

## REVIEW

# Cellular mechanisms of chronic cell-mediated allergic conjunctivitis

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## Introduction

At a European Science Foundation Exploratory Workshop ('Immunotherapeutic Strategies in Chronic Allergic Eye Disease'), held in London during October 2000, ocular allergists from several European centres met to discuss the cellular mechanisms underlying chronic allergic eye disease (CAED) and potential immunotherapeutic approaches. Before discussing the science, the clinical findings and the different approaches to treatment were compared.

'CAED' describes a group of clinically distinct allergic eye disorders, ranging from the milder disorder of giant papillary conjunctivitis (GPC), to the more severe forms of vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC), these latter two being potentially sight-threatening. VKC is often seasonal, occurs in children and young adults and is self-limiting, although loss of vision can occur through damage to the cornea [1]. Many features of this disease suggest an allergic pathogenesis and patients frequently show a strong association with, or a history of, other atopic disease. AKC is a very chronic disorder occurring in adults with systemic atopic disease such as atopic dermatitis or asthma [2]. It is the most severe form of ocular allergy, often causing significant visual impairment due to corneal damage and marked conjunctival scarring for which current treatment is largely inadequate [3]. In contrast, GPC is associated with wearing contact lenses, is not associated with atopy and is relatively benign and reversible [4, 5]. Interestingly GPC is histologically similar to VKC, yet the cornea is never affected, and the reasons for this are unclear.

## Regional differences in clinical features

Comparing the findings from ocular allergy centres across Europe, some regional differences were found in the clinical features and in modes of treatment. In the United Kingdom (UK), for example, VKC usually involves the tarsal conjunctiva, with fewer cases of the limbal form of disease, which tends to occur in deeply pigmented patients (R. J. Buckley, London). The limbal form is more prevalent in Italy and Spain (A. Leonardi, Padua; M. Calonge, Valladolid). In Sweden, VKC is perennial and, due to the lack of heat and pollens in the winter, this form of VKC is likely to have a different aetiology (P. Montan, Stockholm). It has therefore been proposed that ultraviolet (UV) light might be the causal factor, as the sun is known to exacerbate corneal erosions. VKC is more prevalent in Asia and Africa, and recent data demonstrate that Africans

born in Sweden still have an increased prevalence, probably due to genetic factors [6]. AKC is more common than VKC in Sweden, affecting adults, 80% of whom are atopic and all have atopic dermatitis. In Padua, VKC occurs in children and young adults (aged 3–20 years) and, although seasonal exacerbations can occur between March and October, often the disease is perennial. VKC which occurs in Padua can be subdivided into three clinical types, depending on the area of the conjunctiva affected: tarsal, limbal or mixed. The giant papillae might be absent but there will be epithelial thickening and the patients will have positive skin-prick tests. All AKC patients in Padua have atopic dermatitis and 25% of atopic dermatitis patients will demonstrate an ocular involvement. House dust mite is the most commonly associated allergen and a seasonal reactivation depends on the nature of the other allergens involved.

S. Bonini (Rome) described some recently published data suggesting that seasonal and perennial allergic conjunctivitis are more frequently associated with allergic rhinitis, whereas VKC is often associated with asthma. In Rome, the season starts at the end of February and lasts until October, and more often involves grass pollens than tree pollens. In a recent study of 195 VKC patients from central and southern Italy examined over 4 years, the mean age at onset was 11. More than 60% of symptoms were also present 'out of season' and the disease severity correlated with the level of chronicity. In 77% of cases, the most severe clinical manifestations were present in the spring [7].

## Regional differences in therapy

Different treatment modalities are currently used in the ocular allergy centres. In London, mast cell stabilizers and topical steroids are effective in many cases, with sodium chromoglycate (Opticron) being the mast cell stabilizer of choice. Surface-active non-penetrating steroids are less potent and oral steroids are used in severe cases. As skin and conjunctival provocation tests do not always identify the specific allergen, allergen avoidance is difficult to achieve in practice. There are also supportive measures, including cold compresses and placebo drops. Another effective treatment for VKC and AKC is cyclosporin A (CsA) given in the form of a 2% eye drop solution [8, 9].

The clinical management of VKC patients in Stockholm relies on the use of steroids, as antihistamines are not effective and chromones are only weakly effective. Sodium chromoglycate is effective in 50% of cases but not for subtarsal disease. Cyclosporin A, although clinically effective, is only poorly tolerated due to its oiliness, stinging and lid maceration. In contrast to VKC, AKC has a chronic course and CsA is used in Stockholm as an effective treatment for AKC.

In Italy, topical anti-allergic and anti-inflammatory drops have been shown to be effective, and only a few VKC patients require additional systemic antihistamines. However, almost 85% of patients needed to use topical ophthalmic steroids at some point during the course of disease. In almost 16% of cases, the seasonal form of VKC evolved into a chronic, perennial inflammation after 3 years, suggesting a correlation between the duration of disease and chronicity. There was also a positive correlation between the size of the papillae and disease chronicity.

