Late-phase reaction in ocular allergy

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Purpose of review

To determine if the late-phase reaction, which commonly occurs in allergic rhinitis and asthma, is also found in ocular allergy.

Recent findings

Using PubMed, 542 articles were found; 18 articles in the allergy and ophthalmology literature were specifically related to late-phase reaction. Ocular late-phase reaction is clinically seen in 50–100% of allergic rhinoconjunctivitis patients, is associated with progression to systemic atopic disorders that is allergic rhinoconjunctivitis and occurs in several forms including biphasic, multiphasic and a prolonged response.

Summary

The existing literature demonstrates that an ocular late-phase reaction also exists and has implications in the development severity of disease, change of reactivity and progression of the atopic disease state from a localized target organ, such as the nose or eye, to a more systemic atopic disorder. The existence of the clinically relevant allergic late-phase response is not only limited to the nose, skin and lungs but also includes the eyes. The appreciation that the late-phase response may be clinically very important as there is a continuum of ocular mast-cell activation during the waking hours of the day, a better understanding of its clinical impact may be a more appropriate focus in the development of future treatments.

Keywords

allergic conjunctivitis, conjunctival provocation test, eye allergy symptoms, late-phase response, ocular allergy

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Introduction

Although late-phase reaction (LPR) is frequently seen in allergic nasal, respiratory and skin disease [1,2], the clinical impact of LPR in ocular allergy has been questioned. Allergic responses in tissues may vary, partially because of the heterogeneity of mast cells from different tissues [3,4]. Among the different tissues, the eye's anterior surface is easily observed with highly magnifying instrumentation (i.e. slit-lamp microscope or other digital equipment). In addition, mediator release and cellular infiltration can be measured in the immunological fluid that bathes the eye's surface (i.e. tears) and through direct examination of the biopsied conjunctiva, which is easily accessible [5].

While using the conjunctival provocation test (CPT), which was initially employed to study the early-phase response (EPR), researchers discovered that the conjunctiva also exhibited a dose-dependent LPR [6]. As LPR is garnering more attention due to its influence on morbidity and its association with the development of more chronic and systemic forms of atopic disorders, it is becoming important to research the role of LPR in ocular allergy. The CPT is an excellent tool that mimics ocular allergic responses, allowing for the measurement of symptoms, inflammatory mediators, cells and pharmacologic modulation with the use of the contralateral eye for control purposes. The CPT is extremely allergen specific and sensitive [7–10] and has proven to be safe and effective in confirming a diagnosis of allergy, even in cases in which the patient's history and skin testing were doubtful [11] as demonstrated in cases of serologic negativity [negative radioallergosorbent test (RAST)], but with the presence of a positive ocular provocation (positive CPT) [12].

Material and methods/techniques

All journals and review articles were collected using PubMed and by manually searching the major allergy and ophthalmology journals that are listed below. Keywords searched: ocular allergy, eye allergy, LPR, CPT, conjunctival allergen challenge, eosinophil cationic protein (ECP), eosinophil, hyper-reactivity and time course. The search resulted in 542 articles, with 47 articles

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reviewed for this analysis that specifically included LPR data that included 15 review articles and 32 clinical trials with 18 being published since January 2000. In addition to the use of allergens, studies using compound 48/80, platelet-activating factor (PAF) and histamine were also evaluated.

Background

A sensitized individual who comes into contact with a particular allergen at the target site may experience an immediate reaction caused by mast cells, known as the EPR. The mediators that are stored in the mast-cell granules or generated *de novo* by this EPR also lead to a second LPR after 6–24 h [13,14]. LPR is IgE mediated and is dependent on an initial activation of the mast cells by an antigen [1,2]. LPR has been associated with the severity of disease, change of reactivity and progression of the atopic disease state from a localized target organ to a more systemic atopic disorder [15–17]. In many instances, it has been identified that the severity of the disease is determined by eosinophils recruited during LPR [18–22,23••]. Therefore, the study of LPR is essential to understand the mechanism of allergic disease and the therapeutic approaches that are required.

