Conjunctival Allergen Challenge: Models in the Investigation of Ocular Allergy

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Recently, the number of agents to treat ocular allergy has increased dramatically, from three (pheniramine, antazoline, cromolyn) to more than a dozen. A general increase in the incidence of atopy in recent years and the fact that patients are becoming less tolerant of bothersome signs and symptoms have been driving forces in this increase. As visual tasking, such as reading and working on a computer, has become more prevalent, there is an increased awareness of ocular allergy and the impact it has on quality of life and productivity at work and school. With the need for more effective medications, the development of models, such as the conjunctival allergen challenge (CAC), has made the identification of new agents more efficient. In this article, we review the relevant background on the science behind allergen challenges in the eye, how models are designed, and how models are used in the field today.

Introduction

It is estimated that as many as 50 million Americans are affected by ocular allergy—almost 25% of the population [1]. Of the four types of allergic conjunctivitis (atopic keratoconjunctivitis, vernal keratoconjunctivitis, seasonal/perennial allergic conjunctivitis (SAC/PAC), and drug-induced allergic conjunctivitis), the most prevalent forms are SAC, triggered by pollens, and PAC triggered by dust or dander. The bothersome signs and symptoms caused by ocular allergy will cause significant decreases in quality of life and ability to function, sleep problems, decreased ability to visual task, and effects on social interactions, all leading to missed time at work, owing to visits to the doctor's office, and decreased productivity. Therefore, it is important not only that therapeutic modalities be developed for ocular allergic sufferers, but also that the model or methods by which these treatments are identified and tested be accurate and reliable. In the pursuit of effective therapies, the conjunctival allergen challenge (CAC) model has been developed. This model has allowed precise control of confounding factors that are present in the typical environmental study and has helped to evaluate and bring to market effective medications for ocular allergy. The model has also been very useful in elucidating the allergic and inflammatory mechanisms of the ocular surface, in identifying the cells and mediators that are involved, and in identifying targets for novel therapies. In this article, we review the CAC model, compare it with the environmental design, and look at how it has helped contribute further understanding to ocular disease and therapy.

Basic Science of the Conjunctival Challenge Model

Of those who suffer from ocular allergic conditions, at least 90% suffer from SAC/PAC. These diseases are triggered when an allergen comes in contact with conjunctival mast cells containing IgE molecules bound to the cytoplasmic membrane. The cross-linking of pairs of IgE molecules with allergen initiates a cascade of intercellular changes that result in mast-cell degranulation. Understanding the host of substances released, and how they interact, has been driven by use of challenge models.

Various mediators and cytokines are released from the mast cell during degranulation, leading to the clinical signs and symptoms of allergy, and the propagation of the reaction (Table 1). The primary inflammatory mediator released during this process is histamine, as confirmed by a series of studies [2–5,6••]. Instillation of histamine into the eye reproduces in a dose-dependent fashion the signs and symptoms of allergic conjunctivitis: itching, redness, chemosis, tearing, and lid swelling. In fact, histamine is the only mediator that can reproduce the entire clinical allergic condition in the eye [2]. Furthermore, instillation of substances known to induce degranulation of mast cells (secretagogues) and the release of histamine also produce the allergic condition in both animal and human eyes [3]. The collection of histamine in tears is difficult, however, because the enzyme histaminase is also released during mast-cell degranulation and works to break

Table 1. Mediators released by the mast cell

Preformed mediators	
Histamine	Tryptases
Chymases	Serine proteases
Heparin	Carboxypeptidase A
Proteoglycans	
Newly formed mediators	
Leukotriene B4	Thromboxanes
Leukotriene C4, D4, E4	HHT
Prostaglandin D2	HPETE/HETE
Platelet-activating factor	
Mast-cell–derived cytokines/ chemokines	
TNF-α	Eotaxin
IL-1α, IL-1β, IL-3, IL-4, IL-5, IL-6, IL-10	RANTES
Interferon-γ	MCP
Macrophage-inhibitory protein	Granulocyte-macrophage colony-stimulating factor

HETE—hydroxyeicosatetraenoic acid; HHT—hydroxyheptadecatrienoic acid; HPETE-hydroperoxyeicosatetraenoic acid; IL—interleukin; MCP—monocyte chemoattractant protein; RANTES—regulated on activation, normal T-cell expressed and secreted; TNF—tumor necrosis factor.

