



Tolerance mechanisms in allergen immunotherapy

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Purpose of review

Allergen immunotherapy is the only treatment modality which alters the natural course of allergic diseases by restoring immune tolerance against allergens. Deeper understanding of tolerance mechanisms will lead to the development of new vaccines, which target immune responses and promote tolerance.

Recent findings

Successful allergen immunotherapy (AIT) induces allergen-specific peripheral tolerance, characterized mainly by the generation of allergen-specific Treg cells and reduction of Th2 cells. At the early phase, AIT leads to a decrease in the activity and degranulation of mast cells and basophils and a decrease in inflammatory responses of eosinophils in inflamed tissues. Treg cells show their effects by secreting inhibitory cytokines including interleukin (IL)-10, transforming growth factor- β , interfering with cellular metabolisms, suppressing antigen presenting cells and innate lymphoid cells (ILCs) and by cytotoxicity. AIT induces the development of regulatory B cells producing IL-10 and B cells expressing allergen-specific IgG4. Recent investigations have demonstrated that AIT is also associated with the formation of ILC2reg and DCreg cells which contribute to tolerance induction.

Summary

Research done so far, has shown that multiple molecular and cellular factors are dysregulated in allergic diseases and modified by AIT. Studies should now focus on finding the best target and ideal biomarkers to identify ideal candidates for AIT.

Keywords

allergen immunotherapy, B regs, Interleukin-10, innate lymphoid cell regs, immune tolerance, Tregs

INTRODUCTION

Dysregulated immune function has an important role in the development of IgE-mediated allergic diseases including allergic rhinitis, asthma, atopic dermatitis, food allergy, and venom allergy [1]. The IgE-dependent degranulation of mast cells and basophils after cross linking of allergen to their cell surface, causes the release of preformed mediators (i.e. histamine) from the intracellular granules. Together with arachidonic acid metabolites (leukotrienes, platelet-activating factor, prostaglandins), all these factors contribute to allergic inflammation and trigger symptoms when exposed to the allergen [2]. Therefore, primary goal of allergen immunotherapy (AIT) is to restore immune tolerance to the allergens. It is the only guideline approved, curative treatment for allergic diseases which alters the underlying pathophysiology, alters the skewed Th2 response and induces long-term clinical tolerance against allergens [1,3[¶],4–6]. AIT leads to a significant reduction in symptoms and medication use, improves quality of life, and prevents the development of new sensitizations [5,7–9]. Furthermore, AIT prevents the development of asthma in patients with allergic rhinitis

and the progression of prevalent asthma [10^{¶¶}]. The effectiveness of AIT has been confirmed using various allergens such as, grass and tree pollen, house dust mite, insect venom, and animal dander.

Tolerance induction may be affected by both the immunogenicity and allergenicity of the antigen used in immunotherapy. Vaccines containing whole protein allergens cause more side effects, whereas allergoids, peptides, and recombinants are hypoallergic formulations [11,12]. Short-term

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KEY POINTS

- Allergen immunotherapy (AIT) is the only guideline approved, curative treatment for allergic diseases, which alters the underlying pathophysiology and the skewed Th2 response.
- Numerous evidences show that peripheral T-cell tolerance to allergens represents a key step in healthy immune response and successful AIT.
- AIT may reduce inflammatory epithelial responses by inducing the tolerogenic dendritic cells, which can polarize T cells into the Treg phenotype that produces interleukin (IL)-10.
- AIT is associated with the formation of ILC2reg cells, which contribute to tolerance induction.
- Serum level of allergen-specific blocking antibodies increases from the first months of AIT and IgG-related serum IgE inhibitory activity contributes to long-term tolerance

peptide immunotherapies such as, T-cell epitope peptide AIT, peptide hydrolysates from LPP, and very recently B-cell epitope vaccine have been shown to have limited IgE binding and limited cross-linking to FcεR1 on the surface of mast cells and basophils. Therefore, they have fewer side effects and are considered well tolerated and clinically effective alternatives [13[■],14[■],15,16].

Currently, there are two valid immunotherapy routes including; subcutaneous administration (SCIT) which is the reference standard, and sublingual administration (SLIT) [17–19]. In real-life retrospective analysis of patients with allergic rhinitis, it has been shown that in adults and children who take grass pollen SLIT tablets, the progression of disease is slower, the risk of developing new asthma is less frequent, and this effect persists at least 2 years after the end of AIT [7,20[■],21]. Although HDM-SCIT significantly reduced symptoms and medication needs in patients with allergic rhinitis [22[■]], for the first time, a randomized double-blind placebo-controlled trial found SLIT tablets to be effective and safe in pediatric patients with moderate to severe HDM allergic rhinitis [23]. Both of the administration routes are suitable for children and adults in the management of allergic rhinitis and allergic asthma and SCIT is also used for venom allergy [24–28]. Moreover, several novel immunotherapy routes; oral (OIT), intra-lymphatic (ILIT), epi-cutaneous (EPIT), intradermal (IDIT), and local nasal (LNIT) are evaluated in clinical trials [29–34].

