of the prognosis. The diagnosis is generally based on the signs and symptoms of the disease, but in difficult cases can be aided by conjunctival scraping, demonstrating the presence of infiltrating eosinophils. Therapeutic options are many, in most cases topical, and should be chosen on the basis of the severity of the disease. The most effective drugs, steroids, should however be carefully administered, and only for brief periods, to avoid secondary development of glaucoma.

A 2% solution of cyclosporine in olive oil or in castor oil should be considered as an alternative. The long-term prognosis of

patients is generally good; however 6% of patients develop corneal damage, cataract, or glaucoma.

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Introduction

Vernal keratoconjunctivitis (VKC) is a member of a group of diseases classified as allergic conjunctivites including perennial and seasonal rhinoconjunctivitis, atopic keratoconjunctivitis, and giant papillary conjunctivitis.^{1–3} For many years, all allergic conjunctivites were considered (as suggested by Coombs and Gell⁴) the expression of a classical type I IgE-mediated hypersensitivity reaction at the conjunctival level. More recent clinical observations, however, suggest that other tissues of the eye are also involved in the ocular allergic reaction: the lids, with their high content of mast cells, the tear film, with its immunoglobulins, and the cornea, so important for visual function. New discoveries regarding the pathogenesis of ocular allergies have clearly indicated that the participation of the entire ocular surface in allergic diseases is not only the consequence of tissue contiguity but derives from a complex exchange of information between these tissues through cell-to-cell communications, chemical mediators, cytokines, and adhesion molecules. It is also possible that the neural and endocrine systems may influence the ocular allergic response.

The purpose of this paper is to describe the clinical expression of VKC, to discuss its pathogenetic mechanisms, and to suggest novel therapeutic strategies.

Clinical features

VKC is a chronic bilateral inflammation of the conjunctiva characterized by hyperaemia, chemosis, photophobia, and filamentous and

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sticky mucous discharge. The hallmark of the disease is the presence of giant papillae (cobblestone appearance) at the upper tarsal conjunctiva (tarsal form) or at limbus (bulbar form).¹⁻³ Although there are some differences between the two forms, probably related to geographical variations, it seems likely that both represent different clinical manifestations of the same disease.¹ (Figures 1-3).

VKC mainly affects boys in their first decade of life and the sequelae of the disease may be responsible for permanent visual impairment. Despite its name, the disease can be frequently present all year round. Approximately 23% of patients have a perennial form of VKC from disease onset and more than 60% have additional recurrences during the winter.⁵ Furthermore, in almost 16% of the cases, the seasonal (vernal) form evolves into a chronic, perennial inflammation after a mean of 3 years from disease onset, suggesting that the longer patients suffer from VKC, the more apt they are to develop a persistent form of the disease.⁵

Itching, photophobia, burning, and tearing are the major ocular symptoms. Patients also complained of frequent conjunctival redness after exposure to nonspecific stimuli. This finding supports previous reports suggesting the presence of a conjunctival hyper-reactivity when sun, dust, wind, and other general climactic factors or nonspecific stimuli come in contact with the conjunctival mucosa.⁶ This hyper-reactivity, which is also known to be frequently associated with asthma and other allergic diseases, may actually be a distinct clinical entity. At present, the exact mechanism for nonspecific hyper-reactivity is not understood, but it is possible that the release of vasoactive mediators could be involved.⁷

Signs include the presence of giant papillae on the upper tarsal conjunctiva or at the limbus, the presence of aggregates of epithelial cells and eosinophils at the



Figure 1 Giant papillary hypertrophy with the typical cobblestone appearance of the upper tarsal conjunctiva.

