

The eosinophil and the eye

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Personal studies in allergic eye diseases reviewed in this paper indicate that: 1. An increased number and an abnormal distribution of eosinophils is present in conjunctival biopsies of patients with vernal keratoconjunctivitis (VKC). 2. Eosinophil and eosinophil products, such as ECP, are also increased in tears of VKC patients and, in hay fever conjunctivitis, accumulate during the late-phase of allergic reaction following specific allergen challenge. 3. Circulating eosinophils of VKC patients show a typical activation phenotypic profile which is associated with increased serum level of eosinophil cationic protein and eosinophil-derived neurotoxin/protein X. A clinical study of the modulatory effect of cetirizine on the early and late phase of the allergic reaction as well as on the eosinophil activation and tissue recruitment following conjunctival allergen challenge is reported as an example of the need to evaluate eosinophil functions when investigating anti-allergic drugs. Drugs modulating various aspects of eosinophil function could play a primary role in the treatment of allergic eye disease.

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Extensive evidence indicates that eosinophilic inflammation plays a primary pathophysiological role in allergic diseases and asthma (1, 2). In recent years, we focused on the eye, and in particular on vernal keratoconjunctivitis (VKC), as a readily accessible, reliable model with which to study the mechanisms leading to hypersensitivity and symptoms in allergic patients (3, 4). In this paper we review published (5) and more recent data showing that eosinophils have a pivotal role also in conjunctival eye diseases. Consequently, studies on eosinophils and their pharmacological modulation might lead to a new therapeutic strategy in the treatment of allergic diseases.

The role of eosinophil in the pathophysiology of allergic eye diseases

Eosinophil activation and selective recruitment in the human allergic conjunctiva have been demonstrated in studies of conjunctival biopsies, tear fluid and peripheral blood of allergic conjunctivitis patients.

Conjunctival biopsies

An increased number of eosinophils are detectable in conjunctival biopsies of VKC patients both by light microscopy and by immunohistochemistry.

Conjunctival biopsies from the upper tarsal conjunctiva were obtained from seven patients with VKC and four matched controls after anesthesia with 2% mepivocaine hydrochloride. Hematoxylin-eosin staining was used for the evaluation of eosinophils in three randomly selected conjunctival sections and in three different fields for each section (6). The number of eosinophils in VKC patients, as well as that of mast cells and lymphocytes, was significantly increased in VKC patients ($P < 0.01$). However, while mast cells and lymphocytes were also present, albeit in a lower number, in healthy controls, the finding of eosinophils was typical of VKC patients (Fig. 1).

This observation was confirmed in a larger number of VKC patients and controls by using immunocytochemical staining (7). A streptavidin-biotin method was used to look at the inflammatory infiltrate in resin-embedded tarsal and bulbar biopsies of 15 VKC patients and 15 controls. Eosinophils were detected by using a monoclonal antibody against ECP (Clone EG2, Pharmacia, Uppsala, Sweden). Eosinophils were significantly increased ($P < 0.01$) in both bulbar and tarsal conjunctiva of VKC patients. Interestingly, in VKC they could be detected not only in the subepithelium but also in the epithelium, where no eosinophil was ever present in control subjects (Fig. 2).

Tear fluid

An increased number of eosinophils (8) was detected in 2 μ l of tears collected through a capillary in patients with VKC, associated with an increased reactivity to histamine challenge (9). Eosinophil cationic protein was also increased in tears of VKC patients, and was correlated with both signs and symptoms of the diseases (10). In hay fever conjunctivitis patients, whose tears are usually free of eosinophils out of the pollen season, specific allergen challenge caused a dose-dependent selective recruitment of eosinophils in the conjunctival tissue during both the early and, particularly, the late-phase of allergic reaction (11, 12). Eosinophil recruitment after allergen challenge has a different time-course in individual patients (13),

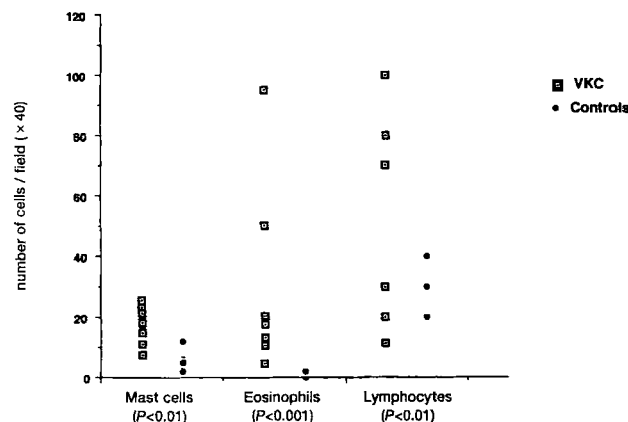


Fig. 1. Inflammatory cells in conjunctival biopsies of seven patients with vernal keratoconjunctivitis (VKC) and in four controls.