down the released histamine, which peaks at 3 minutes. Histaminase levels were found to be lower in patients with vernal keratoconjunctivits resulting in chronically elevated histamine levels, indicating that this condition is allergic in nature [4]. Inactivation of histaminase allows the collection and measurement of tear histamine levels following instillation of allergen in the human eye. Four histamine receptors have been identified in the human body, although two, H_1 and H_2, have been identified in the eye [5]. The binding of histamine to the H_1 receptors on nerve endings leads to itch, and binding to H_1 and H_2 receptors on endothelial vascular smooth muscle leads to dilation (redness) and endothelial gaping (swelling). The blocking of these receptors with selective antagonists results in a decrease in itching and redness. Furthermore, more recently it has been shown that by instilling a potent mast-cell stabilizer into human eyes prior to allergen challenge, histamine levels are reduced, which correlates with reduced signs and symptoms [6••].

The effects of many of the mediators were investigated by instilling each of them onto the eye and observing effects clinically and histologically. For example, platelet activating factor (PAF) was found to be a potent chemoattractant for eosinophils and neutrophils, leading to intravascular margination in the conjunctiva [7]; prostaglandin D_2 resulted in redness, conjunctival chemosis, mucus discharge, and eosinophil infiltrate [8]; and in the human eye leukotriene B4 (LTB4) did not produce vasodilation; however, biopsy revealed infiltration of polymorphonuclear infiltrates (Unpublished data), whereas LTE4 and LTC4 [9] elicited no observable effect. PAF, leukotrienes, and prostaglandins are all newly formed mediators produced in the arachidonic acid pathway during the breakdown of phospholipids from the mast-cell membrane.

Conjunctival challenges have also been used to identify other mediators that are present in allergic patients. Tryptase is a good marker for mast-cell degranulation as the mast cell is the only cell in the body that contains this neuropeptidase. Tryptase levels were found to be increased in patients who were symptomatic with SAC and in patients after challenging the conjunctiva with allergens, compound 48/80, and mechanical rubbing [10]. Implications of this study were twofold: it showed that tryptase is a good indicator of mast-cell degranulation, and it showed that conjunctival challenges can be used to induce mast-cell degranulation. Studies in which the conjunctiva was challenged with allergen have shown increases in histamine, kinins, prostaglandins, albumin, and TAME-esterase (toluene-sulfo-trypsin-arginine methyl ester) [11]; leukotrienes B4, C4, D4, and E4 [12]; eosinophil cationic protein (ECP) [13]; and histaminase [14]. An understanding of the release of histaminase, the enzyme that breaks down the released histamine, following a conjunctival challenge is especially important in understanding the time course of signs and symptoms. The challenge models have also been used to study effects that occur on the epithelium in allergic diseases. For example, it has been shown that conjunctival epithelium expresses intracellular adhesion molecules (ICAM-1) following challenge [15].

During the acute allergic reaction, there are many chemotactic factors released from the mast cell; the actual cellular infiltrate that would be expected to subsequently occur in the eye is more ambiguous. Some of the mediators released from the mast cell, such as PAF, interleukin-5, LTB4, PGD2, and tumor necrosis factor (TNF), will help to recruit leukocytes, lymphocytes, and more mast cells in the conjunctiva. However, usually only high doses of allergen in a challenge test will provoke cellular infiltrate of eosinophils, neturophils, basophils, lymphocytes, and mast cells in selected patients [16], with ranges of 20 minutes to 6 to 24 hours following challenge. Furthermore, not all patients have cellular infiltrate in their environment, and SAC generally occurs in the absence of cellular recruitment [17••,18]. A second peak (or continuation of the acute phase) in symptoms has been demonstrated during this late phase at 6 h [19] following a conjunctival challenge with high doses of allergen. This reaction at 6 h was accompanied by increased histamine and eosinophil cationic protein levels (ECP-released from eosinophils), and upregulated adhesion molecules, as compared with pre-challenge baseline values [20•]. Although mast-cell numbers were increased in this latter study, interestingly tryptase levels were not during this late time point, indicating a potential role for cells other than mast cells (such as basophils) during this late phase. However, it is important to mention that infiltrate in general is not correlated with an increase in clinical signs and symptoms, and although an increase might be seen following CACs on the cellular level, this does not necessarily reach the clinical threshold necessary to induce signs and symptoms. Nonetheless, the study of cellular infiltrate is

very important in the complete understanding of the allergic mechanisms, for severe chronic conditions, and as a surrogate end point for the release of chemotactic factors from mast cells (*ie*, mast-cell degranulation).