Allergic responses in tissues may vary, partially because of the demonstrable heterogeneity of mast cells from different tissues [3,4]. The CPT is an excellent tool that mimics ocular allergic responses, which allows for the measuring of symptoms, inflammatory mediators, cells and pharmacologic modulation. In addition to the use of CPT, we reviewed studies conducted using compound 48/80 (nonimmunologic mast-cell degranulator), PAF, histamine and nitric oxide [10,24–31].

Results

Eighteen studies that involved animal model and human studies supported the concept that LPR is clinically relevant as measured by signs and symptoms, as well as cytological and immunohistochemical changes.

Conjunctival provocation test: signs and symptoms

The use of CPT in the evaluation of LPR was seen in eight of the 18 studies. A study conducted by Bonini *et al.* [32] showed that only with a high allergen dose (320 000 BU/ml) challenge, symptoms were noted after 6 h in seven out of 11 (64%) patients along with EPR at 20 min. Interestingly, cytologic changes occurred at all doses even in the absence of clinical symptoms in the EPR and LPR. In the study by Bacon *et al.* [15], allergic sign and symptoms were graded by a scoring system. All 18 (100%) CPT challenged atopic patients had a median allergic sign and symptom score of 12 (range, 7–16) at 20 min, 13 out of 18 (72%) patients had a score of 9 (range, 4–13) at 40 min and 18 of 18 (100%) patients had clinical score of 8 (range, 4–14) at 6 h. In the study by Montan *et al.* [13], all 15 patients had allergic symptoms in the challenged eye after 10 min; five out of 15 (33%) patients had a second increase in symptoms and signs, at 8 and 24 h, and 12 out of 15 (80%) patients reported itching at 12 h. In the study conducted by Bonini *et al.* [33], an incremental dose of allergen induced increasingly greater clinical reactions at 20 min in the early phase; however, only the highest dose (320 000 BU/ml) produced clinical reactions at 6 h after the CPT.

In animal studies, Calonge *et al.* [34] observed the clinical signs in actively sensitized guinea pigs for up to 48 h. Lid swelling, lid redness and conjunctival redness peaked at 30 min and decreased until 4 h after the challenge. However, there was a second rise from 5 to 8 h after the challenge, which was less intense. All of the animals exhibited an early rise of their clinical scores, but 75% presented with a second peak of clinical observed signs and symptoms of LPR. No animals exhibited an isolated late rise of their clinical scores. Of the animals that experienced a second response, 47% were biphasic, 6% were prolonged and 47% were multiphasic. Leonardi *et al.* [35] observed immunized guinea pigs after hapten dinitrophenylated (DNP)-lysine allergen challenge. The total mean clinical score was measured up to 24 h. The LPR was noticed from 4 to 8 h after the challenge and in one-third of the experimental eyes, clinical signs waxed and waned, another one-third showed biphasic response and the remaining demonstrated progressively decreasing reaction patterns that lasted for 9–12 h.

Conjunctival provocation test: cytological review

Bonini *et al.* [32] measured cells from conjunctival scrapings and tears at different concentrations of CPT. The study showed elevated neutrophils in 20 min, eosinophils in 6 h and neutrophils, eosinophils and lymphocytes in 12–24 h. When the CPT concentrations were increased, there was an increase in the cell count in the tears. Bacon *et al.* [15] showed an increase in mast cells, neutrophils, macrophages, eosinophils, basophils, CD4+ and CD8+ cells in a bulbar tissue biopsy of the substantia propria at 6 h compared to the control eye that was not challenged in all (nine of nine) atopic patients. In the study by Bonini *et al.* [33], it was noted that there was no significant increase in eosinophils and lymphocytes when challenged with lower doses (i.e. 32 000 and 100 000 BU/ml) at 6 h. However, with the high-dose allergen (320 000 BU/ml), there was an increase in eosinophils and lymphocytes compared to the controls in 10 out of 11 patients.

