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Immune Regulatory Mechanisms in Allergic Conjunctivitis:

Insights from Mouse Models

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Abstract

Purpose of review—This review highlights recent findings regarding the immune regulation of allergic conjunctivitis (AC). Mouse models have facilitated prospective studies that have not been possible in patients. The availability of gene knockout mice and the wealth of monoclonal antibodies have permitted exquisite dissection of the pathophysiology and immune regulation of AC.

Recent findings—New insights have emerged in three areas: a) role of costimulatory molecules in the induction of Th2 immune responses; b) crucial role of interferon- γ (IFN- γ) in the expression of AC; and c) the function of T regulatory cells in shaping conjunctival inflammation once the immune response has been initiated.

Summary—Allergic conjunctivitis involves early phase and late phase reactions. The early phase reaction (EPR) is IgE antibody-dependent, while the late phase reaction (LPR) is IgE-independent and is mediated by inflammatory cells, especially eosinophils. Recent studies in mouse models of AC have provided important insights into the immune regulation of both the EPR and LPR of AC. Mounting evidence suggests that IFN- γ is crucial for optimum expression of AC. Costimulatory molecules influence the induction of Th2 immune responses and the EPR while regulatory T cells shape the expression of the LPR of AC.

Keywords

Allergic conjunctivitis; Costimulatory molecules; Hygiene hypothesis; IL-10; T regulatory cells

Introduction

Allergic conjunctivitis (AC) encompasses a variety of ocular inflammatory diseases that are elicited by immediate hypersensitivity responses to environmental agents. Studies have reported that between 20% and 30% of the population in industrialized countries such as the United States have experienced allergies, with 50% of these individuals reporting ocular allergies[1,2]. The incidence of allergies, including AC, has increased steadily over the past 30 years. AC can occur as a mild, transient inflammation, such as seasonal AC or as more severe chronic forms such as atopic keratoconjunctivitis (AKC) or vernal keratoconjunctivitis (VKC). AC is initiated by the preferential activation and polarization of the immune response to environmental agents that culminates in the generation of a Th2-dominated immune response and the preferential generation of IgE antibodies.

The early phase of AC occurs when allergens at the ocular surface engage their specific IgE antibodies that are bound to Fc ϵ RI receptors on conjunctival mast cells. The cross linking of IgE antibodies triggers the degranulation of mast cells resulting in the release of variety of

mediators including vasoactive amines such as histamine. This early phase response is characterized by vasodilatation, increased vasopermeability, and itching. The early phase reaction (EPR) is followed by a late phase reaction (LPR) that begins 6-12 hr later, and involves the infiltration of inflammatory cells, especially eosinophils. Antigen-specific T cells initiate eosinophil infiltration into the conjunctiva, which can result in tissue damage[3]. Severe allergic inflammation of the conjunctiva is mediated primarily by infiltrating eosinophils.

The past 2 to 3 years have witnessed significant new perspectives regarding the regulatory pathways that influence the early and late phases of AC. These insights are largely the result of prospective studies in mouse models of AC.

