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T helper Subsets in Allergic Eye Disease

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Abstract

Purpose of review—Ocular allergy is an IgE-mediated disease that results in inflammation of the conjunctiva and, in more severe cases, the cornea. This is driven by an immediate hypersensitivity response via mast cells, followed by a late phase response mediated by eosinophils—both of which are indeed dependent on T helper (Th) lymphocyte activity. Here we provide an update on Th subsets (Th1, Th2, Th17, T regulatory (Treg) and their relevance in ocular allergy.

Recent findings—Recent evidence in ocular allergy points to an involvement of other Th subsets, in addition to Th2. However, how these subsets are respectively activated and their role in mediating the different clinical forms is poorly understood. Novel mouse models may facilitate addressing such unknowns, and future challenges will involve how to translate such findings into more effective and “patho-specific” treatments.

Summary—Ocular allergy, especially in severe forms, involves subsets other than Th2. Th1 cells have been detected in mild and severe forms, and recent evidence points to a possible role for IL-17 in severe disease. Tregs, on the other hand, dampen pathogenic Th cell function and allergy immunotherapy is associated with Treg augmentation in disease management. Further understanding of Th biology is warranted and may lead to better therapies.

Keywords

Ocular allergy; Allergy; Allergic conjunctivitis; T helper subsets; Keratoconjunctivitis

INTRODUCTION

Ocular allergy ranges from mild/acute (i.e. seasonal and perennial allergic conjunctivitis (SAC and PAC)) to severe/chronic (i.e. atopic and vernal keratoconjunctivitis (AKC and VKC)). SAC occurs seasonally and is elicited by grass, trees, and weed pollens, while PAC occurs year round due to allergens such as dust mites and animal dander (1, 2). AKC and VKC can cause severe damage to the ocular surface, leading to corneal scarring and vision

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CONFLICTS OF INTEREST

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loss (3, 4). Giant papillary conjunctivitis constitutes a third category of ocular disease caused by repeated mechanical irritation, but no IgE-dependent allergic reaction occurs (5).

Ocular allergy is dependent on CD4⁺ T helper cells, of which four major subsets exist: Th2, Th1, Th17, and T regulatory (Treg). Th2 cells are the main mediators of allergic responses (6–8), although the initiating signals remain unknown. There is also evidence that other pathogenic subsets may be involved in mediating ocular allergy, such as Th1 and Th17. Furthermore, Treg may also be involved in ocular allergy, albeit in dampening allergic immune responses, as seen in antigen-specific immunotherapy.

The aim of this review is to provide an update on the Th subsets and their role in the pathobiology of ocular allergy.

OCULAR ALLERGY BACKGROUND

The development of ocular allergy consists of an early and a late phase reaction. The early phase reaction occurs within minutes of allergen exposure and is initiated when the allergen cross links specific IgE antibodies bound via FcεRI receptors on the surface of ocular mast cells, resulting in the release of mediators such as histamine (9, 10). In contrast, the late phase occurs six to twelve hours following antigen exposure and involves the infiltration of inflammatory cells, primarily eosinophils, into the conjunctiva.

The allergic response in mild ocular allergy is mostly in the conjunctival tissues and characterized by itching, tearing, redness, chemosis, and lid swelling (5, 11). Mast cells, and to a lesser extent, eosinophils are the hallmark cells in mild ocular allergy (12). Patients with severe ocular allergy present with similar clinical manifestations (13, 14); however, more chronic symptoms exist (15). These symptoms include thickening of the lid, blepharitis, and meibomian gland obstruction, among others (3, 5, 16–18). The massive infiltration of eosinophils and the chronic exposure to inflammatory mediators can cause signs of keratopathy (15, 19). Additionally, anterior subcapsular cataracts can develop due to the steroid treatment required for this disease (16). Similarly, VKC patients experience more severe symptoms that include giant papillae, trantas dots, and corneal epitheliopathy that develop into shield ulcers (20–23), compromising vision in these patients as well.

Animal models have played a significant role in advancing our understanding of ocular allergy pathobiology (24). Unlike the spectrum of ocular allergy in humans, which are distinct in their pathophysiological manifestations, current animal models mimic the more mildforms. These models are limited with respect to the clinical significance of more severe allergy. Our lab has recently developed a mouse model for severe ocular allergy (25).