Clinical tolerance refers to a state of unresponsiveness of the immune system to an allergen. After entering the body through the respiratory tract, gut,

disrupted skin, or insect sting allergens induce tolerance naturally in the majority of the individuals [35,36]. But, how natural tolerance to allergens appeared is not fully understood. To a large extent, it has been interpreted from studies with patients receiving AIT or naturally exposed to high dose allergens such as beekeepers and cat owners [35,37,38]. When natural tolerance fails to develop, clinical tolerance may be acquired through AIT. It depends on many factors, such as the patient's age, duration of treatment, type of allergic disease, the route of allergen delivery, and the allergen itself [37]. Successful AIT induces allergen specific peripheral tolerance which compromises several cellular and molecular interactions and persists after AIT is stopped [27,39] (Fig. 1). As an early response, a decrease in mast cell, basophil activity and degranulation, and a reduction in inflammatory responses by eosinophils in inflamed tissues are seen. Secondly, Tregs and Bregs are generated and suppress the allergen-specific effector T-cell subsets. Afterwards, immune suppressive mediators such as interleukin (IL)-10, transforming growth factor-β, and allergen-specific blocking antibodies like IgG4 and IgA are released. After several months, as a late response; number of mast cells, eosinophils and their mediators decrease in the tissues [25,27,36,40]. It is obvious that Tregs play a pivotal role in the induction and the maintenance of the tolerance [40,41]. Subcutaneous, sublingual or oral administration of the allergen has been shown to induce allergen-specific Tregs [28,42]. Furthermore, IL-10, a critical suppressive mediator, has been shown to be released by other immune cells other than Tregs, especially innate immune cells and Bregs [43,44]. Additionally, accumulating data supports the role of mast cell, basophils, B-cells, dendritic cells, innate lymphoid cells (ILCs), and macrophages in this process [32,39,45,46]. Despite those cellular and molecular changes, there is currently no valid biomarker to predict and monitor the effectiveness of AIT to perform personalized therapy [18,33,47]. In this review, we discussed the role of cellular and immunological mechanisms in the tolerance induction and maintenance (Fig. 2).

BASOPHILS AND MAST CELLS

Allergen immunotherapy has been shown to have two phased effect on mast cells and basophils, very-early desensitization effects, and late responses in tissues. Clinical desensitization starts just after the first injection of AIT with lower responsiveness to the allergens despite the elevated allergen specific IgE levels (sIgE), and reduced susceptibility to degranulation of the mast cells and basophils [25,30]. Late responses are related to the reduction of tissue inflammation and mediator release of mast

