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Review

Allergy and allergic mediators in tears

Andrea Leonardi*

Department of Neuroscience, Ophthalmology Unit, University of Padua, via Giustiniani 2, 35128 Padova, Italy

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ABSTRACT

The identification of inflammatory mediators in the tear fluid have been extensively used in ocular allergy to find either a 'disease marker', to better understand the immune mechanisms involved in the ocular surface inflammation, or to identify potential targets for therapeutic interventions. While the clinical characteristics allow a relatively convincing diagnosis of ocular allergic diseases, in the initial, non active phases, or in the chronic stages, the diagnosis may not be clear. Although not highly specific, total tear IgE can be measured with local tests by inserting a paper strip in the lower meniscus. The measurement of tear specific inflammatory markers, such as histamine, tryptase, ECP, IL-4, IL-5 and eotaxin, may be useful for the diagnosis or monitoring ocular allergy. New technologies such as multiplex bead assays, membrane-bound antibody array and proteomic techniques can characterize the distribution of a wide range of bioactive trace proteins in tears. Dozens of mediators, cytokines, chemokines, growth factors, angiogenic modulators, enzymes and inhibitors were thus identified in small tear samples using these techniques, providing the possible identification of specific biomarker for either specific disease or disease activity. However, to date, there is no a single specific laboratory test suitable for the diagnosis and monitoring of allergic conjunctivitis.

1. Introduction

Approximately one third of the world population is affected by some form of allergic disease and ocular involvement is estimated to be present in 40–60% of this population. Allergic conjunctivitis is a localized allergic condition frequently associated with rhinitis but often observed as the only or prevalent allergic sensitization. This disease ranges in severity from mild forms, which can still interfere significantly with quality of life, to severe cases characterized by potential impairment of visual function.

The term allergic conjunctivitis refers to a collection of hypersensitivity disorders that affects the lid and conjunctiva. Various clinical forms are included in the classification of ocular allergy each of them requires a differential diagnosis that is usually clinical, yet can be substantiated by objective laboratory parameters (Leonardi et al., 2012). While their clinical characteristics allow a relatively convincing diagnosis of ocular allergy both in the initial or chronic stages, there can be some confusion as to what form of allergy is present. At times, pseudo-allergic forms, with clinical manifestations similar to allergy but with a non-allergic equivocal pathogenesis, are difficult to distinguish from allergic forms, with their precisely defined pathogenic mechanisms. In fact, several ocular surface diseases, including tear film dysfunction, blepharitis, subacute and chronic infections, toxic and mechanical conjunctivitis may mimic the clinical pictures of ocular allergy. To date, there is no specific laboratory test suitable for the diagnosis and monitoring of allergic conjunctivitis. Ancillary tests, such as skin prick and the identification of serum specific IgE, can be useful for diagnosis and treatment, however it is well known that the results are often not correlated with the ocular disease. The identification of inflammatory mediators in the tear fluid have been extensively used in ocular allergy to find either a 'disease marker', to better understand the immune mechanisms involved in the ocular surface inflammation, or to identify potential targets for therapeutic interventions.

1.1. Tear collection

Tear samples can be easily obtained from the ocular surface using different methods and techniques making mediator search an attractive tool in ocular allergy. There different methods for sampling of tears: the microcapillary tube method, the filter paper and ophthalmic sponges. It is preferable to collect tears from both eyes since in many cases of allergic conjunctivitis there is a unilateral predominance.

Aspiration of tears by glass capillary tubes or pipettes can yield volumes of 20–50 μl, but collecting is tedious, time-consuming,

* Fax: +39 049 875 5168.
E-mail address: andrea.leonardi@unipd.it.

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sometimes uncomfortable for patients and children, and may provoke the production of reflex tearing by touching the conjunctiva with the tube. Tears can be recovered from a Schirmer strip but tear reflection is very common due to strong irritation by the strip. Various sponges and extraction buffers can be used, making it difficult to assess the feasibility of the protocols and to compare the results. Moreover, some cytokines bind tightly to the sponges, and diffusing cytokines out of the sponges during the extraction procedure can be difficult. With all these methods, tear reflection may occur easily diluting factors that are going to be evaluated. Thus the ultimate outcome could be also affected by the tear collection method chosen and the consistency of the extraction protocol (Inic-Kanada et al., 2012). In our experience, the best method for avoiding reflex tearing is the capillary tube.

2. Ocular allergy: classification and clinical phenotypes

The current allergic nomenclature is based on pathophysiology according to the different hypersensitivity mechanisms still commonly used in the clinical practice. Ten years ago the European Academy of Allergy and Clinical Immunology and the Nomenclature Review Committee of the World Allergy Organization proposed to distinguish allergic from non allergic hypersensitivity reactions dividing them into IgE- and non-IgE-mediated hypersensitivities (Johansson et al., 2004, 2001). According to this view and to better understand ocular pathology in the future, a new classification of ocular allergy has been recently proposed dividing ocular surface allergic- from non-allergic hypersensitivity disorders (Leonardi et al., 2012). However, single clinical entities maintain the traditional names.