BASIC PRINCIPLES OF CD4⁺ T HELPER CELL SUBSETS

Adaptive immune responses, such as in allergy, are elicited by the activation of T cells by DCs, and this has been demonstrated in ocular allergy as well (25–28). DCs accomplish this by undergoing a maturation process that up-regulates MHC class II and co-stimulatory molecules, i.e. CD40 and CD80/86. Once activated, naïve CD4⁺ T differentiation is primarily determined by the cytokine milieu present, as well as cognate antigen and TCR

affinity (29). CD4⁺ T cells are categorized based on their cytokine profile as being Th2, Th1, Th17, or Treg (Figure 1).

Th2

CD4⁺ T cells differentiate into Th2 cells in the presence of IL-4 and IL-2 during the time of antigen presentation (30, 31). Th2 cells are characterized by the production of IL-4, IL-5, and IL-13 and the expression of the transcription factor GATA3. GATA3 activation is dependent on IL-4 activation of STAT6 (32, 33); and Th2 differentiation is abolished in the absence of GATA3 or STAT6 (33, 34). Th2 cells mediate humoral immune responses and are critical for expelling extracellular pathogens such as parasitic infections (35, 36).

Th1

By contrast, Th1 cells are involved in the clearance of intracellular pathogens. Differentiation of Th1 cells occurs when IL-12 and IFN- γ are present at the time of stimulation (37, 38). Th1 cells are characterized by the production of IFN- γ and the expression of T-bet transcription factor. Tbet induces Th1 cells by remodeling the *Ifng* gene and up-regulating IL-12R β 2 expression (39); therefore, it is not surprising that Tbet deficient mice, *Tbx21*^{-/-}, have severe defects in Th1 differentiation (40). In addition to intracellular pathogens, Th1 cells have been linked to many autoimmune diseases (41–43).

Th17

Autoimmune diseases have also been linked to Th17 cells, as well as clearance of extracellular pathogens (44–47). Th17 cells are characterized by the expression of the transcription factor ROR γ T and by the secretion of IL-17A, IL-17F, IL-21, and IL-22 (48). Their differentiation is dependent on TGF- β and IL-6. It is important to highlight the plasticity of Th17 cells, as Th17 responses can be lost quickly and shift to a Th1 phenotype (49). Recent work has shown that the Th17 phenotype is stabilized by IL-23 and endows them with pathogenic effector functions. Interestingly, these pathogenic effector functions are indicated by the production of both IL-17 and IFN- γ (50).

T regulatory cells (Tregs)

As the name describes, Tregs is the subset responsible for immune regulation. Tregs, which make up 5–10% of the CD4⁺ T cell population, are characterized as being CD4⁺CD25⁺ and by the expression of transcription factor Foxp3 (51). Tregs are categorized as being either natural or induced (52). Natural Tregs develop in the thymus, have a TCR that is specific for autoantigens, and are implicated in preventing autoimmunity (53). By contrast, induced Tregs are generated in the periphery from CD4⁺CD25⁻ T cells under immunosuppressive cytokine TGF- β and are implicated in alloantigen tolerance and allergy (54). Tregs may assert their effect through the production of suppressive cytokines like IL-10 and TGF- β and through the direct contact with DCs via the CD28 family member, cytotoxic T-lymphocyte antigen-4 (CTLA-4). (55–57). This interaction inhibits DC maturation by reducing the expression of CD80/86, and thus, preventing naïve T cell differentiation (Figure 2).

T HELPER CELLS IN OCULAR ALLERGY

Ocular allergy is classically Th2-mediated (9), but recent evidence suggests that other pathogenic subsets may be involved in mediating this disease.

Th2: The Canonical Role in Allergic Immune Responses

In ocular allergy the Th2 cytokine IL-4 induces B cell activation and IgE production (6). Mice that are IL-4 deficient have reduced clinical symptoms, eosinophil infiltration into the conjunctiva, and serum IgE levels (58). IL-5 activates and promotes the maturation of infiltrating eosinophils (59), while IL-13 is involved in mucus production (60). In severe ocular allergy such as VKC, IL-13 is involved in giant papillae formation through conjunctival fibroblast activity (61–63). Similarly, AKC is attributed to Th2 cytokines that include: IL-2, IL-4, and IL-5 (16, 18, 64, 65), where IL-5 levels have been shown to correlate with disease severity (66).