In animal studies, Magone *et al.* [36] investigated the role of IL-4, IFN-γ and IL-12 in the LPR using cytokine knockout mice. The study showed that IL-12 knockout mice had low cellular levels in the conjunctiva, whereas

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IFN-γ knockout mice had a prolonged infiltration into the conjunctiva during 30 min to 120 h. This study suggested that IL-12 plays a role in development of the late-phase pathological features of ocular allergy. IFN-γ suppresses the development of LPR and may be used to control the chronic phase of allergic disease. The study by Leonardi *et al.* [35] on the conjunctival substantia propria after challenge showed a maximal increment of inflammatory cells observed at 3 h for macrophages and neutrophils, and at 24 h for eosinophils and lymphocytes. A study by Ozaki *et al.* [23••] discussed the role of Th2 cells in LPR. Transfer of allergen-specific IgE into normal rats induced the clinical signs of the EPR, but not eosinophil infiltration in the eye. It was noted, however, that the transfer of allergen-primed Th2 cells induced eosinophil infiltrate as well as clinical symptoms of LPR.

Conjunctival provocation test: immunohistochemistry review

Bacon *et al.* [15] conducted a study in which inflammatory mediators and tissue adhesion proteins were measured in tear samples and tissue biopsies. Twenty minutes after the CPT, there was an increase in histamine and tryptase levels in the tears. At 6 h, a second increase in histamine and ECP, but not tryptase, were measured. There was also an increase in E-selectin and intercellular adhesion molecule-1 (ICAM-1), but not vascular cellular adhesion molecule-1 (VCAM-1), in the tissue biopsy after 6 h in eight atopic patients. In the study by Montan *et al.* [13], the ECP in tears was increased in the challenged eye when compared to the unchallenged eye at 6, 8 and 24 h. The increasing symptoms of the challenged eye correlated with the increased levels of tear ECP. During the study by Ozaki *et al.* [23••], 15 min after ragweed challenge to sensitized mice showed clinical signs of allergic conjunctivitis. Additionally, 24 h after challenge there was massive infiltration of eosinophil in the eye on biopsy and an increased level of IL-4, IL-5 and IL-13 in regional lymph nodes.

Conjunctival provocation test with compound 48/80 and platelet-activating factor: signs and symptoms

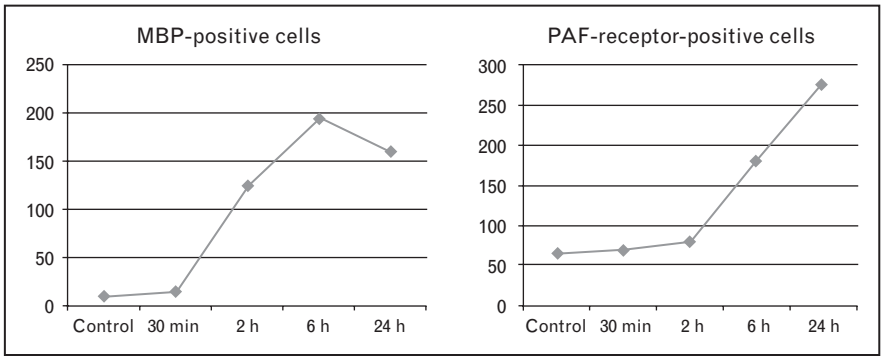
Zinchuk *et al.* [28••] conducted a study in which following PAF instillation in the eye, severe edema of the lids, conjunctival redness and chemosis occurred after 30 min, reaching its peak at 2 h. By 6 h, the signs began to decrease and by 24 h, the signs were minimal.

Conjunctival provocation test with compound 48/80 and platelet-activating factor: immunohistochemistry review

According to Zinchuk *et al.* [28••] after the instillation of PAF in the rat eye, they measured anti-PAF receptor (PAF-R) and anti-major basic protein (MBP) antibodies to visualize the cells expressing PAF-R and eosinophils. PAF-R-positive cells continued to increase until the 24 h time period when the study stopped. MBP-positive cells (eosinophils) continued to increase until 6 h and at 24 h the number started to decrease (Fig. 1). Okumura *et al.* [29••] found that C16:0-PAF, C16:0-lyso-PAF and C18:0-lyso-PAF in guinea pigs (actively sensitized to ovalbumin) showed an increase until 6 h postchallenge. There was no related increase in the unsensitized guinea pigs. This indicates that PAF may be involved in not only EPR but also LPR.