2.1. Seasonal/intermittent (SAC) and perennial/persistent allergic conjunctivitis (PAC)

Seasonal allergic conjunctivitis, or hay fever, one of the most common allergic diseases, results in significant morbidity and presents an increasing economic burden because of direct health expenditures, as well as less evident cost factors, such as lost work time. SAC and PAC are IgE-mediated allergic disease usually associated with exposure to airborne allergens. SAC is characterized by seasonal/intermittent ocular itching, commonly associated to rhinitis, and non specific signs such as conjunctival redness and eyelid edema, which arise and subside with the patient's exposure to the offending allergen(s). PAC is related to allergens that are present year round, such as dust mites, animal dander and molds or to multiple allergen sensitizations. It is a chronic condition, with persistent, frequently mild symptoms, enhanced by higher or longer exposure to allergen and exacerbated by non-specific irritating substances. It is characterized by ocular itching, burning, eye watering and conjunctival redness, conjunctival and eyelid edema and small tarsal conjunctival papillae. In both forms, the only pathognomic symptom is itching.

2.2. Vernal keratoconjunctivitis (VKC)

VKC is a persistent and severe form of ocular allergy affecting children and young adults, particularly in warm climate. VKC usually appears between age 4 and 12 and usually heals after puberty. The disease is more frequent in boys (sex ratio, 3/1) before puberty. The IgE-mediated mechanism found in approximately 50% of the patients does not explain completely the severity and the clinical course of this disease, which is probably related also to T cell-mediated responses, massive eosinophil attraction and activation, and non-specific hyper-reactivity.

Intense itching, tearing and photophobia are the classical symptoms of VKC patients. Disease exacerbation can be triggered by either allergen re-exposure or, more frequently, by non-specific stimuli such as sun light, wind, and dust. The tarsal form of the disease is characterized by irregularly sized hypertrophic papillae, leading to a cobblestone appearance of the upper tarsal plate. The limbal form is characterized by transient, multiple limbal or conjunctival gelatinous yellow-gray infiltrates with white points on the top, known as Horner–Trantas's dots, and papillae at the limbus. Punctate keratitis, epithelial macroerosions or ulcers and plaques are signs of corneal involvement, which resolves with different levels of subepithelial scarring.

2.3. Atopic keratoconjunctivitis (AKC)

AKC is a persistent inflammatory condition involving the eyelid skin, the conjunctiva and possibly the cornea, and that can be defined as the ocular manifestation of atopic dermatitis. The hallmark sign of AKC is an eczematous, erythematous, exudative lesion of the lids often associated with chronic blepharitis and meibomian gland dysfunction. The conjunctival hyperaemia and chemosis affect predominantly the inferior fornix and palpebral conjunctiva. The limbus may also be involved. The disease often leads to cornea lesions and can be complicated by conjunctival fibrosis, *Staphylococcus aureus* colonization of the eyelid, herpes simplex keratitis, keratoconus, retina detachment, cataract with consequence sustained vision deterioration.

2.4. Giant papillary conjunctivitis (GPC)

GPC is an adverse ocular reaction to contact lenses, ocular prostheses, post-operative sutures or some irregularities of the eyeball surface. GPC is a non-allergic hypersensitivity inflammation of the external ocular surface that may overlap with other forms of ocular allergy. The early stages of GPC may be asymptomatic but the initial signs can be observed by slit lamp examination. Increasing intolerance to contact lenses may lead to their discontinuation. Clinically it is characterized by small to giant papillae of the upper tarsal conjunctiva, redness and discharge.

2.5. Contact blepharoconjunctivitis (CBC)

CBC is a result of allergic/irritant reactions after contact with different substances usually applied to the eyelid skin or the conjunctival sac. It is characterized by edema, eyelid skin redness, eczema or lichenification, conjunctival redness and papillae.

3. Allergic inflammation

In sensitized patients, the immediate hypersensitivity associated with ocular allergy is characterized by allergen-mediated cross-linking of IgE on mast cells, leading to degranulation and release of mediators localized in specialized granules, including histamine, tryptase, leukotrienes, cytokines, and platelet-activating factors and the *de novo* synthesis and secretion of cytokines, chemokines and eicosanoids. These mediators stimulate nerve endings, dilate blood vessels, and recruit inflammatory cells to the reaction site, causing immediate clinical symptoms such as itching, redness, and lid and conjunctival edema (early phase) (Fig. 1). The late phase reaction is associated with an accumulation of inflammatory cells in the conjunctiva. This immediate or early response lasts clinically 20–30 min as demonstrated by the specific conjunctival provocation test (CPT). In fact, CPT reproduces signs and symptoms of the allergic reaction and induces enhanced tear levels of histamine, tryptase, prostaglandins, leukotrienes, and the