The Late Phase Response is Sustained by T Cell-Dependent Processes

Allergic conjunctivitis, like other allergic diseases, is initiated when the offending allergen cross-links allergen-specific IgE that is bound to Fc receptors on mast cells. The ensuing activation and degranulation of mast cells culminates in the release of amines that produce the vascular changes characteristic of the EPR. However, mast cell activation also results in the release of pro-inflammatory molecules such as eosinophil chemotactic factor (ECF) and platelet-activating factor (PAF), which recruit and activate eosinophils. Thus, the segue from EPR to LPR requires mast cell activation and degranulation. However, studies in murine models of allergic conjunctivitis suggest that once initiated, the LPR can proceed in the presence of little or no detectable allergen-specific IgE antibody [4,5]. Passive transfer of IgE antibodies specific for short ragweed pollen (SRW) produces only mild LPR in mice challenged topically with SRW pollen[5]. Moreover, in an ovalbumin (OVA) model of AC, the LPR occurs in the absence of detectable anti-OVA IgE [4]. Importantly, LPR can readily be induced by adoptively transferring T cells from allergen-sensitized donors to naïve recipients prior to challenging the ocular surface with the relevant allergens [4,5]. Interestingly, in mouse models of AC, adoptively transferred T cells produce significant quantities of IFN- γ in addition to Th2 cytokines. These results are consistent with a recent study, which showed that SRW-induced AC was milder in IFN- γ knockout (KO) mice or wild-type mice treated with anti-IFN- γ antibody [6]. A recent study on AC and VKC patients lends further support to the notion that the Th1 cytokine, IFN- γ , contributes to the pathogenesis of AC. Leonardi et al. examined cytokine levels in tear samples collected from AKC and VKC patients and found that IFN- γ was the dominant cytokine in as many as 25% of the VKC patients[7]. Moreover, IFN- γ was present in the tears of over 80% of the VKC patients and over 40% of the AKC patients. The inferred role of IFN- γ in AC and VKC is counterintuitive when one considers the widely recognized capacity of this cytokine to cross-regulate the generation of Th2 cytokines. However, the production of IFN- γ in patients with AKC and VKC and in mice with experimental AC begs the question as to how IFN- γ might contribute to conjunctival inflammation. Recent studies using the SRW model of AC have shed light on this. IFN- γ KO mice and wild-type mice treated with anti-IFN- γ antibody displayed milder AC than wild-type mice[6]. Interestingly, mice deficient in IFN- γ failed to express vascular cell adhesion molecule-1 (VCAM-1) on their conjunctival blood vessels. VCAM-1 is an important cell adhesion molecule that is a co-ligand for very late antigen-4 (VLA-4), which is expressed on eosinophils and facilitates their emigration into inflammatory sites [8-10]. If IFN- γ facilitates the full expression of the LPR in AC by promoting VCAM-1 expression, it should be possible to mitigate AC by blocking VLA-4/VCAM-1 interactions. This in fact occurred when SRW-sensitized mice were treated with anti-VLA-4 or anti-VCAM-1 antibody prior to ocular challenge with SRW pollen[11**]. These results remind us of the pleiotropic nature of IFN- γ and demonstrate its capacity to influence the end stages of both Th1- and Th2-based inflammation.

Costimulatory Molecular Interactions Affect the Induction of Th2 Immunity and the Late Phase Response in Allergic Conjunctivitis

The activation and expansion of naïve T cells requires the delivery of two signals. The first signal is transmitted to the T cell receptor by cognate antigen presented on MHC molecules on antigen presenting cells (APC). The second signal is provided by interaction between costimulatory molecules expressed on APC and their ligands on T cells.

Members of the CD28 family of costimulatory molecules include B7-1 and B7-2, which are widely recognized as key costimulatory molecules for T cell activation. Recent studies using SRW pollen model of AC demonstrated the blockade of the B7-1 and B7-2 costimulatory molecules on APC at the time of initial sensitization to SRW pollen resulted in reduced T cell production of IL-5, a key Th2 cytokine necessary for eosinophil recruitment to the conjunctiva [12*]. This in turn was associated with a significant reduction in eosinophil inflammation of the conjunctiva.

In addition to the CD28 family of costimulatory molecules, members of the TNF receptor family also contribute to T cell activation [13]. OX40 (CD134) is a member of the TNF receptor family that is expressed on CD4⁺ T cells and has been shown to facilitate the generation of Th2 cells [14,15]. *In vivo* studies using the SRW model of AC demonstrated that OX40/OX40L interactions were critical for the induction of Th2 immune responses and the clinical manifestations of AC [16*]. That is, mice treated with an agonistic OX40 antibody developed more severe clinical symptoms of AC and displayed more intense eosinophil inflammation in the conjunctiva compared to control mice. Splenocytes from mice treated with agonistic anti-OX40 produced increased quantities of IL-5 and less IFN- γ . In a similar vein, treatment with a blocking antibody to OX40L resulted in milder AC, decreased production of IL-5, and elevated IFN- γ .