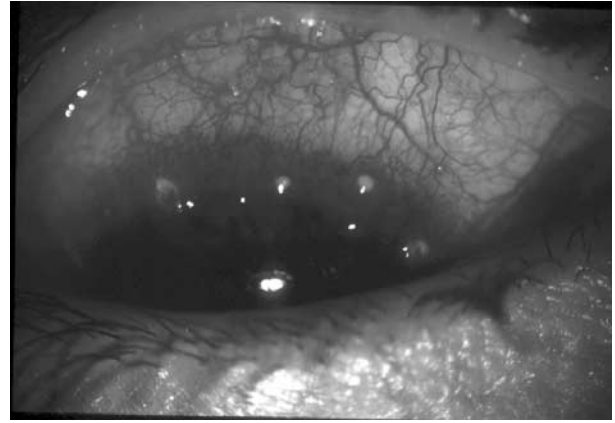


Figure 2 Trantas' dots in the superior limbus represent an aggregation of epithelial cells and eosinophils.

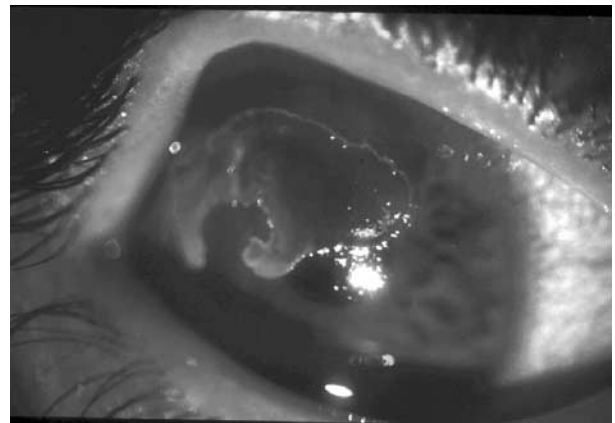


Figure 3 Corneal shield ulcers (Togby's ulcers) are mainly located in the upper paracentral cornea.

limbus (Trantas' dots), and marked conjunctival hyperaemia. Subconjunctival fibrosis, symblepharon, and conjunctival keratinization can develop. The cornea is almost always damaged with a superficial keratopathy, or the presence of corneal shield ulcers (occurring in approximately 3-11%) and neovascularization.^{5,8,9} A blepharitis is frequently associated and eczema or maceration of the lid can be observed. Cataract and steroid-induced glaucoma are the major ocular complications.^{5,9}

Immunologic mechanisms

Patients with VKC have a family history of atopic diseases in 49% of cases. These patients may also have a medical history of other atopic conditions including asthma (26.7%), rhinitis (20%), and eczema (9.7%).⁵ Data regarding the frequency of sensitisation are conflicting: Ballou and Mendelson¹⁰ reported 19% of positive

sensitisation, Easty *et al*¹¹ reported 80%. In a large series of 195 patients, we found a positive response to skin tests and RAST in 57 and 52%, respectively.⁵ Total IgE has been shown to be elevated in the serum, and local production of IgE in tears has been postulated.^{10–14} However, levels of tear IgE were low, at 18.5 ± 19.2 U/ml, in our patients and we never observed a positive RAST in tears in the presence of negative results in the serum.¹⁵

Recent studies suggest a more complex non-IgE-dependent pathogenic mechanism. A multitude of cells and mediators have been detected in the serum, conjunctiva, and tears of patients with VKC, which may have a relevant role in the pathogenesis of the disease.^{13,16–19} Although many features of VKC suggest an allergic pathogenesis,^{3,12,20} this eye disease can no longer be considered a classical type I IgE-mediated disease, as included in the classification by Gell and Coombs.^{16,21} In fact, skin tests and RAST are often negative in VKC and several patients do not have a personal or family history of atopy.⁵ On the basis of challenge studies as well as immunohistochemical and mediator studies, we recently suggested a Th2-driven mechanism and a definition, similar to that of asthma, of 'an allergic inflammatory disease of the conjunctiva with mast cells, eosinophils, and lymphocytes'.²¹ Supporting this definition are the findings that T-cell clones derived from VKC tissues are mainly of the Th2-type and that in the CD4 areas of VKC biopsies, there is an increased *in situ* hybridization signal for IL-5 associated with increased IL-5, but not IL-2, levels in tears, suggesting Th2 rather than Th1 activity.^{18,22–25} It is possible that the pathogenesis of VKC is characterized by a Th2 lymphocyte alteration, while the exaggerated IgE response to common allergens is an inconsistent and, perhaps, secondary event. Th2 lymphocytes are responsible both for hyperproduction of IgE (interleukin 4, IL-4) and for differentiation and activation of mast cells (IL-3) and eosinophils (IL-5). Mast cells and basophils cause the immediate reaction (through the release of histamine) and the recruitment of inflammatory cells (lymphocytes and eosinophils). This cell recruitment (favoured by an overexpression of adhesion molecules) results in the release of other toxic cell mediators (such as eosinophil cationic protein, EDN/EPX) with corneal epithelial damage.^{18,26–28} Indeed, several inflammatory and epithelial cells may induce fibroblast proliferation and collagen production leading to the characteristic conjunctival findings.