Papathanassiou *et al.* [37•] studied the effect of topical cysLT-receptor antagonist, zafirlukast on the compound 48/80-induced nitric oxide release in the rat conjunctiva. After compound 48/80 challenge, the nitrite level in the conjunctival lavage fluid increased to 220 and 230% ($n = 4$, $P < 0.01$) compared to the control at 6 h. However,

Figure 1 Major basic protein positive and platelet-activating factor receptor positive cells in platelet-activating factor induced rat conjunctivitis



After PAF 1% solution instillation into rat conjunctiva, intact conjunctiva was obtained from the rat eye. The number of MBP (a marker of eosinophils)-positive cells and PAF-receptor-positive cells were measured with immunostaining. Values at each time were compared to values at all other time points ($P < 0.01$). PAF, a major mediator in allergic conjunctivitis caused recruitment of eosinophils during LPR. MBP, major basic protein; PAF, platelet-activating factor. Reproduced with permission [28••].

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treatment with disodium cromoglycate before the challenge and with zafirlukast or levocabastine after the challenge attenuated nitrite levels at 6 h after the compound 48/80 challenge to 150, 121 and 54%, respectively. There was no decrease in the nitrite levels with the unchallenged conjunctiva. In addition, zafirlukast had no significant effect on the histamine content measured at 45 min in either the unchallenged or challenged conjunctiva (compound 48/80).

Conjunctival provocation test treatment models: signs and symptoms

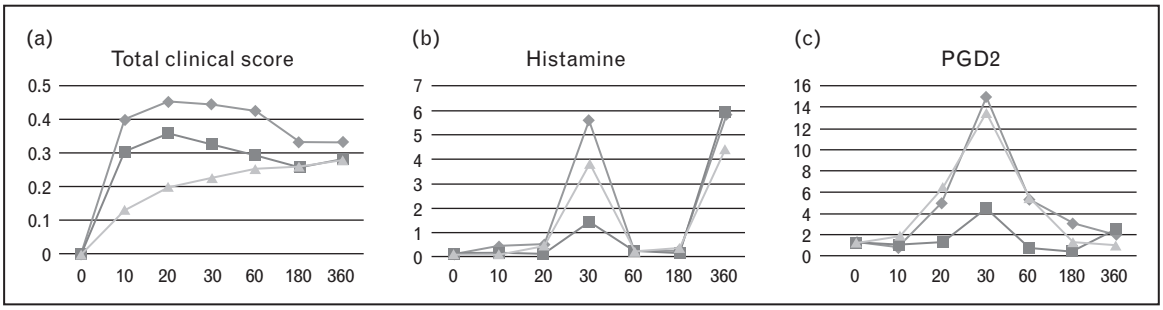
In clinical studies evaluating the impact of treatment in a CPT model, the study by Leonardi *et al.* [38] demonstrated that at 6 h after challenge, the signs and symptoms score was lower than at 15 and 30 min, but they were present. Both the single dose and the 48 h pretreatment with desonide reduced the severity of the immediate allergic reaction and the occurrence of the clinical late phase, but the 48 h pretreatment with desonide was more effective than the single dose before CPT. This study suggests that the treatment with a low-dose steroid can inhibit or attenuate the allergic reaction phase that initiates the transition from early acute to the chronic inflammatory response. Desonide provided rapid relief of the symptoms during seasonal allergic conjunctivitis (SAC) with significant improvement observed in the first week of treatment.

Leonardi and Abelson [39••] measured the symptoms and signs after 15 min and 5 h after CPT with and without olopatadine. Olopatadine reduced the itching and redness score compared to the placebo group throughout the time course. Ahluwalia *et al.* [40] measured the signs and symptoms after CPT with rye grass. In the placebo group, the symptom score increased from 0 to 6/15 in 10 min, peaked at 7/15 at 20–30 min and stayed at 5/15 at 180–360 min. Both nedocromil and levocabastine lowered the symptom scores significantly during first 60 min (Fig. 2).