The earlier discussion was not intended to give a full review of the allergic mechanisms in the eye; however, the compilation of research highlights ways in which conjunctival challenge models have been used to understand the pathophysiology of the ocular surface. The clinical relevance of the conjunctival challenge is validated by the similarities seen between the reactions following a challenge with the reactions seen in symptomatic atopic patients with allergic conjunctivitis.

Environmental Model for Studying Allergic Conjunctivitis

The environmental model for testing the effectiveness of anti-allergy agents has been used extensively throughout the world, and was the original manner in which ocular allergy was studied. In fact, the "environmental" concept is used throughout the medical research field to study almost all diseases. The idea is that a patient can be given the medication to use at home and either maintains a diary, or returns to the office for follow up visits. A study using the environmental model might be conducted during the course of several weeks to months. In ocular allergy, the patient can be given a diary to record severity of symptoms (itching) and perceived signs (redness) on a daily basis. Generally, patients are given scales to use as a reference in grading. At predetermined time intervals, the patients return to the office for examinations by the investigator. These office visits serve as safety visits—to determine efficacy and to review compliance with dosing and record keeping in the diary. Compliance can also be monitored utilizing telephone contacts made by study staff between office visits.

Factors Affecting Data in the Environmental Model

Although this type of study design most accurately reflects what would occur in a clinical setting in the individual patient, several confounding factors might interfere with the analysis and combination of data from patients within the same office and those seen at different sites in multicenter studies. Particularly in studying an acute condition such as allergic conjunctivitis, the viability and variability of the results and interpretation of the data might be difficult. These issues relate to five main concepts: 1) enrollment of sensitized atopic individuals; 2) exposure to offending allergens; 3) reliance on subjective data and compliance; and 4) placebo effect.

The environmental model relies on the fact that the patients enrolled suffer from the condition that is being studied. Therefore, patients enrolled in environmental ocular allergy studies need to be atopic, and specifically allergic in the eye. If they are not, there is no way to ensure that the individual will be allergic to the particular allergens that are in season. Often, skin testing is performed to qualify patients, and it is assumed they will have ocular allergy. However, in our experience, we have found an approximately 60% to 70% correlation between positive skin tests and positive reaction to allergen instilled in the eye; therefore, if skin testing is solely relied on, some patients will be enrolled who might not have allergy to the pollen in season. Others have also seen a similar correlation [21]. Often, entry criteria require a patient to present in the office with a positive skin test and positive clinical signs and symptoms of ocular allergy. In this case, it is important to ensure that standard diagnostic criteria are being followed.

The second, and most obvious, problem associated with the environmental model is the inability to regulate each participant's exposure to various allergens. Each individual is exposed to various degrees and types of allergens owing to differences in work habits; life style; natural variation in pollen counts between home and workplace; indoor pets or plants; use of air conditioning, fans, or ventilation ducts that would move airborne allergens throughout the home/office; density of plants outside; and natural variations in pollen counts. Additionally, some behavioral modifications, such as avoidance of allergen during the allergy season, might further complicate the issue. If the patient is not experiencing significant signs and symptoms, it is more difficult to identify a drug effect. Alternatively, if a patient reports to the office with few signs or symptoms, it could be due to a lack of exposure to offending allergens.

The scheduled office visits that are included in the study design to ensure a degree of objectivity are problematic owing to the unlikelihood of having patients whose worst allergic symptoms are timed synchronously with the predetermined scheduled visit. Patient diaries can be used to track signs and/or symptoms daily, and the patient's assessment of exposure to the outdoors and pollen counts are recorded within the geographic area of the study site by a pollen-counting station. But, patients might be allergic to indoor allergens or exposed to other irritants. It is questionable, therefore, whether a regional pollen count (or patient-recorded exposure) is a true measure of personal allergen exposure. Interestingly, clinical signs and symptoms are not always exactly correlated with the absolute values of pollen counts [22]. Pollen counts can vary even within the same area and will differ based on the exact location of the counter itself. Perhaps the fact that pollen-counting stations are not validated by standard criteria between sites might also play a role.