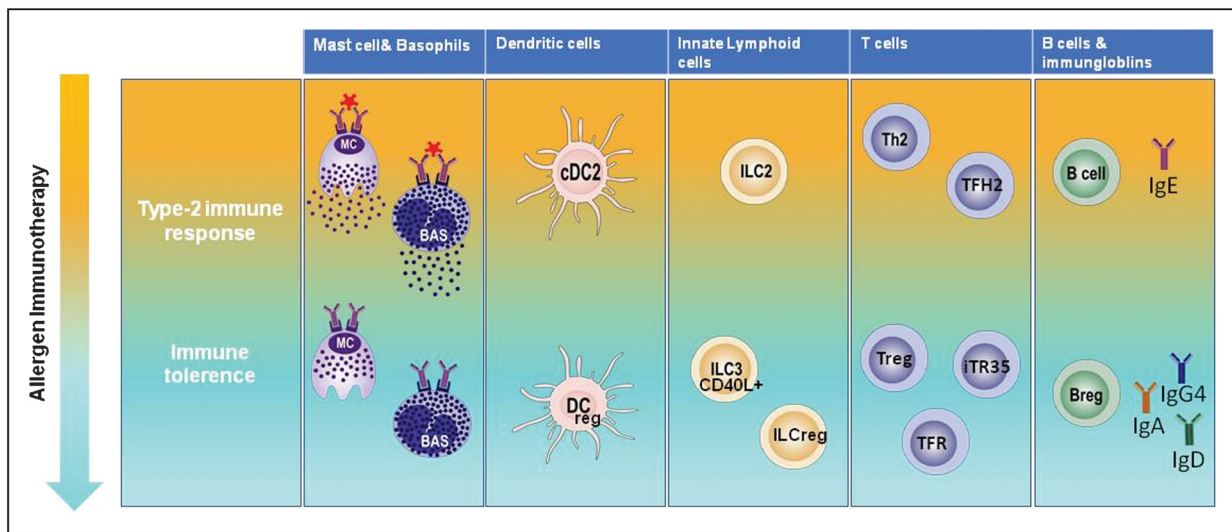


FIGURE 1. Change of function in type-2 inflammatory cells in response to AIT. With the induction of allergen immunotherapy type-2 immune response cells such as mast cells, basophils, DC2 cells, ILC2s, Th2 and TFH2 cells, and B cells producing IgE decrease in number and function. At the same time, regulatory subsets such as DCregs, CD40L⁺ ILC3s, regulatory ILCs, Tregs, iTR35 and follicular regulatory T cells, Bregs, and B cells producing IgA, IgG4, or IgD are induced and increase in number and function.

cells and basophils. One of the possible mechanisms underlying successful suppression of mast cells and basophils is the suppression of FcεR1 activity on mast cells and basophils with high expression of low affinity IgG receptors (FcγRIIa and FcγRIIb) [48–50]. Frequency of basophils expressing FcγRII in peripheral blood has been reported to increase significantly after 1 year of AIT [48]. Another one is the rapid upregulation of HR2. It has been demonstrated that histamine and leukotrienes are released during AIT without inducing systemic anaphylaxis. Histamine is the main soluble factor released from mast cells and basophils after allergen challenge and mediates its effects via histamine receptors. Four different HR types have been described and HR2 has been shown to take place in tolerogenic immune responses [25,51]. Rapid upregulation of HR2 might be highly relevant to early desensitization effect by suppressing the FcεR1 mediated activation and degranulation of mast cells and basophils [52]. This early allergen tolerance has been demonstrated in venom AIT [52]. On the other hand, mast cells may inhibit the generation of Tregs by blocking gene transcription of Foxp3 with IL-4 secretion. Therefore, with AIT, Treg formation can be promoted by suppressing the activation of mast cell [53]. Furthermore, early improvement in basophil sensitivity has been shown to correlate to symptom relief in grass pollen SCIT [54,55]. Through HR2, histamine acts directly on immature dendritic cells and induces IL-10 production [56] and Th2 cells [57] and increases the suppressive activity of TGF-β on T cells [58]. From the basophil point of view, basophils may be able to reduce the development of allergy during

AIT, which can be explained as basophil anergy [59]. Despite the individual differences and the risks for developing systemic anaphylaxis, inhibition of mast cells and basophils continues through the course of AIT with the changes in other immune parameters.

ROLE OF ANTIGEN PRESENTING CELLS IN THE TOLERANCE INDUCTION

Dendritic cells play a crucial role during the course of AIT. As a specialized antigen presenting cell, dendritic cells either initiate or hamper the allergenic inflammation depending on their maturation phase, location, and associated local cytokine milieu [60–63]. Five molecular markers usually expressed by peripheral blood dendritic cells reflect changes in regulatory (C1Q, FcγRIIIa) or proallergic responses (CD141, GATA3, RIPK4) and these markers can be used to monitor the effectiveness of AIT at an early stage with a sensitivity of 90.8% and a specificity of 61.6% [60,64,65]. In atopic subjects while aeroallergens pass through the inflamed epithelium, IL-25, IL-33, and TSLP are expressed from damaged epithelial cells. These epithelial cytokines favor development of a pro-allergic dendritic cell (DC2) phenotype and promotes their migration to the regional lymph nodes to differentiate naive T cells into Th2 cells. AIT may reduce these inflammatory epithelial responses by inducing the tolerogenic dendritic cell phenotype (DCregs), which can polarize T cells into the Treg phenotype that produces IL-10. In a recent study, oral CD103⁺ CD11b⁺ classical dendritic cells has shown to be involved in the transportation of sublingual allergens to regional lymph

