

Animal models of ocular allergy

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Purpose of review

This review focuses on reports on animal models of ocular allergy published within the past year. A number of animal models are currently being used to clarify the pathophysiology of ocular allergy and to improve the therapeutic interventions for this disease.

Recent findings

Published literature examined the role of cytokines and other effector molecules which drive the immunopathology of ocular allergies in several animal models. Animal models were also used to compare the safety and efficacy of currently available drugs, and were utilized in initial trials of novel therapeutic agents. Novel therapeutic options being studied include DNA immunizations and recombinant peptides that block enzymes involved in the inflammatory processes.

Summary

Several animal models are currently being used in the study of ocular allergy. These include different strains within the mouse, rat, guinea pig, rabbit and dog species. Continuing investigations are needed to elucidate the complex molecular and cellular processes involved in the pathogenesis of ocular allergies. A better understanding of the interplay of effector cells, cytokines, adhesion molecules and a number of other inflammatory mediators will broaden our knowledge of the pathophysiology of ocular allergy and allow improved therapeutic options for this disease.

Keywords

animal models, ocular allergy

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Abbreviations

IFN- γ interferon- γ
OVA ovalbumin
PLA₂ phospholipase A₂

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Introduction

Ocular allergy is an immunopathological reaction of the ocular surfaces that come into contact with the external environment. Allergic diseases, including ocular allergies, appear to be increasing: in 1997, it was estimated that as much as 30% of the US population has been reported to have nasal and ocular symptoms for at least 1 week per year [1]. This figure may be underestimated, as symptoms of allergic conjunctivitis are often under-reported. Nonetheless, the direct cost due to allergic rhinoconjunctivitis has been estimated to be US\$5.9 billion per year, with 25% of this being related to the purchase of symptom-alleviating medications [2].

Ocular allergy as a disease complex spans a clinical spectrum that ranges from mild seasonal allergic conjunctivitis to the more severe, sight-threatening atopic keratoconjunctivitis and vernal keratoconjunctivitis. Clinical features of the disease vary from person to person, although ocular allergy is often characterized by itching, burning and redness of the conjunctiva. The most common site of allergic inflammation in the eye is the conjunctiva; hence, allergic conjunctivitis may often appear synonymous with ocular allergy. However, it is important to remember that other parts of the eye, such as the cornea or the eyelid, may also be involved in the disease process.

The basic cellular and molecular mechanisms of ocular allergic disorders are complex and require further investigation. To this end, many allergic conjunctivitis animal models that mimic the cellular and humoral parameters of human disease have been developed. Animals that have been used in these models include the mouse [3], the rat [4–6], the guinea pig [7–10], the rabbit [11,12] and the dog [13,14], as summarized in Table 1 [3,4,6,8–12,14•,15,16•,17–22,23••]. Over the years, the use of different allergenic agents has evolved from the non-specific mast-cell degranulator compound 48/80 (Sigma, St. Louis, MO, USA), to protein allergens such as ovalbumin and bovine serum albumin, and environmental allergens including ragweed pollen and cat dander. In our laboratory, we use each of these reagents in our model of ocular allergy, depending upon the scientific question that is being asked.

Mechanistic studies

Animal models of allergic disease have furthered our understanding of the complex interaction between the