M. Calonge (Valladolid) explained that AKC in Spain is most commonly associated with skin eczema, but there are also associations with asthma, rhinitis, drug, food or airborne allergens, urticaria or other atopic disease [10]. In more chronic cases, cicatrizing conjunctivitis is observed, which is not seen in VKC. Biopsies are required to eliminate pemphigoid, with 76% having a severe corneal pathology and 53% a milder version. Limbal disease is very marked, with persistent epithelial defects being a serious complication. Unlike VKC, *Staphylococcus aureus* is the main secondary infection in AKC, with ocular rosacea seen in 18% of AKC cases in Valladolid. Treatment is multidisciplinary, including control of environmental exposure, modulation of the immune response, treating the blepharitis and controlling the epithelial cell defects [11].

## Cellular mechanisms

In acute forms of allergic conjunctivitis, antihistamines and/or mast cell stabilizers are highly effective, as the main infiltrating cell type is the mast cell. In contrast, the cells infiltrating the conjunctiva during active CAED are predominantly CD4<sup>+</sup> T lymphocytes and eosinophils, with increased numbers of mast cells, neutrophils, activated macrophages and Langerhans cells. Therefore mast cell stabilizers alone are not an effective therapy in CAED, and usually corticosteroids and cyclosporin are required. To enable future therapies to be identified, it is important to understand the basic immunopathogenic mechanisms involved and in the second part of the workshop our current understanding of T cells, eosinophils and cytokines was discussed, with particular reference to CAED, as well as some recent immunotherapeutic approaches that might be applicable to CAED.

## T cells

To phenotype the infiltrating T cells in CAED, studies have mainly involved immunostaining and in tarsal conjunctival tissue from GPC, VKC and AKC there is an increased expression of activated CD4<sup>+</sup> T cells and HLA-DR when compared with normals [12]. The T cells were found mainly in the sub-epithelial layer of the affected tissue. Others have isolated the conjunctival T cells and expanded them *in vitro*, and cloned conjunctival T cells from patients with VKC were found to be Th2-like, expressing IL-4 and providing help for B cells but producing little or no IFN- $\gamma$  [13]. Further evidence for a role of Th2 cytokines was obtained using *in situ* hybridization histochemistry, demonstrating an up-regulation of IL-3, IL-4 and IL-5 mRNA in all three chronic conjunctival disorders as compared with normals, in areas of maximum T-cell infiltration

[14]. IL-2 mRNA was only significantly up-regulated in AKC and there was also enhanced expression of IFN- $\gamma$  by CD4<sup>+</sup> T cells in AKC. These results suggested that the cytokine patterns differed depending on the clinical form of CAED, with IFN- $\gamma$  expressed in the more severe disease. However, use of this technique could not conclusively demonstrate T cells as the main producers of the cytokines as mast cells, basophils and eosinophils are also capable of producing many Th2 cytokines [15–17]. Comparing conjunctival T cell lines derived from the different forms of CAED, it was found that in VKC there is a predominant Th2 profile, whereas in AKC, IFN- $\gamma$  was significantly upregulated and in GPC cytokines were produced at lower levels [18], in agreement with the tissue studies. In T cells derived from VKC tear samples, intracellular cytokine staining was done to demonstrate the presence of Th2 cells expressing IL-4 and IgG in 67% of VKC tear specimens [19].

## Eosinophils

Evidence for a role for eosinophils in CAED is based on their presence in high numbers within the affected conjunctiva [20], with raised serum IgE levels. Eosinophil products have been detected at significantly increased levels in tear specimens in VKC and are a good marker of clinical disease activity [21, 22].

During allergic and parasitic responses there is an increase in IgE and eosinophil numbers and the important role of eosinophils in protection against infection was discussed by M. Capron (Lille). Due to their release of major basic protein (MBP), eosinophil peroxidase (EPO) and eosinophil cationic protein (ECP), eosinophils are effective responders following binding to IgE [23]. Eosinophils are also important mediators of tissue damage due to the toxicity of their secreted molecules [24]. In addition to IgE, IgA can bind to specific receptors on eosinophils, resulting in the release of ECP, eosinophil-derived neurotoxin (EDN), EPO and IL-5. Thus IgA, especially secretory IgA (sIgA), is a potent stimulus for eosinophil degranulation, and this has not yet been investigated in allergic eye disease [25].

Recent *in vitro* evidence supports an additional role for eosinophils in the regulation of the immune response due to their ability to secrete a range of pro-inflammatory cytokines, growth factors and chemokines (IL-3, GM-CSF, IL-5, MIP-1 $\alpha$ , RANTES and eotaxin [26]). More recently, however, human eosinophils have been demonstrated to produce both Th1 and Th2 cytokines (IFN- $\gamma$ , IL-2, IL-4, IL-5, IL-10, IL-12 and IL-13 [27]). Interestingly, the pattern of cytokines produced depends on the nature of the stimulus. For example, if eosinophils are stimulated via their IgE or IgA receptors, this leads to the secretion of IL-4, IL-5 and IL-10 [28]. In the presence of the costimulatory molecule CD86 and absence of CD80, Th2 cytokines are produced, suggesting a role for eosinophils in polarization towards Th2 responses. In contrast, Th1 cytokines are produced by eosinophils when immobilized anti-CD28 antibody is used as the stimulus. Addition of soluble IgA (sIgA) with anti-CD28 antibody results in a decrease in IL-2 and IFN- $\gamma$ , suggesting that sIgA is down-regulatory for Th1 cytokines. It is as yet unclear whether there are subtypes of eosinophils, based on their cytokine profiles, as there are for T lymphocytes.