B7-H3 is another costimulatory molecule that is expressed on T cells and has been shown to provide a positive signal for Th1 cell development [17]. Studies using the same mouse model of SRW-induced AC demonstrated that T cells from mice treated with anti-B7-H3 antibody produced significantly higher levels of IL-5, and IL-13 [18*]. Moreover, anti-B7-H3-treated mice also displayed more intense eosinophil infiltration of the conjunctiva. Thus, blocking the B7-H3 costimulatory molecule blunts Th1 immune responses and presumably, releases Th2 responses from Th1 cross-regulation thereby resulting in an exacerbation of AC.

Do Regulatory T Cells Shape the Expression of Allergic Conjunctivitis?

Regulatory T cells (T_{regs}) influence the expression of immune-mediated inflammation of the ocular surface and the survival of corneal allografts [19-23]. Recent studies in mouse models of AC suggest that T_{regs} also influence the LPR of AC. NKT cells are a heterogeneous population of lymphocytes that co-express the T cell receptor (TCR) and NK markers and have the capacity to act as “first responders” in the immune surveillance of malignancies and microbial infections [24-26]. NKT cells also display immunoregulatory properties and are crucial for the induction of some forms of immune tolerance [27]. The synthetic glycolipid, α -GalCer, stimulates NKT cells to express immunoregulatory functions and has been used to mitigate experimental autoimmune diseases [28]. With this in mind, Fukushima and co-workers tested the effect of α -GalCer on SRW-induced AC in mice [29*]. Their findings revealed that treatment with α -GalCer at the time of ocular challenge with SRW pollen resulted in a significant mitigation in AC that coincided with a 10-fold increase in the expression of Foxp3 in CD4⁺CD25⁺ T cells; that is, a steep increase in putative T_{regs}. The α -GalCer-induced attenuation of AC was most likely due to the generation of CD4⁺CD25⁺Foxp3⁺ T_{regs} and not a result of cross-regulation of Th2 cells by IFN- γ , as a similar

effect was found in IFN- γ KO mice. One of the mechanisms utilized by CD4⁺CD25⁺Foxp3⁺ T_{regs} utilize is through their production of anti-inflammatory cytokines, TGF- β and IL-10. It is noteworthy that in addition to its anti-inflammatory properties, IL-10 has recently been shown to stabilize mast cells and reduce their degranulation *in vitro* and as a result attenuate allergic conjunctivitis in a mouse model of AC [30**].

The Hygiene Hypothesis and Allergic Conjunctivitis

The past 30 years have witnessed a steep increase in the incidence of allergic diseases. Although many explanations have been offered to account for this, none has captured more attention and provoked more discussion than the “hygiene hypothesis”, which was formulated by Strachan almost 20 years ago[31]. In its simplest form, the hygiene hypothesis proposes that reduced exposure to infections in early childhood reduces the incidence of allergic diseases by some unknown mechanism. The emergence of Th1 and Th2 cell paradigm in the 1980’s provided a simple mechanistic explanation for the increased incidence of allergic disease in industrialized societies that were noted for their high standards of hygiene. In such societies antibiotic use is widespread in patients and even in livestock and poultry. This results in reduced exposure to microbial infections. By contrast, in developing countries, microbial infections are more commonplace and trigger Th1 responses that are characterized by the elaboration of IFN- γ , which is known to down regulate the emergence of Th2 cells and the production of Th2 cytokines. Thus, frequent microbial infections might create a persistent cytokine milieu that discourages the generation of Th2 immune responses, resulting in milder, or less frequent atopic diseases. However, this explanation has fallen to the wayside with numerous observations indicating that helminth infections, which are notorious for provoking robust Th2 immune responses, were associated with a steep reduction in allergic diseases [32-34]. Moreover, the incidence of Th1-mediated autoimmune diseases is significantly lower in undeveloped countries and the severity of experimental autoimmune diseases is remarkably milder in animals bearing helminth infections[34]. A number of appealing propositions have been offered to explain the hygiene hypothesis. Among these is the notion that microbial infections, especially parasitic infections, induce the generation of T_{regs} that dampen both Th1 and Th2 immune responses and thus, reduce the incidence and severity of allergic diseases and autoimmune diseases.