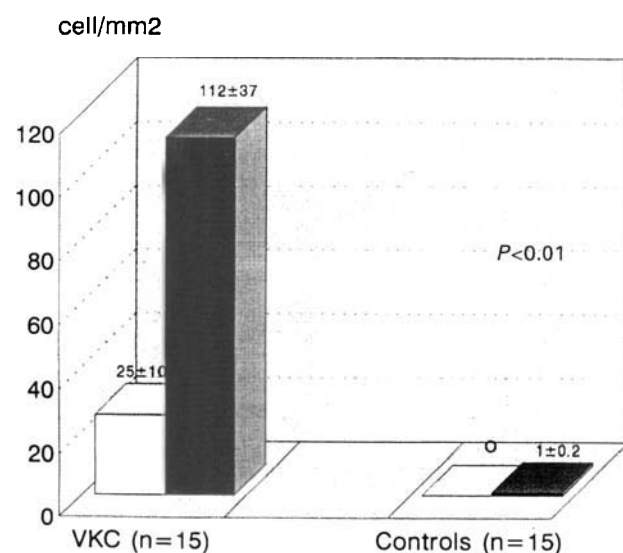


Fig. 2. EG2-positive cells in the epithelium (white column) and subepithelium (black column) of vernal keratoconjunctivitis and normal conjunctiva.

but a significantly increased number of eosinophils and increased tear levels of ECP are usually found 6 hours after provocation (3, 11–13).

We recently showed that IL-5 is also increased in tears of VKC patients (14). Although eosinophils have been shown to be able to produce and secrete IL-5 (15, 16), it is still questionable whether CD4+ lymphocytes with a functional Th2 profile or eosinophils or mast cells represent the major source of IL-5. However, independently of its cellular source, the increased IL-5 tear levels in VKC patients can be responsible for a selective recruitment, activation and survival of eosinophils and conjunctival tissues (17).

Peripheral blood

In VKC patients, eosinophils and eosinophil products not only accumulate at tissue level but are also detectable in enhanced amounts in peripheral circulation. Flow cytometry of 4% paraformaldehyde fixed and 0.74% n-octyl-beta-D-glucopyranoside-permeabilized circulating eosinophils from 14 patients with VKC and 25 matched controls showed an increased number of cells reacting with EG2 monoclonal antibodies as well as a peculiar activation phenotype using monoclonal antibodies against various adhesion molecule antigens (Fig. 3) (18).

Taken together the data reported above clearly show that an abnormal number and function of eosinophils is a feature also of the allergic eye, as it is in other allergic diseases, and may play a primary pathophysiological role in effector mechanisms that lead to tissue damage and symptoms.

Pharmacological modulation of eosinophilic inflammation of the eye

In view of the potential effector role of eosinophils in inducing inflammation and symptoms in the eye, their pharmacological modulation could be a valuable

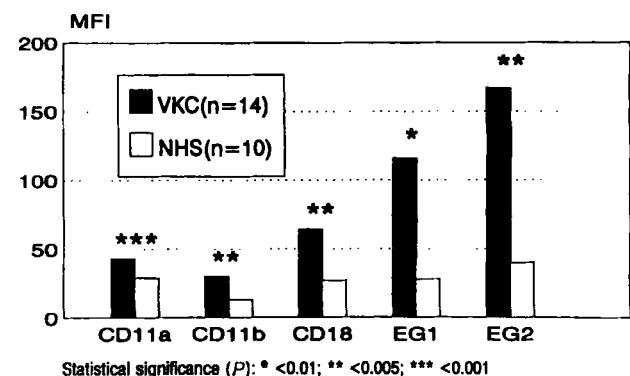


Fig. 3. Flow cytometry of circulating eosinophils in 14 vernal keratoconjunctivitis patients and in 25 control subjects.

therapeutic approach for the treatment of allergic eye diseases. Systemic and topical steroids have been shown to represent a very effective tool for controlling late phase events following allergen challenge, eosinophilic inflammation and clinical symptoms in several allergic diseases such as asthma and rhinitis. However, their use in allergic eye diseases is limited by the possible appearance of severe side-effects.

Cetirizine, a potent selective histamine anti-H1 receptor antagonist (20), was successfully used for treatment of allergic rhinoconjunctivitis in both adults and children (20, 21). In addition to its anti-histaminic activity, the drug was shown to have anti-allergic properties and to modulate eosinophil activities both *in vitro* and *in vivo* (22-23). In fact, Leprevost and co-workers were able to show that the administration of a single dose (10 mg) of cetirizine inhibited *in vitro* the chemotaxis of eosinophils in response to PAF-acether and to FMLP. Moreover, cetirizine was also shown to inhibit eosinophil PAF-induced hyperadherence to HUVEC. Finally, eosinophil migration was also inhibited *in vivo* in subjects sensitive to grass and challenged at skin-site (24).

Given these findings supporting the anti-allergic and anti-eosinophil properties of cetirizine, we were prompted to look at the effect of the drug on the conjunctival provocation test. In our experience, this test is a very valuable tool for the evaluation of the inhibitory effects of various drugs on the pathophysiological changes and clinical symptoms induced by allergen challenge (3).