Conjunctival provocation test treatment models: cytological review

The study by Miyazaki *et al.* [41], using short ragweed (SRW)-sensitized mice, pollen challenge and evaluating the effect of administering the immune-stimulatory sequence oligodeoxynucleotides (ISS-ODN) as a single dose 3 days before the challenge intraperitoneally, demonstrated that the total clinical score after conjunctival SRW challenge increased to 10/16 (63%) compared to 3/16 (19%) with placebo at 20 min, whereas the injection of ISS-ODN before the challenge decreased the clinical score to 4/16 (25%) at the same time point, that is 20 min. The study also showed that 24 h after SRW challenge, the eosinophil count increased from 20 to 95%, whereas after intraperitoneal ISS-ODN treatment before the challenge, the eosinophil count decreased from 95 to 20%. Similarly, the neutrophil count increased from 15 to 60% 24 h after challenge with SRW and decreased from 60 to 10% after ISS-ODN injection. In another animal model, Murata *et al.* [14] conducted an experiment on ovalbumin-sensitized guinea pigs and observed the effect of secretory leukocyte protease inhibitors (SLPI) on eosinophils during the LPR. The antigen conjunctival challenge induced an increase in eosinophils starting at 30 min, eventually reaching its peak at 6–12 h and decreasing slowly by 24 h. SLPI instillation given 10 min before the challenge effectually decreased eosinophil infiltration at 6–12 h. However, there was no effect seen at 24 h. In addition, the percentage of degranulated eosinophil increased from 0 to 60% at 6 h after challenge and it stayed at 60% until 24 h. After treatment with SLPI

Figure 2 Clinical scores and mediators measured after conjunctival provocation test compared with nedocromil-treated and levocabastine-treated group



Ocular challenge was performed with 10 μl of ryegrass extract, followed up until 360 min. Individuals were divided into three groups: placebo, $n = 12$; nedocromil sodium (2%) received group, $n = 14$; levocabastine (0.05%) received group, $n = 22$. (a) Total symptom: total symptom score divided by maximum total symptom score of 15 (itching + hyperemia + lacrimation + chemosis). Data are median scores. Statistically significant ($P < 0.05$) from placebo, nedocromil sodium. (b) Histamine level in tears after challenge: statistically significant ($P < 0.05$) from placebo. (c) PGD2 level in tears after ocular challenge: statistically significant ($P < 0.05$) from placebo. PGD2, prostaglandin D2. (-◆-) After placebo; (-■-) after nedocromil; (-▲-) after levocabastine. Reproduced with permission [40].

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inhibitors, there was also a decrease in the percentage of the degranulated eosinophil from 6 to 24 h. It should also be noted that there was no effect on the clinical signs of EPR by SLPI.