## Immunotherapies

The use of cytokine-directed immunotherapy for various autoimmune diseases was reviewed (M. Feldmann, London). The hypothesis that all inflammatory diseases exhibit similar pro-inflammatory cytokine networks has been investigated over many years. One of the benefits of investigating diseases such as rheumatoid arthritis (RA) is tissue accessibility and it was found that almost all cytokines are produced in RA synovium. Following an analysis of cytokine regulation, it was concluded that RA is not due to a lack of anti-inflammatory mediators but a slight excess of pro-inflammatory mediators and that TNF- $\alpha$  is central to the inflammatory process [29]. As a result of clinical trials, the anti-TNF- $\alpha$  monoclonal antibody (Infliximab) and TNF-receptor/Fc fusion protein (Etanercept) have both been demonstrated to be effective immunotherapies for RA [30,31]. In contrast in other clinical trials targeting cytokines, anti-IL-6 was moderately successful but anti-IL-1 was not, and anti-IL-10 and anti-IL-4 treatments had to be withdrawn due to toxicity and thrombocytopenia. In a recent trial of a combination of low-dose anti-TNF- $\alpha$  antibody with low-dose methotrexate (ATTRACT), the duration of clinical benefits exceeded the effective drug concentration and this is thought to be due to an overall reduction (60%) of leucocyte trafficking, resulting in a normalization of the inflammatory response [32]. Anti-TNF- $\alpha$  therapy is also being used successfully in the treatment of Crohn's disease [33], severe psoriasis [34] and ankylosing spondylitis [35]. The use of TNF- $\alpha$ -directed immunotherapy as a potential treatment for AKC was discussed, and future research should focus on understanding the cytokines involved during active CAED.

The use of conventional allergen-specific immunotherapy (SIT), and its associated drawbacks, were described by A. Frew (Southampton). The modified allergen vaccines can be formalin-inactivated allergens, T cell peptides, recombinant allergens, bioengineered vaccines or naked DNA vaccines. The overall aim of this form of therapy is to anergize the responding T cells to render them functionally non-responsive. This can be done either by ligation of the T cell receptor without the costimulatory signal or using an altered peptide ligand or by adding excess antigenic peptide. Due to the long course of treatment needed, it can only be done in specialized centres and there can be adverse side-effects, either locally or systemically, which may be fatal. It is unclear as to the duration of efficacy and the standardization of the allergen extracts is technically difficult. Several published studies using the different approaches were discussed, including grass pollen allergens [36], cat dander [37] and the Allervax cat trials [38], in which low, medium or high doses of cat peptides were given and the main clinical benefits were observed in the lungs. Interestingly, a very different T cell response is seen *in vitro* depending on whether Fel d 1 peptides or the whole cat dander allergen is applied. Therefore although peptide immunotherapy (PIT) is partially effective and probably safer than SIT, the duration of the protective response is as yet unknown. In conclusion, SIT and PIT therapies are only useful when the target allergen is known, and therefore currently could only be applied to allergic conjunctivitis where the allergen has been identified.

## Conclusion

Currently available therapy for CAED is inadequate and key areas of research are required to further understand the immunopathogenic mechanisms involved, which hopefully will determine future therapeutic approaches. The involvement of resident conjunctival cells, including fibroblasts, epithelial cells and Langerhans cells, should be investigated, to determine how these cells contribute to the tissue damage and conjunctival scarring that occurs during CAED. For example, the relationship with infection needs to be determined, as it is possible that, as a result of inflammatory-induced receptor expression, these could become ligands for viruses, as is known to occur in other diseases, which would then exacerbate the disease. There are some regional differences due to the prevailing allergens and the diverse genetic backgrounds across Europe. The importance of these genetic and environmental influences needs to be explored, as has been done for VKC in Stockholm.

The clinical definitions for each form of disease are broadly similar. However, for multicentre studies there is a need to establish a database or register to define the criteria for clinical diagnosis, preferably through the use of flow charts for accurate disease phenotyping, including markers for severity.

During the European Science Foundation Exploratory Workshop, it was agreed that a 'European Network in Ocular Allergy' would be established, with the three main objectives being as follows:

- To make an inventory of all major European centres working in the area of ocular allergy, as it was felt that some countries were not represented, including Turkey, Belgium, France and Eastern Europe.
- To define a common plan of research priorities in the area of ocular allergy, representing the basis for a virtual centre of scientific excellence.
- To establish an on-line registry and possibly a biological bank for rare diseases such as VKC and AKC.

If anyone is interested in finding out more about the European Network in Ocular Allergy, details can be obtained from the author or by emailing <http://www.esf.org>.

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