Nineteen asymptomatic patients with allergic conjunctivitis sensitive to grass or *Parietaria officinalis* were studied out of the pollen season. Ten of them were treated with cetirizine (10 mg b. i. d. for one week) and nine with placebo in a random double-blind trial. Thirty minutes after the last administration of drug/placebo, one eye was challenged with specific allergen (320,000 BU/ml) and the contralateral eye with diluent. A clinical score (from 0 to 18) was assigned by adding the clinical score of three major symptoms (redness, tearing, and itching), each one graded from 0 to 6, before challenge as well as 20 minutes and 6 hours after challenge. At the same time points, cells present in 2 μ l of tears were also counted after hematoxylin-eosin staining and tear ECP was measured in pools obtained from the two differently treated-groups both from eyes challenged with allergen and from eyes challenged with diluent.

There was a significant reduction of both the early and the late phase clinical reaction in patients treated with cetirizine vs patients treated with placebo. In fact, changes in clinical score 20 minutes after challenge were absent or modest in the

drug-treated group, while they were modest to very marked in the nine patients treated with placebo (Fig. 4). At 6 hours after challenge no change in clinical score was observed in six patients treated with cetirizine while only modest changes were observed in the other four treated patients. Differently, there was a modest to relevant change in the clinical score in seven out of the nine patients treated with placebo, while only two patients did not show any change in their clinical picture after provocation (Fig. 5).

A significant cellular reaction and eosinophil recruitment in the allergen challenged eye vs the diluent challenged eye was observed in only two out of nine patients treated with cetirizine and in

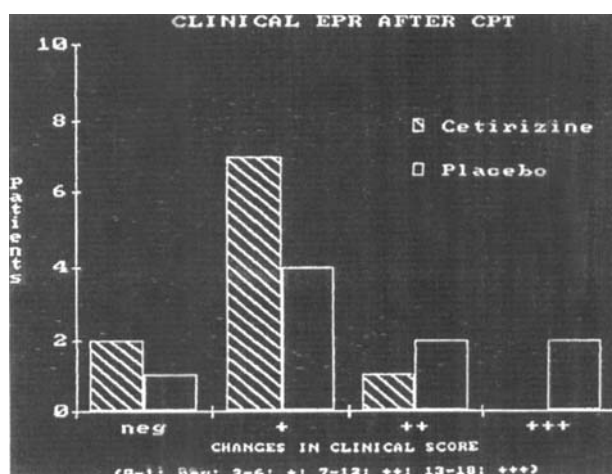


Fig. 4. Changes in clinical score 20 minutes after specific conjunctival allergen challenge in ten hay fever conjunctivitis patients treated with cetirizine (10 mg b. i. d. for seven days) and in nine placebo-treated patients.

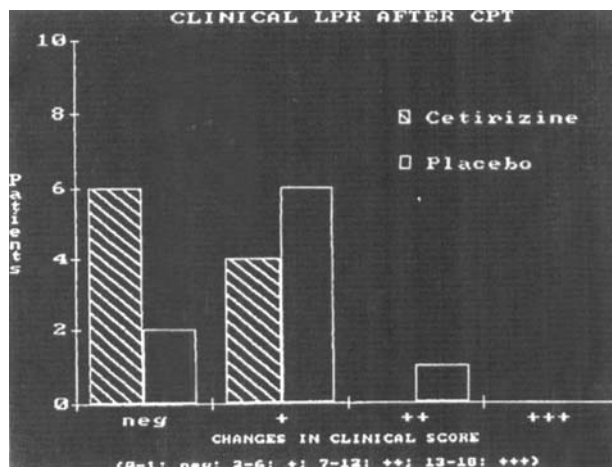


Fig. 5. Changes in clinical score 6 hours after specific conjunctival allergen challenge in ten hay fever conjunctivitis patients treated with cetirizine (10 mg b. i. d. for one week) and in nine placebo-treated patients.

two out of seven patients treated with placebo. Therefore, although the mean numbers of inflammatory cells and eosinophils were lower in the cetirizine-treated group vs the placebo-treated group both during the early and the late-phase of allergic reaction, the drug did not modify the total number of inflammatory cells and or the number of eosinophils recruited by the allergen challenge. Cetirizine did not affect the increase of tear ECP observed 6 hours after challenge in the eyes challenged with allergen.

Concluding remarks

Eosinophils are involved in several eye diseases (25). The data presented here indicate that eosinophils and eosinophil products play a relevant pathophysiological role in allergic diseases of the eye, as in other allergic diseases. Interestingly, in allergic eye diseases eosinophil activation is also detectable at peripheral level, thus suggesting a systemic involvement of eosinophils even in diseases apparently confined to a single organ or tissue. While new therapeutic approaches should be designed aimed at modulating eosinophil production, activation, recruitment and survival as their primary therapeutic focus, the clinical data presented here on the efficacy of cetirizine in allergic eye diseases suggest that the effects on eosinophil function should be taken into account when investigating new anti-allergic drugs.

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