Table 1. Animal models of allergic conjunctivitis

Animal	Agent studied	Parameters measured	Experimental outcome	Species limitations	References
Mouse	Compound 48/80 (Sigma, St. Louis, MO)	Clinical symptoms, histopathology	Conjunctival inflammation with cellular infiltrates; response varied with different mouse strains	Small conjunctival tissue size; different strain susceptibilities	[15]
	Ovalbumin	Clinical symptoms, histopathology, enzyme assay	Eosinophilic infiltration in allergic conjunctivitis followed histamine binding of histamine H ₁ receptors		[16•]
	Ragweed	Cellular proliferation, cytokine production, antigen-specific antibody production	Conjunctival inflammation with cellular infiltrates plus humoral (IgG1 and IgE) response to allergen and Th2 cytokine production by activated lymphocytes; systemic or conjunctival administration of immunostimulatory DNA significantly inhibited allergic response		[3,17,18]
	<i>Felis domesticus</i> antigen 1 (Fel d1)	Clinical symptoms, histopathology, chemokine production	Interleukin-1R antagonist reduced allergic conjunctivitis through a decrease in chemokine production		[19,20]
Rat	Compound 48/80	Clinical symptoms, histopathology	Conjunctival inflammation with cellular infiltrates	Immunological reagents not as extensive compared with mouse or human; relatively small tissue size	[4]
	Ovalbumin	Clinical symptoms, histopathology, IgE antibody and cytokine production	Significant lymphocytic infiltration at 48 h compared to compound 48/80 challenge; appears to be specific to antigen and not due to mast-cell degranulation <i>per se</i> ; IFN- γ inhibited the induction phase of allergic conjunctivitis		[6,21]
Guinea pig	Ovalbumin	Clinical symptoms, histopathology	Monoclonal antibody to very late activation antigen-4 (VLA-4) significantly inhibited conjunctival infiltration of eosinophils in sensitized animals; iodine supplement in feed decreases chemosis and eosinophilic infiltration of allergic conjunctivitis	Limited immunological reagents; not many inbred strains	[9,22]
	Ragweed	Clinical symptoms, histopathology, enzyme-activity assay	Sensitized animals showed conjunctival redness and edema, along with decreased goblet cells and increased mast cell degranulation in the conjunctiva; TGase2 inhibitors inhibit allergic conjunctivitis		[10,23••]
	Japanese cedar pollen	Clinical symptoms, histopathology	Conjunctivitis noted in sensitized animals, with increased histamine content in the conjunctiva		[8]
Rabbit	Compound 48/80, codeine phosphate, lipopolysaccharide	Clinical symptoms, histopathology	Mast cell degranulation, increased intraocular pressure with compound 48/80, nitric oxide implicated in pathogenesis of allergic conjunctivitis	Limited reagents for immunological studies; expensive; fewer inbred strains	[11,12]
Dog	Ovalbumin	Clinical symptoms, histopathology, IgE antibody-production skin testing	Conjunctival/pulmonary challenge testing is a more sensitive marker for atopy than serum allergen-specific IgE levels	Limited reagents for immunological studies; lack of inbred strains; very expensive	[14•]

ocular tissues and infiltrating inflammatory cells. The conjunctival allergen model is now being used on both human and animal subjects.

In severe cases of allergic conjunctivitis, both eosinophilic and lymphocytic infiltration have been noted. To understand the mechanism of allergic conjunctivitis development, Fukushima *et al.* [21] established an experimental model of blepharoconjunctivitis in Brown Norway rats. In rats actively immunized with ovalbumin (OVA), the injection of interferon- γ (IFN- γ) after immunization diminished eosinophilic but not lymphocytic infiltration. When these rats were subsequently passively immunized with OVA-specific T cells and later challenged with OVA, the introduction of either IFN- γ or anti-IFN- γ had no effect on the disease process. Interestingly, IFN- γ appeared to exert its effect only during the induction phase, by suppressing eosinophilic infiltration, perhaps by effecting a decrease in interleukin-5 expression.

To understand the mechanism of eosinophilic infiltration in the late-phase reaction of allergic conjunctivitis, Izushi *et al.* [16•] challenged wild-type and histamine H₁ receptor-deficient C57BL/6 mice with OVA and later measured eosinophil peroxidase activity in the conjunctiva. The histamine H₁ receptor-deficient mice did not show any elevation in eosinophil peroxidase levels after challenge, in contrast to the wild-type mice. Interestingly, pretreatment of wild-type mice with an anti-P-selectin antibody caused a significant decline in conjunctival eosinophil peroxidase levels after challenge. Therefore, the authors concluded that eosinophilic infiltration in the late-phase reaction of allergic conjunctivitis is due to P-selectin expression of endothelial cells following histamine binding of H₁ receptors. An important point to remember is that histamine can also bind to the H₂ and H₃ receptors as well as to the H₁ receptor. Therefore, a role for the H₂ and H₃ receptors in eosinophil infiltration still remains to be elucidated.

Surrogate markers of atopy were examined by Zemmann *et al.* [14•] in the allergic dog model. The authors found conjunctival/pulmonary challenge testing in their dog model to be more sensitive in assessing allergic predisposition than OVA-specific serum IgE levels. Serum allergen-specific IgE levels decrease within 4–6 weeks after challenge, while positive skin tests in the same dogs remain for up to 10 weeks after the last allergen exposure. Allergic pulmonary tissue responses, which include altered measurements of airway resistance, and a decrease in lung dynamic compliance, were still present after 15 weeks. This study showed that in this model, tissue-bound IgE has a longer half-life than serum IgE.

Therapeutic studies

Therapy for allergic conjunctivitis has evolved from the use of systemic agents to more specific topical ocular preparations. Animal models have been used to assess therapeutic candidates, before being tested on human subjects using the conjunctival allergen challenge. The newer topical medications, such as the antihistamine levocabastine, and agents with multiple actions, such as ketotifen and olopatadine, have been tested on animal models. Improved therapy for the more severe and sight-threatening forms of ocular allergy is needed to avoid the harmful side-effects of steroid use, e.g. increased intraocular pressure leading to glaucoma. There has been a recent review of the newest Food and Drug Administration-approved ocular allergy treatments [24•], so an extensive review of such treatments is not included here.