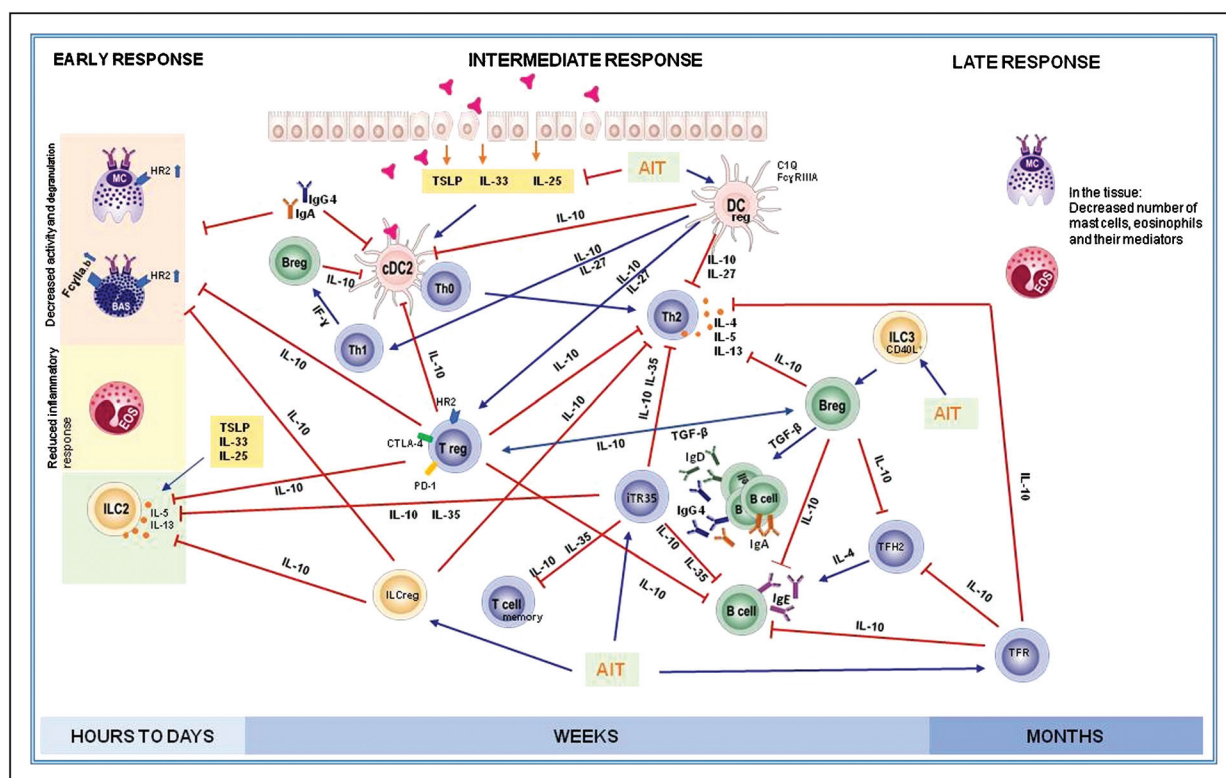


FIGURE 2. Immune tolerance mechanisms to an allergen induced by AIT. As an early response, a decrease in mast cell, basophil activity, and degranulation, and a reduction in inflammatory responses by eosinophils in inflamed tissues are seen. Rapid upregulation of HR2 might be highly relevant for the early desensitization effect by suppressing the FcεR1-mediated activation and degranulation of basophils. In weeks, regulatory cells (Tregs, Bregs, DCregs, and ILCregs) are generated and suppress the allergen-specific effector T-cell subsets. IL-10 secreting Tregs and Breg cells inhibit allergic inflammation by suppressing mast cells, basophils, eosinophils as well as ILC2s, TFH2, Th2 and IgE secreting B cells. iT35, a subgroup of iTreg cells, also suppresses memory T cells via IL-35 and IL-10. TFR cells have higher capacity to produce IL-10 and they are likely to suppress Th2 responses, IgE producing B cells and have been found to modulate TFH2 responses during AIT. The number of circulating TFR cells and suppressive functions on IgE have been found to improve after 12 months of AIT, but some variations have been observed among patients. AIT induces class switching in B cells and promotes the production of allergen-specific blocking antibodies like IgG4, IgA, and IgD. After several months, as a late response; number of mast cells, eosinophils, and their mediators decrease in the tissues.

nodes and induce antigen-specific Foxp3⁺ Tregs [66]. Peripheral blood sample PCR studies on sublingual grass immunotherapy patients showed a significant increase in the numbers of DCreg phenotype [64[□]]. As predicted from in-vitro studies, an increase in mRNA expression for stabilin and C1Q represents the DCreg signature, and this signature was observed only in responders to AIT [67]. In parallel with this findings in a 1 year follow-up study with SLIT in children with mite allergy, dendritic cells showed an increased capacity to produce IL-10 [68]. Recently, mannan conjugated polymerized allergoids have been reported to target dendritic cells and promote IL-10 producing tolerogenic dendritic cells which stimulates FOXP3⁺ Tregs [69]. In another in-vitro study, IL-27 which is produced by dendritic cells was shown to suppress grass pollen allergen stimulated PBMC proliferation in a dose-dependent