An area that remains poorly understood is identifying the initial signals that allow DCs to favor Th2 responses. Thymic stromal lymphopoietin (TSLP) has been described as inducing inflammatory Th2 differentiation (67). TSLP inhibits the expression of IL-12p40 subunit in DCs (68), thus impairing Th1 differentiation (69). Furthermore, TSLP-treated DCs primed naïve Th cells to produce IL-4, IL-5, and IL-13, while down-regulating IFN- γ and IL-10. Considerable effort has also been focused on defining the function of classical DC subsets, such as CD11b⁺ and CD103⁺ DCs (70). Evidence from our lab has pointed to a key role for CD11b⁺ DCs in ocular allergy pathogenesis (25–28). This is consistent with what has been reported in allergic airway inflammation (71–75). Recent work from Medzhitov and colleagues has suggested a role for IRF4 expression by DCs in mediating Th2 activity, although the exact DC subset governed in this manner is not completely known (76).

Th1: The double-edged sword

In the classic paradigm of immunology, IFN- γ cross-regulates IL-4 (77). Th1 would therefore be expected to suppress allergic Th2 immune responses; however, studies exploring this topic have reported various conclusions. On the one hand, IFN- γ has been shown to have a protective effect in ocular allergy as IFN- γ ^{-/-} mice have more severe allergic responses compared to WT control animals (58). However, Stern and colleagues showed that neutralizing IFN- γ during the efferent phase (i.e. time of ocular challenge) resulted in a reduction in the ocular allergic response (78). Furthermore, IL-12, the Th1 polarizing cytokine, has been found to be important for the development of the late-phase reaction as eosinophil infiltration was reduced in IL-12^{-/-} mice (58).

In humans, tear analysis from allergic rhinitis patients that experience conjunctival responses after allergen exposure revealed that IL-4 was detected in the tears as early as 20 minutes following allergen exposure (79). Interestingly, IFN- γ was detected during the delayed response (24–48 hours post allergen exposure), suggesting that IFN- γ plays a role in the delayed, rather than the early response of ocular allergy. IFN- γ has also been linked as playing a role in the more severe and chronic forms of ocular allergy as VKC patients have higher levels of IFN- γ (22). In addition, IFN- γ is more predominant than Th2 cytokines in

AKC patients, but not in VKC and GPC patients (64, 65). However, some studies have suggested that IFN- γ levels correlates with AKC disease severity (65), although others have not seen this (62, 80). Dissecting the role IFN- γ is playing in severe ocular allergy may be problematic because no animal model exists that mimics these diseases, so while correlations have been established, the underlying mechanism is poorly understood.

Th17: “Are you in or out?”

The role of Th17 in ocular allergy is poorly understood. Fukushima et al. has shown that WT and IL-17^{-/-} mice did not differ in conjunctival eosinophil infiltration (81), suggesting that IL-17 does not play a role in seasonal ocular allergy. This is in contrast to what is observed in severe ocular allergy, where VKC patients are reported to have increased levels of serum IL-17 compared to non-allergic controls (82). However, this does not directly point to a role for Th17 cells as other cells, such as $\gamma\delta$ T cells, are also IL-17 producers (83, 84).