Studies in the SRW model of AC lend suggest that the hygiene hypothesis applies to AC. Chronic infection with the nematode, *Ascaris suum*, resulted in a global increase in IL-10 and an attenuation of SRW pollen-induced AC [35]. Adoptive transfer of CD4⁺CD35⁺ T cells from *A. suum*-infected mice to recipient mice sensitized with SRW pollen and challenged topically with SRW allergens resulted in significant mitigation of AC. Follow up studies found that infection with viable *A. suum* was not necessary for mitigating AC and simply injecting pseudocoelomic fluid from *A. suum* into mice at the time of SRW sensitization protected against AC [36**]. However, protection was IL-10-independent, as mitigation of AC was detected in IL-10 KO mice. The authors suggested that *A. suum* pseudocoelomic fluid modulated AC by down regulating costimulatory molecule (CD86) expression on APC. Thus, the immunomodulatory effects of helminth and microbial infections may involve multiple pathways and are most likely not restricted to a single mechanism such as the generation of CD4⁺CD25⁺Foxp3⁺ T_{regs}. Whatever the mechanisms turn out to be, it is clear that we are at an exciting juncture in understanding the immune regulation of AC and the influence of infectious, and perhaps noninfectious, environmental agents in this process. Unfolding the mysteries of the hygiene hypothesis may yield important new strategies for treating the ever-expanding population of individuals who suffer from allergic diseases.

Conclusions

Animal models have provided enormous insights into the pathophysiology and immunobiology of allergic eye diseases. In the past 2-3 years these studies have demonstrated the role of costimulatory molecules, cytokines, chemokines, and T_{regs} in both the early and late phases of allergic conjunctivitis. These investigations offer hints for novel therapeutic interventions that can be tailored to treat individual stages of the AC cascade. The hygiene hypothesis has provided a fresh and novel perspective for understanding the epidemiology of allergic eye diseases and offers glimpses of novel therapies that can target each phase of ocular inflammation. The next 2-3 years will undoubtedly yield even more intriguing insights into the pathology, immunobiology, and epidemiology of allergic eye disease.

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References and Recommended Reading