manner. Additionally, IL-27 significantly down-regulated IL-4 and IL-5, whereas upregulated IL-10 and IFN-γ [70]. Afterwards, retinoic acid skewed dendritic cells have been shown to express IL-10, IL-27, TGF-β, ALDH1A2, and their ability to induce Tregs has been indicated in-vitro [71]. Recently, a murine model has highlighted the vital role of dendritic cells targeted by IL-10 in establishing allergen-specific tolerance. Moreover, it was observed that loss of IL-10 signaling in dendritic cells resulted in a neutrophil-dominated allergic airway inflammation and a failure of tolerance induction [72[□]].

INNATE LYMPHOID CELLS AND IMMUNE TOLERANCE

Discovery of ILCs and its subsets has changed the understanding of immune tolerance mechanisms by

AIT. ILCs can be subdivided into three groups (ILC1, ILC2, ILC3) based on their surface markers, master transcription factors, and cytokines produced, in sharp correlation with their T helper counterparts. Unlike to T and B cells, ILCs do not express antigen-specific receptors, but they respond to damage induced signals such as cytokines and alarmins. ILC2s are primarily located in tissues with epithelial barrier including lungs, they are dependent on GATA-3 and they express a cytokine profile similar to Th2 [73]. Although, ILC1s are T-bet dependent and produce Th1 type cytokines, ILC3s are ROR- γ t-dependent cells and release IL-17 and IL-22 [74]. Particularly, ILC2s have an essential role in allergic airway inflammation [75,76]. Primarily, an increase in ILC2s has been reported in the sera of patients with cat allergy 4 hours after the cat allergen nasal challenge [77]. Subsequently, in the patients with grass pollen allergy, ILC2s were found to increase during the pollen season. ILC2s, which are predominant in the circulation and the affected tissues of the allergic patients, may play a role in maintaining allergic condition and resistance to AIT [78].

Research about the effects of AIT on ILC2s has been mostly performed in peripheral blood but not in target organs, because it is difficult to identify these cells in tissues [65]. Cross-sectional studies suggest that AIT affects the proportion of circulating ILCs. Increased ILC2/ILC1 ratio has been shown to return normal by a successful AIT in patients with HDM allergies [79,80]. Marked inhibition of seasonal increases in ILC2s was seen after grass pollen SCIT when compared to untreated controls [81]. Recently, IL-10⁺ ILCregs, which are CD25 and CTLA-4 high, CRTH2 and ST2 low, were demonstrated with the induction of ILC2s by retinoic acid [82[■],83[■]]. They can suppress ILC2 and T-cell activation and may have a role in AIT-induced tolerance [82[■]]. Moreover, close contact and interaction with CD40L expressing ILC3s and Bregs is important for maintaining immune tolerance [84]. ILC3s, Bregs, and Tregs colocalize in palatine tonsils. CD40L⁺ILC3s might induce functional immature transitional Bregs in tonsils and these cells can contribute to suppress T-cell responses [84]. It was thought that these mechanisms can also be important in immune tolerance induction during successful SLIT [85].

TOLERANCE INDUCTION AND REGULATORY T AND FOLLICULAR HELPER T CELLS

Numerous evidences show that peripheral T-cell tolerance to allergens represents a key step in healthy immune response and successful AIT [86[■]]. Peripheral T-cell tolerance is mainly characterized by an increase

in Tregs and shifting T-cell response balance from Th2 to Th1 with the contribution of many factors [87,88,89[■],90,91]. However, a new alternative, peptide immunotherapy has been shown to differ from conventional AIT in its mechanism of action [13[■],14[■],15]. Accordingly, there was no decrease in allergen-specific CD4⁺ T cells and Th2 to Th1 shift after peptide immunotherapy [14[■]]. However, surface expression of CRTH2 by allergen-specific CD4⁺ T cells is reduced. Thus, allergen-specific CD4⁺ T-cell recruitment to sites of allergen contact and their production of Th2 cytokines may be decreased [14[■]].

In AIT, Tregs act through at least four different suppressive mechanisms: secreting inhibitory cytokines including IL-10, TGF- β ; affecting cellular metabolisms by CD25, cAMP, adenosine receptor-2, HR2, CD39, and CD73; suppressing antigen-presenting cells and ILCs by PD-1, CTLA-4, or ICOSL; cytotoxicity by granzymes A and B [92]. Data from ex-vivo analysis of human PBMCs have shown that allergen-specific Tregs play a role in maintaining immune tolerance, and these cells increase with the induction of natural tolerance in beekeepers during beekeeping season [93] and the induction of clinical tolerance in patients treated with AIT [89[■]]. Supporting this finding, bee venom has been shown to promote the expansion of Foxp3 expressing CD4 T cells in vivo [94[■]]. While AIT upregulates the activated allergen-specific Tregs, it downregulates ILT3 expressing-dysfunctional Tregs [86[■],89[■]].