While histamine is the main mediator in allergic reactions with a prevalent Type I hypersensitivity mechanism (such as perennial or seasonal allergic conjunctivitis), and in the early phase of allergic reactions (antihistamines are thus effective in these conditions),

other mediators are involved in VKC, such as eosinophilic mediators and substances derived from the metabolism of arachidonic acid (prostaglandins and leukotrienes).^{12,29} In particular, leukotrienes are produced during allergic and inflammatory respiratory diseases by mast cells, macrophages, and neutrophils.^{30,31} They are potent mediators of hypersensitivity and inflammatory reactions.¹⁶ Their activities include smooth-muscle contraction, small vessel dilatation, increase in blood vessel permeability, promotion of glycoprotein secretion from epithelial glands, and increase of nasal blood flow and airway resistance.^{32–36} It has been demonstrated that leukotrienes are also produced in the conjunctiva³⁷ and are detectable in tear fluids of patients affected by allergic conjunctivitis including VKC.^{38–40} Indeed LT concentration in tears increases in allergic subjects following allergen challenge.^{41,42} In addition, conjunctival administration of LTB₄, as well as LTC₄ and LTD₄, induces vessel vasodilatation, oedema, hyperaemia, and leucocyte and eosinophilic infiltration of the conjunctiva.^{43–45} The biological activities of leukotrienes on the conjunctiva may contribute to the presence of the characteristic symptoms observed in VKC, such as mucous secretion, conjunctival hyperaemia, and chemosis.

Neural and endocrine involvement

The existence of a relationship between the central nervous system and the eye is easily established when considering common anatomical and embryogenic origins. This cross-talk is also strictly related to the immune system and is shown by nerve and mast cell interaction. Whether neurotransmitters and neurotrophins may influence conjunctival inflammation is not clear at present. Substance P, a neuropeptide with well-known activity on immune cells, has been detected in tears, and high serum levels have been found in patients with VKC.^{46,47}

Receptors for nerve growth factor have been found in the epithelium and substantia propria of the conjunctiva, and high serum levels of nerve growth factor are detectable in the active form of the disease and are directly related to the number of mast cells in the conjunctival tissue, suggesting that neural influences may have a role in the pathogenesis of allergic diseases.^{48,49}

A role for sex hormones has been postulated in the pathogenesis of the disease. This assumption derives from the observation of a prevalence of males *vs* females and a spontaneous resolution of the disease at puberty. Sex hormones may play a relevant role in the pathophysiology of allergic diseases by reciprocal interactions between the immune and the endocrine

system. Oestrogens and progesterone have been shown to be active players in the ocular immune system, with an already well-established role in another immunological disease, dry eye syndrome.⁵⁰ In a previous immunohistochemical study of patients with VKC, we reported that oestrogen and progesterone receptors were overexpressed on the conjunctiva by eosinophils and other inflammatory cells.⁵¹ These hormones may bind to conjunctival receptors and exert a proinflammatory effect through the recruitment of eosinophils to the conjunctival tissue.^{52,53}

Diagnosis

The typical, characteristic signs and symptoms of this disease render the diagnosis of VKC fairly straightforward, even for the general ophthalmologist. Atypical presentations or incomplete forms of VKC may, however, lead to an underestimation of its incidence. The identification of both the major and minor signs and symptoms of VKC allows an early and accurate diagnosis of this disease.