More is known about the role of IL-17 in allergic asthma, which may be relevant in ocular allergy. The role of IL-17 is in the recruitment and activation of neutrophils directly through IL-8 (85), or indirectly by the production of CXCL8 and colony stimulatory factors (86). Typical of what is seen in the conjunctiva in ocular allergy, in allergic asthma, airway infiltrate is predominantly eosinophils (87, 88); however, in some severe forms of asthma the predominant infiltrate is neutrophils (89, 90). Administration of IL-17A in mice resulted in neutrophilic infiltration and airway hyperresponsiveness (AHR) that was abrogated with anti-IL-6 treatment, a Th17 differentiating cytokine (91). Another study adoptively transferred *in vitro* polarized, OVA-specific Th2 or Th17 cells and assessed disease development. After OVA challenge, not surprisingly, Th2 cells induced AHR, airway inflammation, and eosinophil infiltration into the lung that was reduced upon glucocorticosteroid treatment (92). The transfer of Th17 also resulted in AHR and airway inflammation. Interestingly, however, a neutrophilic rather than eosinophilic infiltrate that was not reduced upon glucocorticosteroid treatment. This correlates with studies by Zhao and colleagues who showed that in patients, steroid-resistant allergic asthma is associated with elevated IL-17 levels and a predominant neutrophil infiltration (93).

Tregs

The control of ocular allergy has been suggested to be a balance between Tregs and pathogenic T cells. Differences in the frequency of CD4⁺CD25⁺ (pathogenic) and CD4⁺CD25⁺Foxp3⁺ (regulatory) T cells in patients with allergic asthma, rhinitis, and dermatitis have been reported (94–96). Tregs have been shown to reduce clinical symptoms, mast cell degranulation, IgE responses, and Th2 cytokines (97–99). These effects are reversed by anti-TGF- β and anti-IL-10 treatment.

The absence of Tregs appears to correlate with exacerbation of ocular disease. In a mouse model of ocular allergy using short-ragweedpollen, depletion of Tregs by anti-CD25 treatment resulted in increased allergic disease, as defined by eosinophil infiltration into the conjunctiva (100, 101). Conversely, reduction in ocular disease seems to be dependent on the presence of Tregs. This becomes evident in the high-dose allergen sensitization model, which leads to augmented Treg activities that blunted Th2 responses and reduced clinical

disease (102–104). These studies suggest that ocular allergy may be a result of impaired Treg activity and that enhancing Treg activity will modulate allergic responses.

Changes in Treg frequencies are reported in patients with ocular allergy. Patients with PAC, for example, had higher frequency of CD4⁺CD25⁺ cells compared to healthy controls upon challenge with *Dermatophagoides pteronyssinus* allergen (105). These cells, however, had reduced Foxp3 expression or were Foxp3⁻, suggesting that PAC patients have higher levels of activated effector CD4⁺ T cells and reduced levels of Tregs. It has also been reported that CD4⁺CD25⁺ putative Tregs from healthy controls and patients with birch pollen allergen were able to suppress T cell proliferation. By contrast, during pollen season, these CD4⁺CD25⁺ T cells were unable to down-regulate IL-5 and IL-13 production (106). This correlates with what is seen in patients with allergic asthma (107).

Allergen-specific immunotherapy (AIT) has been used to treat allergic diseases and is thought to mediate this, at least in part, via augmenting allergen-specific Tregs (108, 109). In a mouse model of ocular allergy using *Dermatophagoides farinae*, sublingual immunotherapy (SLIT) reduced clinical symptoms and the associated Th2 responses (110). Interestingly, SLIT in patients with allergic asthma to the same allergen had reduced symptoms that correlated with reduced Th17 cells and increased Tregs (111).

Innate Lymphoid Cells: The newest kids on the block

More recently, innate lymphocytes (ILCs) have been described as being involved in T cell responses in mucosal tissues. ILCs are classified as ILC1, ILC2, and ILC3, where they share similarities in cytokine profiles and transcription factors with Th1, Th2, and Th17, respectively (112). ILC2, for example, secrete high levels of IL-4, IL-5, and IL-13 in response to helminth infections and allergic disease (113–115). While these studies suggest that ILCs may play a role in ocular allergy, this remains to be explored.

The roles of NKT and $\gamma\delta$ T cells have been studied in ocular allergy. On study administered α -GalCer at the afferent phase, which is known to stimulate NKT cells, and found a significant increase in eosinophil infiltration into the conjunctiva (116). By contrast, if α -GalCer was administered at the efferent phase, there was reduced eosinophil infiltration. A more direct examination of innate T cells was assessed with the use of knockout mice and these studies revealed that NKT and $\gamma\delta$ T cells mediate or amplify the Th2 inflammatory responses in the lymphoid tissue as well as at the ocular surface and are needed for maximal expression of ocular allergy (117, 118).