The third issue is the reliance on patient's diaries to determine drug efficacy. The diaries contain a high level of subjectivity owing to differences in symptom interpretation among people. Although standardized scales can be used, environmental studies rely on data recorded for primary efficacy variables of itching and redness by the patients themselves.

Compliance issues affect the quality of results, as one must assume that in some cases subjects will neglect to enter data in a timely fashion, and then later "back-fill" prior to the next office visit.

Another issue involved with the use of the environmental model is the high rate of placebo effect seen. A placebo drop, many times an artificial tear, can effect allergy treatment. They do this by acting as a barrier to prevent allergen from attacking the conjunctival surface, helping to dilute allergen and mediators in the tear film, and acting as an eyewash. Such environmental studies are known to have placebo effect ratings as high as 50% and 60% [23,24]. Although it is difficult to completely eliminate, the placebo effect is a significant factor, and it can be expected to play a larger role in environmental studies in which it acts as an eyewash, compared with single-drop studies in the CAC model.

The Conjunctival Allergen Challenge Model for Studying Allergic Conjunctivitis

To evaluate anti-allergic agents in a more controlled manner, CACs have been developed. Histamine produces a dose-dependent response when instilled in the eye, and thus has been used as a model for screening anti-allergic drugs. Although such an agent can help evaluate drugs with antihistaminic properties [25], and drugs that actively reduce redness, such as vasoconstrictors [26], this challenge is not directly stimulating mast-cell degranulation, as happens with allergen. Substances such as compound 48/80, which is a secretagogue that induces mast-cell degranulation, have also been used in human challenge tests [10]. However, because the secretagogues do not induce an immunologic reaction via an IgE-mediated pathway, they might not be appropriate for evaluating agents with mast-cell stabilizing activities. The CAC [27] was developed as the most accurate replication of the true allergic reaction, because it is IgE mediated, and results in mast-cell degranulation.

The standard controlled CAC study design includes two baseline visits. The first is a titration visit, and a selected allergen is instilled into both eyes of the patient. Signs and symptoms are then graded on standardized scales. Allergen is instilled into the eyes at increasing concentrations until a prespecified threshold of clinical response is achieved. The threshold scores, however, need to be set considering the reaction that resembles a natural allergic reaction—in other words, one that provides sufficient improvement of drug over placebo, but does not stimulate such a large reaction that it cannot be modulated by the drug. The intent of the study also needs to be considered when evaluating this threshold and allergen used. For example, a high dose of allergen is generally required to stimulate a significant cellular infiltrate and to correlate this infiltrate with clinical signs and symptoms. However, this reaction might be higher than that usually seen in the environment. When critically evaluating data from a study, the methodology and allergen dose used should be considered in determining clinical relevance.

Once the threshold allergen dose is determined in the patient, the patient returns for a confirmation visit. At this visit, the dose that elicited a sufficient reaction at the first visit is instilled in both eyes. This second visit confirms the consistency and reproducibility of the reaction in the patient. Patients who demonstrate a sufficient and reproducible response proceed to a third visit.

Both onset and duration of action of the agent can be evaluated using the CAC model. The patient can be dosed with the study treatment (placebo in one eye and drug in the other; drug in both; or drug A in one and drug B in the contralateral eye) and then challenged with the appropriate dose of allergen in both eyes. The eyes are then evaluated for signs and symptoms, and the appropriate analysis is performed. To evaluate duration of action, the challenge can be performed at a specific time following instillation of treatment. For example, if the patient is challenged 6 hours following instillation of the drug, then it is clear that the drug effects last at least 6 hours. Onset and duration of action are evaluated at separate office visits.

Safety during allergen challenge cannot be emphasized enough, because conjunctival instillation can produce significant nasal, throat, and respiratory reactions. Having trained medical personnel and appropriate emergency equipment on-site is critical.