At present, total and specific IgE determination, as well as skin tests cannot be considered useful additional laboratory tests, because more than 50% of patients with VKC are negative.

In case of a diagnostic dilemma, a conjunctival scraping can be precious in demonstrating the presence of eosinophils infiltrating the conjunctival epithelium.

Therapy

Clinical observation suggests that VKC generally subsides with the onset of puberty, but some therapeutic measures may be required beyond this age to control the course of the disease. In some cases, permanent changes to the ocular surface may occur and be accompanied by permanent visual impairment. Although vernal keratoconjunctivitis generally has a good prognosis, 52% of patients in our cohort had persistent symptoms after a mean follow-up period of approximately 5 years and 6% of patients showed a permanent reduction in visual acuity as a result of corneal damage.⁵

Treatment is symptomatic and topical eye drops are generally preferred as first choice. Cromolyn and the new generation of antiallergic compounds such as alomide, tromethamine, nedocromil sodium, spaglumic acid, and topical antihistamines are effective in reducing signs and symptoms of the disease.⁵⁴ Use of unpreserved solutions may reduce the risk of hypersensitivity to preservatives that are frequently superimposed in these patients. Nonsteroidal anti-inflammatory agents also produce a beneficial effect on the course of VKC.⁵⁵ Topical steroid

preparations are, naturally, the most effective therapy for moderate to severe form of VKC,^{5,9} however their use should be strictly limited and carefully monitored because long-term use of topical steroids is responsible for the 2% incidence of glaucoma in VKC patients.⁵

Therefore, this class of drugs should be used to restore corneal damage induced by epithelial toxic mediators from eosinophils and neutrophils. Once the acute phase of the disease is controlled, steroids should be discontinued and alternative topical treatment (mast cell stabilizers, antihistamine, NSAIDs) should be started.

Cyclosporine A (CsA) from 0.5 to 2% ophthalmic emulsions in olive or castor oil, used four times daily, represents a valid alternative to steroids in severe forms of VKC.

In fact, CsA is effective in controlling ocular inflammation, blocking Th2 lymphocyte proliferation, and IL-2 production. It also inhibits histamine release from mast cells and basophils and, through a reduction of IL-5 production, it may reduce the recruitment and the effects of eosinophils on the conjunctiva.^{56–59} Moreover, the therapeutic efficacy of CsA in VKC, a conjunctival hyperproliferative disorder, seems to be related to the drug's efficacy in reducing conjunctival fibroblast proliferation rate and IL-1 β production.⁶⁰

In a few patients with VKC, a systemic treatment may be required. Oral antihistamines can reduce the generalized hyper-reactivity but have little or no effect on vernal keratoconjunctivitis,⁶¹ while aspirin treatment,^{62,63} as well as oral administration of Montelukast (5–10 mg once/day),⁶⁴ an anti-leukotriene drug usually used in mild asthma, has been demonstrated to be effective in reducing signs and symptoms of VKC.

Outcome

Patients with VKC generally have spontaneous resolution of the disease after puberty without any further symptoms or visual complication. However, corneal ulcers, which are reported to develop in approximately 9.7% of patients, as well as the development of cataract or glaucoma, can produce a permanent visual impairment.^{65,66}

As negative prognostic factor, it has been reported that the size of the giant papillae is directly related to the probability of the persistence or worsening of symptoms and that the bulbar forms of VKC have a worse long-term prognosis than the tarsal forms.⁵

Conjunctival fibrosis observed on the upper tarsal conjunctiva can be considered to be the natural evolution of giant papillae. It is important to note that in the few patients treated with cryogenic surgery to reduce

papillary excrescencies, a marked pemphigoid-like appearance was evident all over the conjunctiva. Thus, it appears that in some VKC patients, any additional inflammation such as that induced by cryogenic therapy may result in an exaggerated tissue response.