Allergen-specific Tregs have several distinct subsets; Foxp3 expressing Helios⁺ CD25⁺CD127⁻ natural regulatory Tregs (nTreg) and inducible Treg cells (iTreg) including Foxp3-expressing iTregs, IL-10 secreting Tr1 cells, and TGF- β producing Th3 cells [83[■],86[■],95]. Natural regulatory T-cells often tend to tolerate self-antigens, exert their suppressive effects directly on cell-cell contact, and also modulate allergen-specific T-cell responses in healthy nonatopic individuals [2,65,96]. Inducible Treg cells have been shown to modulate Th2 responses and cytokine release. During pollen season IL-10 expressing Tregs and TGF- β ⁺ Tregs were found to be increased and this was correlated with increases in serum IgG4 [97,98] and IgA levels [99], respectively. In a mouse model treated with TGF- β 1 mimetic peptide, IL-10 has been shown to be upregulated, Treg cell production induced, and these mice had lower levels of IgE, IgG2, and higher levels of IgA [100]. Human in-vitro studies showed that local nTreg [101–103] and iTreg [104–106] cell responses increased after AIT. At first, these changes were observed with SCIT, but later SLIT, OIT, and EPIT have been shown to induce suppressive effects and long-term Treg production by inducing epigenetic changes in the Foxp3

regions in Tregs [107–110]. Recently, it has been demonstrated that nTregs increased at the end of 10 weeks of SCIT and kept nearly stable levels during the immunotherapy. In addition, IL-10⁺ iTregs are still present at high frequency at the end of SCIT [89[■]]. Moreover, a 5-year SLIT clinical trial indicates that the acquisition and maintenance of Tregs is important in long-term tolerance after immunotherapy [111[■]]. In a longitudinal study consisting healthy subjects and allergic patients, it has been shown that a skew from Th2 to Treg cells occurs during both natural allergen exposure and 3–7 months period of preseasonal SCIT and remains high till the end of a year [64[■]]. All of these findings support the concept that allergen tolerance is based on Tregs in humans [25,86[■]].

IL-35-inducible regulatory T cell (iTR35), a newly identified subset of iTregs have potent immune regulatory properties [112]. These cells reduce memory T-cell proliferation, Th2 inflammation response, proliferation, and cytokine production of ILC2s depending on IL-10 and IL-35 [86[■]]. In addition, IL-35 can impair IgE production from B cells, decrease Th2 cell polarization, dendritic cell-mediated cytokine production, and enhance the production of IFN- γ and IL-10 [112]. More recently, a cross-sectional study has demonstrated that successful SLIT increases the number of iTR35 and levels of IL-35 in the peripheral blood. The percentage of iTR35 and IL-35 production capacity is inversely proportional to the rhinitis symptom score [113[■]]. Another study supporting these findings concluding that the production of IL-35 from Tregs and Bregs by SLIT could be critical for induction of immune tolerance [111[■]].

Increasing knowledge has shown that the regulation of follicular T helper (TFH) cells play an important role in the development of tolerance in AIT [86[■]]. TFH cells are CXCR5⁺ CD4 T-cell subset specialized in providing essential help for B-cell maturation and class switching [65,114,115]. Several reports show that TFH2 cell count increases in patients with allergic rhinitis, and it decreases sharply in response to AIT, which is correlated with improvement in symptom scores [86[■],116]. Recently, a distinct population of CXCR5 expressing Foxp3⁺ Tregs, namely follicular regulatory T (TFR) cells, which suppress T-cell and B-cell responses in the germinal centers, have shown to modulate TFH cells [117,118,119[■]]. It was demonstrated that frequency of circulating TFR cells correlated with the frequency of TFR in germinal centers [120[■]]. TFR cells have higher capacity to produce IL-10 and they are likely to suppress Th2 responses during AIT [115]. In patients with allergic rhinitis circulating TFR cells were decreased and impaired in function.

This TFR cell defect was found in correlation with disease severity and sIgE production [86[■],120[■]]. The number of circulating TFR cells and suppressive functions on IgE production have been found to improve after 12 months of AIT, but some variations have been observed among patients, that may be responsible for the mechanism underlying different clinical responses to AIT [120[■]].