Advantages of the Conjunctival Allergen Challenge Model

The CAC model mimics the signs and symptoms of an ocular allergic response accurately in a controlled setting [28••]. The instillation of the threshold dose in the subject's eyes consistently results in itching and redness.

By enrolling patients based on their response to a CAC, only those patients who actually have ocular allergy are being enrolled. The titration of allergen during the first visit provides a method for obtaining the threshold dose needed for adequate reactivity. The coupling of the titration with the second visit for confirmation ensures reproducibility. The CAC model contains a level of internal control that is not seen in the environmental model because the bilateral instillation of drug and placebo serves as a highly reproducible internal control.

The patient's exposure to offending allergens and certainty that the drug is being tested in an allergic eye is controlled by precisely instilling allergen in the office, in patients who are asymptomatic at baseline when they enter the office. Therefore, variable exposure patterns to allergens typically seen between patients in environmental designs is controlled. By completing the study in the "off-season" (*ie*, not during the pollen season) with allergens that the patients are allergic to, it can be further ensured that any environmental exposure will not confound the results.

By inducing the allergic reaction in the office, a trained, masked examiner can be used to evaluate the primary signs (redness and chemosis). The primary symptoms can also

be evaluated by the patients using standardized scales in the office while being observed by study staff, ensuring grading is done properly and that the patients correctly understand the scales. The CAC allows a timely and concise evaluation for the effects of the investigational drug. Also, with the instillation of the study treatments in the office, compliance is ensured.

Use of the Conjunctival Allergen Challenge Model for Evaluation of Drugs

Owing to the CAC model's high level of internal control, sensitivity, and reproducibility, it can be used in several ways. The CAC model is very applicable for studies involving a comparison of efficacy between drug and placebo [29,30,31•] The CAC model can also be used to compare a drug with an active control. This has been done by many groups using various agents available for eye allergy [32,33,34•,35••,36•]. Using the CAC, precise comparisons of onset of action and duration of action can be measured, which cannot be accurately evaluated in environmental studies. It is important to note that in the challenge studies, in which standardized scales are used, a specified unit change between drug and placebo on that scale can be defined as being clinically significant. This is different from showing statistical significance, which can occur without clinical significance. For example, typically on the 0–4 scale, a unit change is considered by the FDA to be clinically significant. However, even if a drug might not produce a clinically significant response of one full unit, the CAC model is still very useful for evaluating efficacy and in helping to select agents for further testing (*eg*, dose ranging).

Environmental and CAC models can be combined. In this design, patients are first exposed to a CAC. Patients who respond sufficiently to an initial CAC are enrolled into the study with an environmental design. This model helps to ensure that patients who are enrolled are atopic and, more specifically, are sensitive in the eye to the allergen currently in-season, during which the study is conducted. This hybrid model has successfully been used to study the mast-cell stabilizer pemirolast [35••].

A unique use of the CAC is to study effects of drugs on nasal signs and symptoms. Inflammatory mediators, released during the allergic reaction in the conjunctiva, and/or allergen itself, can drain through the nasolacrimal duct into the inferior turbinate of the nose and produce clinically significant nasal itching, sneezing, congestion, and rhinorrhea. Similar to mediators, topical drugs can also drain from the eye into the nose. In fact, we have seen an effect of potent allergy eye drops on nasal signs and symptoms, in both challenge models and environmental studies [36•,37•].

Conclusions

We can see how the CAC model has been a useful tool for the development of new agents for ocular allergy, and to help further our understanding of the pathophysiology of ocular allergy. The controls afforded by the use of this type of model lead to more reliable results and help to mitigate many of the issues we see with standard environmental studies.

Challenge tests have been used for years in the fields of asthma and allergic rhinitis. The ophthalmic division at the FDA has been a leader in accepting the CAC model, and has helped our field tremendously by giving us an efficient study design in which to evaluate the condition and to pave the way for the development of novel pharmaceuticals. With the recognition of the significance of using the model for the drug development process, as a pathway for drug approval, we are actually now seeing agents being developed first specifically for the eye, as a proof of concept for other indications. A thorough understanding of the model is required to ensure that accurate interpretations are made from the results, and that the study is still designed appropriately, matching the pharmacology of the agent, clinically relevant mechanisms of the disease process, and the objectives of the study.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

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