Conclusion

Recently, new reports have improved the knowledge of the pathogenetic mechanisms of VKC. The typical male pattern of incidence significantly decreases with age, with disparity diminishing greatly in patients after puberty. It was shown to be a perennial and not a seasonal disease in the majority of patients, with a tendency to become chronic in long-lasting forms. VKC was shown to be not solely IgE-mediated, but its pathogenesis is multifactorial, mediated by Th2 lymphocytes, eosinophils, IgE, mast cells, and a complex network of interleukins and cell mediators. The presence of increased serum levels of cytokines, enzymes, eosinophil-derived mediators, neuropeptides, and neurotrophins also suggests that this is a systemic, and not just a local, disease.^{67–69} The long-term prognosis of the VKC patient is generally good; however approximately 6% of the patients develop a visual impairment owing to corneal damage, cataract, or glaucoma.⁵

References

- 1 Beigelman MN. *Vernal Conjunctivitis*. University of Southern California Press: Los Angeles, 1950.
- 2 Donshik PC, Ehlers WH. Clinical immunologic diseases. In: Srolin G, Thoft RA (eds). *The Cornea*, 3rd ed, chap. 12. Little, Brown & Company: Boston, 1994.
- 3 Brody JM, Foster CS. Vernal conjunctivitis. In: Pepose JS, Holland GN, Wilhelmus KR (eds). *Ocular Infection and Immunity*, Chap. 27. Mosby: St Louis, 1996.
- 4 Coombs RRA, Gell PGH. The classification of allergic reactions underlying disease. In: Gell PGH, Coombs RRA (eds). *Clinical Aspects of Immunology*. Chap. 13. Blackwell Scientific Publications: Oxford, 1962.
- 5 Bonini S, Bonini S, Lambiase A, Marchi S, Pasqualetti P, Zuccaro O et al. Vernal keratoconjunctivitis revisited: a case series of 195 patients with long-term followup. *Ophthalmology* 2000; **107**: 1157–1163.
- 6 Bonini S, Bonini S, Schiavone M, Centofanti M, Allansmith MR, Bucci MG. Conjunctival hyperresponsiveness to ocular histamine challenge in patients with vernal conjunctivitis. *J Allergy Clin Immunol* 1992; **89**: 103–107.
- 7 Togias AG, Naclerio RM, Proud D, Fish JE, Adkinson Jr NF, Kagey-Sobotka A et al. Nasal challenge with cold, dry air results in release of inflammatory mediators. Possible mast cell involvement. *J Clin Invest* 1985; **76**: 1375–1381.
- 8 Cameron JA. Shield ulcers and plaques of the cornea in vernal keratoconjunctivitis. *Ophthalmology* 1995; **102**: 985–993.
- 9 Tabbara KF. Ocular complications of vernal keratoconjunctivitis. *Can J Ophthalmol* 1999; **34**: 88–92.
- 10 Ballou M, Mendelson L. Specific immunoglobulin E antibodies in tear secretions of patients with vernal conjunctivitis. *J Allergy Clin Immunol* 1980; **66**: 112–118.
- 11 Easty DL, Birkenshaw M, Merrett T, Merrett J, Entwistle C, Amer B. Immunological investigations in vernal eye disease. *Trans Ophthalmol Soc U K* 1980; **100**: 98–107.