A clinical trial in recent years has reported that pathogenic Th2 cell subsets such as HDM-reactive ST2⁺CD45RO⁺CD4⁺ and IL-5⁺IL-13⁺CD27[−]CD161⁺CD4⁺ have significantly decreased after 1 year of SLIT and these can be used as a biomarker to reflect the clinical efficacy of SLIT [121].

B REGULATORY CELLS

An increasing body of evidence demonstrates that Breg cells have an essential role in the induction of allergen tolerance in AIT. Several studies on how Bregs support tolerance induction in AIT include stimulation of Tregs, suppression of effector CD4 T cells either directly or indirectly by the inhibition of dendritic cell maturation, modulation of TFH responses, and production of IgG4 antibodies [46,122]. The major phenotypes of regulatory B cells in humans are: IL-10 producing Breg subsets including; CD19⁺CD5⁺CD1d⁺ and CD19⁺CD24⁺CD38⁺ Bregs, which suppress Th1 cells, CD19⁺CD25⁺CD71⁺Cd73[−] Bregs (BR1) that may play a role in allergen-specific immune tolerance [83[■],123–125], plasmablasts, and immature B cells [126[■]]. AIT induces the development of IL-10 producing regulatory B cells and allergen-specific IgG4 expressing B cells [46]. Knowledge on the role of B cells in tolerance induction was obtained mainly from researches on beekeepers and patients under venom immunotherapy [127]. BR1 cells produce significantly increased levels of IgG4 therefore, these cells appear to support immune tolerance during AIT via IL-10 and IgG4 [123,127]. Recently, it was demonstrated that BR1 cells produce IL-1RA in addition to IL-10 [128[■]].

PLA2-specific B cells that have increased production of IL-10 and IgG4 were identified in bee venom exposed beekeepers or subjects on VIT [123]. Recently, after high dose of allergen exposure, PLA2-specific B cells have shown to upregulate CCR5 expression, which is also expressed by Tregs to migrate to inflamed tissues [124]. Furthermore, a study was shown that both allergic patients undergoing VIT and nonallergic beekeepers exposed naturally to high doses of bee venom have increased frequencies of plasmablasts, PLA-specific memory B cells and IL-10 secreting BR1 cells [2]. After exposure to bee venom, PLA-specific IgG4⁺ memory B cells and PLA-specific CCR5 expressing B cells were

observed to expand [32,124]. In a follow up study, during AIT in patients with HDM allergies, the number of IgG4⁺ and IgA⁺ DerP1-specific B cells, plasmablasts and IL-10⁺IL-1RA⁺BR1 cells expanded significantly especially in responders [128^{***}]. Therefore, it can be said that increased numbers of IL-10⁺IL-1RA⁺ Bregs, IgA⁺/IgG4⁺ B cells and plasmablasts might be associated with successful treatment [128^{***}]. With the increase in IL-1RA, the inhibitory effect of IL-10 is enhanced and these linkage was first shown in the IL-10 overexpressing B cells [129]. These cells acquired a momentous immunoregulatory profile including upregulation of SOCS3, GARP, CD25, and PD-L1 [129]. IL-10 overexpressing B cells secrete less IgE, strongly suppress proinflammatory cytokines and dendritic cell maturation and augmented the production of IL-1RA and VEGF [129].

In a cohort study of 75 HDM allergic patients undergoing SCIT, it has been shown that the number of allergen-specific IgD producing B cells increases and this is associated with symptom control to support the hypothesis that allergen specific IgD may play a role in immune tolerance induction and maintenance in AIT [130]. Recently, it has been shown that IgD binds to CD44 on basophils and actively inhibit IgE-mediated degranulation [131^{***}]. In addition to IL-10, Breg cells can secrete other regulatory molecules such as TGF- β and IL-35 [132]. An important role for TGF- β is its capacity to support conversion of naive CD4⁺ T cells to Treg cells [30]. And IL-35, can induce Treg proliferation and suppress Th17 responses [119^{***}].