1. Bielory L. Allergic and immunologic disorders of the eye. Part II: ocular allergy. *J Allergy Clin Immunol* 2000;106:1019–1032. [PubMed: 11112882]
2. Stahl JL, Barney NP. Ocular allergic disease. *Curr Opin Allergy Clin Immunol* 2004;4:455–459. [PubMed: 15349048]
3. Leonardi A, DeFranchis G, Zancanaro F, et al. Identification of local Th2 and Th0 lymphocytes in vernal conjunctivitis by cytokine flow cytometry. *Invest Ophthalmol Vis Sci* 1999;40:3036–3040. [PubMed: 10549670]
4. Fukushima A, Ozaki A, Fukata K, et al. Ag-specific recognition, activation, and effector function of T cells in the conjunctiva with experimental immune-mediated blepharoconjunctivitis. *Invest Ophthalmol Vis Sci* 2003;44:4366–4374. [PubMed: 14507881]
5. Fukushima A, Ozaki A, Jian Z, et al. Dissection of antigen-specific humoral and cellular immune responses for the development of experimental immune-mediated blepharoconjunctivitis in C57BL/6 mice. *Curr Eye Res* 2005;30:241–248. [PubMed: 15823916]
6. Stern ME, Siemasko K, Gao J, et al. Role of interferon-gamma in a mouse model of allergic conjunctivitis. *Invest Ophthalmol Vis Sci* 2005;46:3239–3246. [PubMed: 16123425]
7. Leonardi A, Fregona IA, Plebani M, et al. Th1- and Th2-type cytokines in chronic ocular allergy. *Graefes Arch Clin Exp Ophthalmol* 2006;244:1240–1245. [PubMed: 16538446]
8. Elices MJ, Osborn L, Takada Y, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 1990;60:577–584. [PubMed: 1689216]
9. Sagara H, Matsuda H, Wada N, et al. A monoclonal antibody against very late activation antigen-4 inhibits eosinophil accumulation and late asthmatic response in a guinea pig model of asthma. *Int Arch Allergy Immunol* 1997;112:287–294. [PubMed: 9066516]
10. Weg VB, Williams TJ, Lobb RR, et al. A monoclonal antibody recognizing very late activation antigen-4 inhibits eosinophil accumulation in vivo. *J Exp Med* 1993;177:561–566. [PubMed: 8381157]
- 11*. Fukushima A, Yamaguchi T, Ishida W, et al. Role of VLA-4 in the development of allergic conjunctivitis in mice. *Mol Vis* 2006;12:310–317. [PubMed: 16617298]
12. This study demonstrates that blocking the interactions between cell adhesion molecules and their ligands on leukocytes dampens eosinophil inflammation into the conjunctiva. This may also explain why cytokines such as interferon- γ , which upregulate cell adhesion molecule expression, are crucial for the full expression of allergic conjunctivitis.
- 12*. Sumi T, Fukushima A, Fukuda K, et al. Differential contributions of B7-1 and B7-2 to the development of murine experimental allergic conjunctivitis. *Immunol Lett* 2007;108:62–67. [PubMed: 17109973]