- 12 Abelson MB, Schaefer K. Conjunctivitis of allergic origin: immunologic mechanisms and current approaches to therapy. *Surv Ophthalmol* 1993; **38**: 115–132.
- 13 Montan PG, van Hage-Hamsten M. Eosinophil cationic protein in tears in allergic conjunctivitis. *Br J Ophthalmol* 1996; **80**: 556–560.
- 14 Aalders-Deenstra V, Kok PT, Bruynzeel PL. Measurement of total IgE antibody levels in lacrimal fluid of patients suffering from atopic and non-atopic eye disorders. Evidence for local IgE production in atopic eye disorders? *Br J Ophthalmol* 1985; **69**: 380–384.
- 15 Bonini S, Lambiase A, Juhas T, Bonini S. Allergic conjunctivitis. *Dev Ophthalmol* 1999; **30**: 54–61.
- 16 Bonini S, Bonini S, Bucci MG, Berruto A, Adriani E, Balsano F et al. Allergen dose response and late symptoms in a human model of ocular allergy. *J Allergy Clin Immunol* 1990; **86**: 869–876.
- 17 Bonini S, Tomassini M, Adriani E, Magrini L, Rumi C, Bussa S et al. Markers of eosinophilic inflammation in allergic diseases. *Allergy* 1993; **48**: 133–137; discussion 143–145.
- 18 Trocme SD, Aldave AJ. The eye and the eosinophil. *Surv Ophthalmol* 1994; **39**: 241–52.
- 19 Fujishima H, Shimazaki J, Takeuchi T, Saito I, Tsubota K. Interleukin-4 and IgE in seasonal allergic conjunctivitis. *Ophthalmologica* 1996; **210**: 325–328.
- 20 Bielory L, Frohman LP. Allergic and immunologic disorders of the eye. *J Allergy Clin Immunol* 1992; **89**: 1–15.
- 21 Bonini S, Bonini S. IgE and non-IgE mechanisms in ocular allergy. *Ann Allergy* 1993; **71**: 296–299.
- 22 Bonini S, Bonini S, Lambiase A, Magrini L, Rumi C, Del Prete G et al. Vernal keratoconjunctivitis: a model of 5q cytokine gene cluster disease. *Int Arch Allergy Immunol* 1995; **107**: 95–98.
- 23 Maggi E, Biswas P, Del Prete G, Parronchi P, Macchia D, Simonelli C et al. Accumulation of Th-2-like helper T cells in the conjunctiva of patients with vernal conjunctivitis. *J Immunol* 1991; **146**: 1169–1174.
- 24 van Leeuwen BH, Martinson ME, Webb GC, Young IG. Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5. *Blood* 1989; **73**: 1142–1148.
- 25 Metz DP, Bacon AS, Holgate S, Lightman SL. Phenotypic characterization of T cells infiltrating the conjunctiva in chronic allergic eye disease. *J Allergy Clin Immunol* 1996; **98**: 686–696.
- 26 Bagnasco M, Pesce G, Fiorino N, Riccio AM, Ciprandi G, Buscaglia S et al. In situ hybridization analysis of ICAM-1 (CD54) mRNA on conjunctival epithelium during allergic inflammation. *Clin Exp Allergy* 1997; **27**: 737–743.
- 27 Tomassini M, Magrini L, De Petrillo G, Adriani E, Bonini S, Balsano F et al. Serum levels of eosinophil cationic protein in allergic diseases and natural allergen exposure. *J Allergy Clin Immunol* 1996; **97**: 1350–1355.
- 28 Gill KS, Yannariello-Brown J, Patel J, Nakajima N, Rajaraman S, Trocme SD. ICAM-1 expression in corneal