The histamine H₁ receptor antagonist olopatadine hydrochloride was tested on the allergic rat and guinea-pig model [25•,26]. Oral and topical administration of the drug inhibited symptoms of rhinoconjunctivitis in sensitized animals. Inhibition of the release of inflammatory mediators such as histamine, platelet-activating factor and thromboxane A₂, and infiltration by eosinophils, were also observed in these models. Olopatadine also appears to inhibit phospholipase A₂ (PLA₂), but this finding needs to be clarified in further experiments. For a review of the pharmacological and clinical properties of olopatadine, the reader is encouraged to refer to the review article by Ohmori *et al.* [25•].

Ozturk *et al.* [27] compared the mast-cell stabilizer lodoxamide tromethamine with a similar drug, sodium cromoglycate, in the treatment of allergic conjunctivitis in New Zealand albino rabbits. The animals were challenged with bovine serum albumin and treated with either lodoxamide, cromoglycate, or dexamethasone. Ocular inflammation was quantified with a blood-conjunctival permeability index, using radioactivity and by measuring the weight of the conjunctiva. Lodoxamide appeared to be superior to cromoglycate, but was not more effective than the corticosteroid dexamethasone. Since long-term topical use of steroids may have potentially serious complications, including the development of glaucoma, lodoxamide may prove to be an alternative therapy for prolonged use.

Novel therapeutic strategies, such as the use of recombinant peptides to block the inflammatory pathways, were recently examined [23•] in a guinea-pig model of allergic conjunctivitis. Both transglutaminase 2 and PLA₂ promote inflammation. The mechanism these molecules use in the inflammatory process is that transglutaminase 2 activates PLA₂ and activated PLA₂ hydrolyzes phosphoglycerides – the first step in the eicosanoid

cascade that generates arachidonic acid. Arachidonic acid is later converted to either prostaglandins or leukotrienes, which have been proven essential in allergic inflammation. Consensus sequences from antinflamins, which are competitive inhibitors of PLA₂, and pro-elafin, which is a competitive inhibitor of transglutaminase 2, were determined. Recombinant peptides were created to act as competitive inhibitors for both transglutaminase 2 and PLA₂, and were instilled into the eyes of the guinea pigs before and after ragweed-pollen application. These peptides were shown to abolish the transglutaminase 2-catalyzed activation of PLA₂ *in vitro*, and to reduce the allergic conjunctivitis inflammation *in vivo* (according to clinical and histological evaluation). Excitingly, some peptides were shown to reduce eosinophil infiltration of the conjunctiva better than either the commonly used drug dexamethasone or the H₁ receptor antagonist levocabastine, suggesting that recombinant-peptide therapy might prove to be an effective treatment for allergic conjunctivitis.

Finally, the anti-allergic effect of iodine in a guinea-pig allergic conjunctivitis model was studied [22]. In these experiments, guinea pigs were given three different concentrations of iodine in their feed, and either passively sensitized with OVA antibody or actively sensitized with OVA. As the amount of iodine increased systemically, there was reduced chemosis and eosinophilic infiltration in the conjunctiva, concurrent with lowered systemic histamine. However, eosinophilic infiltration induced by platelet activating factor in non-sensitized guinea pigs was not inhibited. Platelet activating factor is known to be an important non-specific mediator of allergic inflammation, stimulating the synthesis of cysteinyl leukotrienes and thromboxane A₂, aside from being a chemotactic factor and a potent activator of granulocytes. The authors concluded that iodine was exerting its anti-allergic effect on mast cells; this requires further investigation.

Conclusion

While a number of animal models have been used historically in the study of ocular allergic disorders, there is a paucity of such work in the literature published during the past year. Insights into the role of cytokines and adhesion molecules in eosinophilic infiltration were reported. Topical and systemic therapeutic interventions were studied, including a comparison of the efficacies of different classes of anti-allergic drugs. A new topical drug in the form of recombinant peptides that block the inflammatory pathway shows promising results in guinea pigs, but will need to be investigated in clinical trials. In addition, immunostimulatory DNA sequences, which skew the response of allergy-inducing Th2 helper T lymphocytes to Th1 helper T lymphocytes, have been reported in the past year, and show promise in the

murine model [28,17,18]. Phase 1 clinical trials have been started to test these molecules [29]. Finally, the cellular and molecular basis of ocular allergy is complex and requires further investigation. The existence of numerous animal models that have been developed for such work should encourage further research in this field. In our own laboratory, we have been using these models in transgenic and knock-out mice to assess the role of genetic manipulation in the allergic response. Further studies will increase our knowledge of the basic immunopathology of this disease and will enable researchers to tackle the different forms of allergic eye disease with better drugs possessing increased safety and efficacy.

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