14. This investigation demonstrates that blocking costimulatory molecules hampers the induction of Th2 immunity and as a result, attenuates allergic conjunctivitis.
13. Watts TH. TNF/TNFR family members in costimulation of T cell responses. *Annu Rev Immunol* 2005;23:23–68. [PubMed: 15771565]
14. Flynn S, Toellner KM, Raykundalia C, et al. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. *J Exp Med* 1998;188:297–304. [PubMed: 9670042]
15. Ohshima Y, Yang LP, Uchiyama T, et al. OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human CD4(+) T cells into high IL-4-producing effectors. *Blood* 1998;92:3338–3345. [PubMed: 9787171]
- 16*. Fukushima A, Yamaguchi T, Ishida W, et al. Roles of OX40 in the development of murine experimental allergic conjunctivitis: exacerbation and attenuation by stimulation and blocking of OX40. *Invest Ophthalmol Vis Sci* 2006;47:657–663. [PubMed: 16431965]
19. This study demonstrates that it was possible to manipulate the induction of Th2 immune responses and the clinical expression of allergic conjunctivitis by blocking the costimulatory molecule OX40 with its ligand. These investigators also show that agonistic antibody to the OX40 ligand produces an adjuvant like effect and promotes a more intense Th2 response.
17. Chapoval AI, Ni J, Lau JS, et al. B7-H3: a costimulatory molecule for T cell activation and IFN- γ production. *Nat Immunol* 2001;2:269–274. [PubMed: 11224528]
- 18*. Fukushima A, Sumi T, Fukuda K, et al. B7-H3 regulates the development of experimental allergic conjunctivitis in mice. *Immunol Lett* 2007;113:52–57. [PubMed: 17825429]
22. Blocking Th1-inducing costimulatory molecule, B7-H3, diminishes Th1 cytokine production and exacerbates allergic conjunctivitis in mice. Blocking the Th1 costimulatory molecule of T cells presumably releases Th2 immune responses from cross-regulation by Th1 cytokines.
19. Nieder Korn JY. Immunology and immunomodulation of corneal transplantation. *Intern Rev Immunol* 2002;21:173–196.
20. Nieder Korn JY. The immune privilege of corneal grafts. *J Leukoc Biol* 2003;74:167–171. [PubMed: 12885932]
21. Nieder Korn JY. See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nat Immunol* 2006;7:354–359. [PubMed: 16550198]
22. Nieder Korn JY, Stern ME, Pflugfelder SC, et al. Desiccating stress induces T cell-mediated Sjogren's Syndrome-like lacrimal keratoconjunctivitis. *J Immunol* 2006;176:3950–3957. [PubMed: 16547229]
23. Streilein JW. Ocular immune privilege: therapeutic opportunities from an experiment of nature. *Nat Rev Immunol* 2003;3:879–889. [PubMed: 14668804]
24. Jameson J, Witherden D, Havran WL. T-cell effector mechanisms: $\gamma\delta$ and CD1d-restricted subsets. *Curr Opin Immunol* 2003;15:349–353. [PubMed: 12787763]
25. Seino K, Taniguchi M. Functionally distinct NKT cell subsets and subtypes. *J Exp Med* 2005;202:1623–1626. [PubMed: 16365145]
26. Terabe M, Berzofsky JA. NKT cells in immunoregulation of tumor immunity: a new immunoregulatory axis. *Trends Immunol* 2007;28:491–496. [PubMed: 17964217]
27. Stein-Streilein J. Invariant NKT cells as initiators, licensors, and facilitators of the adaptive immune response. *J Exp Med* 2003;198:1779–1783. [PubMed: 14676293]
28. Hammond KJ, Kronenberg M. Natural killer T cells: natural or unnatural regulators of autoimmunity? *Curr Opin Immunol* 2003;15:683–689. [PubMed: 14630203]
- 29*. Fukushima A, Sumi T, Fukuda K, et al. Modulation of murine experimental allergic conjunctivitis by treatment with α -galactosylceramide. *Immunol Lett* 2006;107:32–40. [PubMed: 16930723]
34. NKT cells display immunoregulatory properties and as shown here, stimulating NKT cells with a synthetic glycolipid resulted in a 10-fold increase in the expression of Foxp3 transcription factor that is associated with T regulatory cells and resulted in the mitigation in allergic conjunctivitis in mice.
- 30*. Bundoc VG, Keane-Myers A. IL-10 confers protection from mast cell degranulation in a mouse model of allergic conjunctivitis. *Exp Eye Res* 2007;85:575–579. [PubMed: 17765892]
36. IL-10 is known for its anti-inflammatory properties that mitigate allergic conjunctivitis. This study demonstrates that IL-10 also attenuates allergic conjunctivitis by preventing mast cell degranulation.

31. Strachan DP. Hay fever, hygiene, and household size. *Bmj* 1989;299:1259–1260. [PubMed: 2513902]
32. Maizels RM. Infections and allergy - helminths, hygiene and host immune regulation. *Curr Opin Immunol* 2005;17:656–661. [PubMed: 16202576]
33. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002;296:490–494. [PubMed: 11964470]
34. Zaccane P, Fehervari Z, Phillips JM, et al. Parasitic worms and inflammatory diseases. *Parasite Immunol* 2006;28:515–523. [PubMed: 16965287]
35. Schopf L, Luccioli S, Bundoc V, et al. Differential modulation of allergic eye disease by chronic and acute ascaris infection. *Invest Ophthalmol Vis Sci* 2005;46:2772–2780. [PubMed: 16043850]
- 36**. McConchie BW, Norris HH, Bundoc VG, et al. *Ascaris suum*-derived products suppress mucosal allergic inflammation in an interleukin-10-independent manner via interference with dendritic cell function. *Infect Immun* 2006;74:6632–6641. [PubMed: 16966410]
43. Although nematode infections provoke robust Th2 immune responses, injecting nematode pseudocoelomic fluid from a roundworm, *Ascaris suum*, reduces the severity of allergic conjunctivitis by an IL-10-independent mechanism.