SPECIFIC IGE, SIGG4, AND SIGA RESPONSES

Regardless of administration route and the type of allergen, an early but transient increase is seen in allergen-specific IgE, which may be because of memory responses [133,134]. Overtime, serum levels of allergen-specific IgE tend to decline that may foster long-term clinical tolerance [133–135]. Specific IgG competes with IgE to bind to the epitopes in allergens and decreased IgE/IgG ratio has been shown to correlate with amelioration in allergy symptoms [135–138]. In a human/mouse chimeric model, it was shown that this improvement in clinical symptoms is associated with AIT-induced blocking antibodies and occurs independently of AIT-induced cellular changes [139]. On the other hand, a decrease in IgE/IgG4 ratio during AIT may indicate a shift from Th2 to Treg cell and Breg cell dominance [25,86^{*}]. Inhibition of IL-4 and IL-13 during experimental AIT demonstrates immune-tolerant effects by enhancing and stabilizing Tregs [140^{***}]. In addition, because IL-4/IL-13 receptor signaling plays a critical role in

IgE class switching, blocking IL-4R α may prevent IgE formation and enhance IgA and IgG response [140^{***}]. Both SCIT [99,141] and SLIT [104,142–144] have been shown to induce allergen-specific IgG1, IgG4, and IgA. This is clinically important to achieve the tolerant effect in AIT [133]. Serum level of allergen specific blocking antibodies (i.e. IgG1, IgG4) increases from the first months of AIT [145]. A randomized placebo-controlled study showed that serum IgG4 levels increased after 3 months of SCIT and most patients treated with at least six months of immunotherapy, tolerated the allergen 50 times more than baseline [146].

More recently, increased specific IgG2 serum levels have been demonstrated after three consecutive courses of SLIT. IgG2 and IgG4 with anti-inflammatory properties, alongside with increased frequency of IgG2⁺, IgG4⁺ memory B cells can be induced as a result of class switching of allergen specific IgG1⁺ memory B cells upon repeated allergen exposure [147^{*}]. Studies have reported 10-fold to 100-fold increase in IgG4 during AIT and this was found to be correlate with the clinical response [142,148,149]. For instance, increased level of antivenom IgG in venom allergy is found to be associated with the protection provided by immunotherapy [150]. In a retrospective analysis of patients with clonal mast cell disorders, sIgG4 antibodies were found to be increased over the course of VIT and inversely correlated with the severity of re-sting reactions [151]. Allergen-specific IgG4 suppresses mast cell and basophil degranulation by blocking of IgE cross-linking to allergens [152].

Moreover, IgG4-mediated Fc γ RIIb stimulation leads to the conversion of M2a macrophages to M2b followed by IL-6, IL-10, and CCL1 production [153^{*},154]. In addition, IL-10 supports class switching to B cells producing IgG4, whereas CCL1 recalls Foxp3⁺ Tregs from the periphery [153^{*}]. Although, IgG4 levels decrease about 80–90% within 1 year after stopping AIT, IgG-related serum IgE inhibitory activity continues for few more years and contributes to long-term tolerance [141,142]. Following grass pollen SLIT, IgE facilitated allergen binding (IgE-FAB) reduced which is associated with an increase in sIgG1, sIgG4, and sIgA [3^{*}]. In addition, the results of recent studies have confirmed that the inhibition of IgE-FAB rather than merely serum IgG4 concentration also correlates well with the clinical effectiveness of AIT [64^{***},98,102,142,155,156]. Moreover, a novel subcutaneous peptide immunotherapy (LPP) has been found to be clinically effective just before the pollen season [15] and may have a more positive effect on FAB inhibition and induction of sIgG4 than conventional SCIT [16]. This response was seen as early as a month after the

initiation of treatment and caused a shift of IgE/sIgG4 ratio, which indicates the development of tolerance [138,157]. Similarly, in a murine model, treatment with purified protein or peptide vaccines is assumed to induce immune tolerance at least as much as whole body HDM SCIT [158].

Allergen-specific IgA represents the first-line defense on mucosal surfaces. It has been reported that IgA secretion is enhanced with the local TGF- β production and can induce IL-10 production from monocytes [99,159]. Thus, IgA prevents IgE-mediated reactions and reduces allergic inflammatory responses [133]. After AIT, IgA levels increase in parallel with IgG4 suggesting that these antibodies might be regulated by common factors and alter both systematic and mucosal immunologic reactivity [104].

CONCLUSION

Significant improvements in the mechanisms of allergen-specific immune responses have provided a better understanding of the tolerance mechanisms in AIT in recent years. Immune tolerance induced in AIT includes several cellular and molecular events responsible for inhibition of both early-phase and late-phase responses to allergen. AIT has been associated with a skew from T helper 2 to T regulatory cells. Increased proportion of Th1 and Treg cells and cytokines released from these cells are thought to downregulate the Th2 response, and inhibitory activity of allergen-specific IgG4. In addition to these basic mechanisms it has been shown that, dendritic regulatory cells, ILCregs, iTregs, follicular regulatory T cells, and Bregs have important roles on the development of immune tolerance. Although significant progress has been made in understanding the mechanisms of AIT, further research is needed to gain biomarkers for the development of new treatments as well as to identify the best candidates for AIT.

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Authors declare that they have no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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