- epithelium of a patient with vernal keratoconjunctivitis: case report. *Cornea* 1997; **16**: 107–111.
- 29 Baraniuk JN. Pathogenesis of allergic rhinitis. *J Allergy Clin Immunol* 1997; **99**: S763–S772.
- 30 Wardlaw AJ, Hay H, Cromwell O, Collins JV, Kay AB. Leukotrienes, LTC4 and LTB4, in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. *J Allergy Clin Immunol* 1989; **84**: 19–26.
- 31 Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. *N Engl J Med* 1999; **340**: 197–206.
- 32 Creticos PS, Peters SP, Adkinson Jr NF, Naclerio RM, Hayes EC, Norman PS *et al*. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N Engl J Med* 1984; **310**: 1626–1630.
- 33 Bisgaard H, Olsson P, Bende M. Effect of leukotriene D4 on nasal mucosal blood flow, nasal airway resistance and nasal secretion in humans. *Clin Allergy* 1986; **16**: 289–297.
- 34 Knapp HR. Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor. *N Engl J Med* 1990; **323**: 1745–1748.
- 35 Marom Z, Shellhamer JH, Kaliner M. Effects of arachidonic acid, monohydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways *in vitro*. *J Clin Invest* 1981; **67**: 1695–1702.
- 36 Reiss TF, Altman LC, Chervinsky P, Bewtra A, Stricker WE, Noonan GP *et al*. Effects of montelukast (MK-0476), a new potent cysteinyl leukotriene (LTD4) receptor antagonist, in patients with chronic asthma. *J Allergy Clin Immunol* 1996; **98**: 528–534.
- 37 Orning L, Kaijser L, Hammarstrom S. *In vivo* metabolism of leukotriene C4 in man: urinary excretion of leukotriene E4. *Biochem Biophys Res Commun* 1985; **130**: 214–220.
- 38 Nathan H, Naveh N, Meyer E. Levels of prostaglandin E2 and leukotriene B4 in tears of vernal conjunctivitis patients during a therapeutic trial with indomethacin. *Doc Ophthalmol* 1994; **85**: 247–257.
- 39 Kulkarni PS, Srinivason BD. Synthesis of slow reacting substance-like activity in rabbit conjunctiva and anterior uvea. *Invest Ophthalmol Vis Sci* 1983; **24**: 1079–1085.
- 40 Sengor T, Irkec M, Gulen Y, Taseli M, Erker H. Tear LTC4 levels in patients with subclinical contact lens related giant papillary conjunctivitis. *CLAO J* 1995; **21**: 159–162.
- 41 Proud D, Sweet J, Stein P, Settipane RA, Kagey-Sobotka A, Friedlaender MH *et al*. Inflammatory mediator release on conjunctival provocation of allergic subjects with allergen. *J Allergy Clin Immunol* 1990; **85**: 896–905.
- 42 Bisgaard H, Ford-Hutchinson AW, Charleson S, Taudorf E. Detection of leukotriene C4-like immunoreactivity in tear fluid from subjects challenged with specific allergen. *Prostaglandins* 1984; **27**: 369–374.
- 43 Bisgaard H, Ford-Hutchinson AW, Charleson S, Taudorf E. Production of leukotrienes in human skin and conjunctival mucosa after specific allergen challenge. *Allergy* 1985; **40**: 417–423.
- 44 Spada CS, Woodward DF, Hawley SB, Nieves AL. Leukotrienes cause eosinophil emigration into conjunctival tissue. *Prostaglandins* 1986; **31**: 795–809.
- 45 Woodward DF, Ledgard SE. Effect of LTD4 on conjunctival vasopermeability and blood-aqueous barrier integrity. *Invest Ophthalmol Vis Sci* 1985; **26**: 481–485.
- 46 Lambiase A, Bonini S, Micera A, Tirassa P, Magrini L, Bonini S *et al*. Increased plasma levels of substance P in vernal keratoconjunctivitis. *Invest Ophthalmol Vis Sci* 1997; **38**: 2161–2164.
- 47 Fujishima H, Takeyama M, Takeuchi T, Saito I, Tsubota K. Elevated levels of substance P in tears of patients with allergic conjunctivitis and vernal keratoconjunctivitis. *Clin Exp Allergy* 1997; **27**: 372–378.
- 48 Lambiase A, Bonini S, Bonini S, Micera A, Magrini L, Bracci-Laudiero L *et al*. Increased plasma levels of nerve growth factor in vernal keratoconjunctivitis and relationship to conjunctival mast cells. *Invest Ophthalmol Vis Sci* 1995; **36**: 2127–2132.