CONCLUSION

Ocular allergy has been established as being mediated by Th2 cells; however, what triggers Th2 responses remains poorly understood. Additionally, there is evidence that other T cell subsets (i.e. Th1 and Th17) may be involved in ocular allergy pathobiology (Table 2). Studies assessing the role of IFN- γ in ocular allergy have yielded varying results (58, 78, 119). IFN- γ has also been detected in patients with severe ocular allergy (64, 65, 78), but its role in severe disease remains incompletely understood. It also appears that IL-17 may be playing a role in the pathobiology of severe ocular allergy (82), but not in mild disease (81);

however, whether this IL-17 is produced by Th17 cells or innate lymphoid cells requires further investigation. While animal models typically mimic mild ocular allergy, the development of a model for severe ocular allergy (25) now allows for the examination of other T cell subsets in this disease. By contrast, Tregs are implicated in dampening ocular allergic disease and allergen-specific immunotherapy is aimed at increasing their frequencies. A better understanding of the T cell subsets that mediate ocular allergy, specifically the severe and more chronic forms, is crucial.

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KEY POINTS

- Allergic immune responses are mediated Th2 cells, although the initiating signals that induce Th2 immunity remain poorly understood.
- Th1 cells play a pleiotropic role in mild ocular allergy and have been detected in severe disease, suggesting that Th subsets other than the classically described Th2 are involved in ocular allergy.
- IL-17 has also been detected in severe ocular allergy patients; however, whether this IL-17 is produced by Th17 cells or by innate lymphoid cells remains unknown.
- By contrast, immunotherapies aim to generate Tregs that dampen ocular allergy and continued efforts are needed to increase Treg frequencies and function.
- With the use of established and newly described models, studies dissecting the role that the different Th subsets are playing in ocular allergy pathobiology can now be performed.

Figure 1. CD4⁺ Th subsets. Th cells are categorized based on their cytokine profiles as being: Th1, Th2, Th17, or Treg. Naïve Th cells differentiate into the various subsets based on the stimulatory conditions that influence transcription factor expression. Once differentiated, the cytokine pattern that characterized the individual subset dictates their effector functions.

Figure 2.

Treg suppressive functions. Tregs mediate their suppressive function in part via CTLA-4 interaction with CD80/86 on DCs. This inhibits DC maturation and prevents naïve T cell differentiation. In addition, Tregs secrete suppressive cytokines such as TGF- β and IL-10 that inhibit effector T cell functions.

Table 1

List of Abbreviations

Defined Term	Abbreviations
T helper	Th
T regulatory cell	Treg
Seasonal allergic conjunctivitis	SAC
Perennial allergic conjunctivitis	PAC
Atopic keratoconjunctivitis	AKC
Vernal keratoconjunctivitis	VKC
Giant papillary conjunctivitis	GPC
Dendritic cell	DC
T cell receptor	TCR
Cytotoxic T-lymphocyte antigen-4	CTLA-4
Thymic stromal lymphopoietin	TSLP
Airway hyperresponsiveness	AHR
Ovalbumin	OVA
Wild-type	WT
Sublingual immunotherapy	SLIT
Interferon regulatory factor 4	IRF4
Innate lymphocytes	ILCs
Natural killer T cells	NKT cells

Table 2

T cell subsets and their role in allergic responses.

T cell subset	Cytokines	Effector functions	Involvement in Ocular Allergy	SAC/PAC	VKC	AKC
Th2	IL-4 IL-5 IL-13	IgE production Eosinophil maturation and recruitment Mucus production	Immediate hypersensitivity, late phase reaction, and chronic disease	+ ⁵⁸	++ ^{64,65}	++ ^{64,65}
Th1	IFN-γ	Delayed-type hypersensitivity	Chronic disease	+ ^{58,78}	++ ^{64,65}	+++ ^{64,65}
Th17	IL-17A/F	Neutrophil recruitment	Detected in severe disease	- ⁸¹	? ^{82*}	?
Treg	IL-10 TGF-β	Suppression of effector T cells	Frequencies are reduced	↓ ^{100,101,102,105}	?	?

* Source of IL-17 not determined.