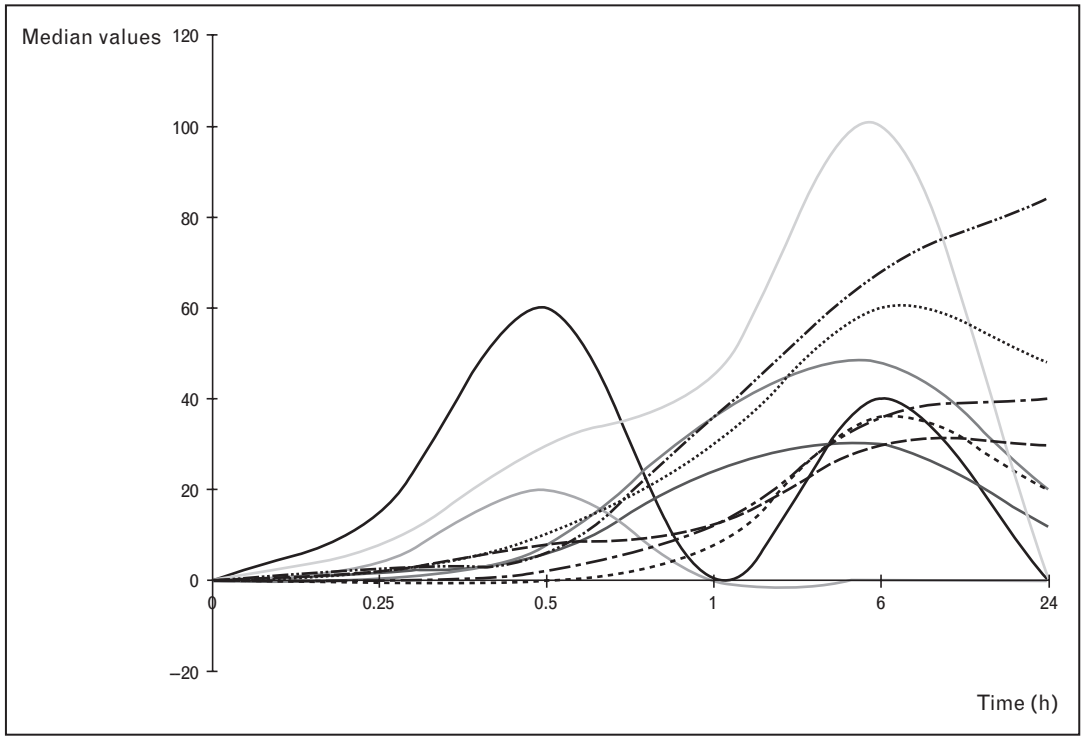
Sengoku *et al.* [42•] used FK506 (tacrolimus hydrate) to show its effect on LPR in ocular allergy. Twenty-four hours after an egg albumin challenge, histological analysis was performed on egg-albumin-sensitized rats. Compared to the normal rats, the clinical inflammation score, T-cell infiltrate and eosinophil count were significantly increased. FK506 decreased all three levels in a dose-dependent manner. Betamethasone and fluorometholone eye drops also decreased the level of T-cell infiltrate and eosinophil count but did not decrease the clinical inflammation score in comparison to tacrolimus.

Interestingly, the study by Leonardi and Abelson [39••] showed a reduced count of neutrophils and total cells at 30 min and decreased the number of eosinophils, neutrophils, lymphocytes and the total cells at 5 h with a formulation of a multiple action agent, olopatadine.

Conjunctival provocation test treatment models: immunohistochemistry review

A study completed by Leonardi *et al.* [43] measured the level of histamine at 20 min (EPR) and at 6 h (LPR). One tear sample was treated with perchloric acid to inhibit all enzymatic activity including histaminase. At 20 min (EPR), histamine in both the untreated and treated group increased in correlation with the sign and symptom after CPT. Post-treatment with lodoxamide showed that the tear histamine level during EPR was lower than before the CPT. At 6 h (LPR), only the histamine in the treated sample increased. With post-treatment with lodoxamide, histamine levels were low, but not significantly in the treated and untreated group. The low histamine level during LPR can be attributed to the dominant cells in LPR, which are neutrophils, eosinophils rich in histaminase activity. The dominant cells in EPR, which are the

Figure 3 Ocular allergic diseases are characterized by specific activation of conjunctival mast cells with subsequent release of preformed and newly formed mediators



Mast-cell numbers on the ocular surface are increased in all forms of allergic conjunctivitis. Mast-cell activation plays a central role in the development of the ocular allergic reaction, which can be divided into an early and a late inflammatory phase. Mast-cell mediators have been measured in tears of patients suffering from various forms of allergic conjunctivitis, and in sensitized patients after specific ocular allergen challenge. Histamine, tryptase, prostaglandins, leukotrienes, ECP, as well as cellular infiltrates including neutrophils, eosinophils, basophils and macrophages are some of the most studied mediators in tears of allergic patients. Recent studies have expanded the evaluations to include cytokines, such as IL-4, TNF-α, fibroblast growth factor, as well as various adhesion molecules are also produced and released by various conjunctival cells (including mast cells) and most probably play a role in the immunoregulation on the ocular surface allergic and other immune disorders affecting the conjunctival surface. ECP, eosinophil cationic protein; ICAMP, intercellular adhesion molecule; TNF-α, tumor necrosis factor alpha. (——) Histamine; (——) leukotrienes; (— -) prostaglandins; (——) tryptase; (——) neutrophils; (·····) eosinophils; (- - - -) basophils; (— —) macrophages; (—··—) ICAM; (——) ECP.

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