- 49 Bonini S, Lambiase A, Bonini S, Angelucci F, Magrini L, Manni L *et al*. Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci USA* 1996; **93**: 10955–10960.
- 50 Kramer P, Lubkin V, Potter W, Jacobs M, Labay G, Silverman P. Cyclic changes in conjunctival smears from menstruating females. *Ophthalmology* 1990; **97**: 303–307.
- 51 Bonini S, Lambiase A, Schiavone M, Centofanti M, Palma LA, Bonini S. Estrogen and progesterone receptors in vernal keratoconjunctivitis. *Ophthalmology* 1995; **102**: 1374–1379.
- 52 Tchernitchin A, Roorijck J, Tchernitchin X, Vandenhende J, Galand F. Dramatic early increase in uterine eosinophils after oestrogen administration. *Nature* 1974; **248**: 142–143.
- 53 Grunert G, Porcia M, Neumann G, Sepulveda S, Tchernitchin AN. Progesterone interaction with eosinophils and with responses already induced by oestrogen in the uterus. *J Endocrinol* 1984; **102**: 295–303.
- 54 Bonini S, Schiavone M, Bonini S, Magrini L, Lischetti P, Lambiase A *et al*. Efficacy of lodoxamide eye drops on mast cells and eosinophils after allergen challenge in allergic conjunctivitis. *Ophthalmology* 1997; **104**: 849–853.
- 55 D'Angelo G, Lambiase A, Cortes M, Sgrulletta R, Pasqualetti R, Lamagna A *et al*. Preservative-free diclofenac sodium 0.1% for vernal keratoconjunctivitis. *Graefes Arch Clin Exp Ophthalmol* 2003; **241**: 192–195.
- 56 Secchi AG, Tognon MS, Leonardi A. Topical use of cyclosporine in the treatment of vernal keratoconjunctivitis. *Am J Ophthalmol* 1990; **110**: 641–645.
- 57 BenEzra D, Matamoros N, Cohen E. Treatment of severe vernal keratoconjunctivitis with cyclosporine A eyedrops. *Transplant Proc* 1988; **20**: 644–649.
- 58 Pucci N, Novembre E, Cianferoni A, Lombardi E, Bernardini R, Caputo R *et al*. Efficacy and safety of cyclosporine eyedrops in vernal keratoconjunctivitis. *Ann Allergy Asthma Immunol* 2002; **89**: 298–303.
- 59 Bielory L, Bonini S, Bonini S. Allergic eye disorders. *Clin Allergy Immunol* 2002; **16**: 311–323.
- 60 Leonardi A, Borghesan F, DePaoli M, Plebani M, Secchi AG. Procollagens and inflammatory cytokine concentrations in tarsal and limbal vernal keratoconjunctivitis. *Exp Eye Res* 1998; **67**: 105–112.
- 61 Allansmith MR. *The Eye in Immunology*. St Louis: Mosby, 1980.
- 62 Abelson MB, Butrus SI, Weston JH. Aspirin therapy in vernal conjunctivitis. *Am J Ophthalmol* 1983; **95**: 502–505.
- 63 Chaudhary KP. Evaluation of combined systemic aspirin and cromolyn sodium in intractable vernal catarrh. *Ann Ophthalmol* 1990; **22**: 314–318.
- 64 Lambiase A, Bonini S, Rasi G, Coassin M, Bruscolini A, Bonini S *et al*. Montelukast, a leukotriene receptor antagonist, in vernal keratoconjunctivitis associated with asthma. *Arch Ophthalmol* 2003; **121**: 615–620.

- 65 Neumann E, Gutmann MJ, Blumenkrantz N, Michaelson IC. A review of 400 cases of vernal conjunctivitis. *Am J Ophthalmol* 1959; **47**: 166–172.
- 66 Cameron JA. Shied ulcers and plaques of the cornea in vernal keratoconjunctivitis. *Ophthalmology* 1995; **102**: 985–993.
- 67 Abu El-Asrar AM, Geboes K, Missotten L, Emarah MH, Maudgal PC, Desmet V. Cytological and immunohistochemical study of the limbal form of vernal keratoconjunctivitis by the replica technique. *Br J Ophthalmol* 1987; **71**: 867–872.
- 68 Abu el-Asrar AM, Van den Oord JJ, Geboes K, Missotten L, Emarah MH, Desmet V. Immunopathological study of vernal keratoconjunctivitis. *Graefes Arch Clin Exp Ophthalmol* 1989; **227**: 374–379.
- 69 Leonardi A, DeFranchis G, Zancanaro F, Crivellari G, De Paoli M, Plebani M *et al*. Identification of local Th2 and Th0 lymphocytes in vernal conjunctivitis by cytokine flow cytometry. *Invest Ophthalmol Vis Sci* 1999; **40**: 3036–3040.