Diverse immune mechanisms of allergen immunotherapy for allergic rhinitis with and without asthma

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Allergen immunotherapy (AIT) is an effective treatment for allergic rhinitis, inducing long-term clinical tolerance to the sensitizing allergen. Clinical tolerance induction can be achieved when AIT is administered for at least 3 years. AIT is associated with the modulation of innate and adaptive immune systems. This comprises inhibition of IgE-dependent activation of mast cells and basophils in the local target organ, suppression of T_H2 cells, immune deviation toward T_H1 cells, induction of T and B regulatory cells, and production of allergen-neutralizing antibodies. However, recent developments in their underpinning mechanisms have revealed that AIT, administered subcutaneously or sublingually, induces immune regulation through novel cell targets and molecular mechanisms. This comprehensive review discusses how immune tolerance driven by subcutaneous immunotherapy and sublingual immunotherapy is associated with the induction of a novel regulatory subset of innate lymphoid cells and suppression of proinflammatory T_H2, allergen-specific T_H2 (T_H2A), and T follicular helper cells. Moreover, they are associated with exhaustion of T_H2A cells and differential expression of nasal and systemic IgA antibodies. Uncovering the underpinning mechanisms of a successful AIT and immune tolerance induction will allow the development of targeted therapeutics for allergic rhinitis with and without asthma. (J Allergy Clin Immunol 2022;149:791-801.)

Key words: *Allergen immunotherapy, antibody response, innate lymphoid cells*

Abbreviations used

AIT:	Allergen immunotherapy
AR:	Allergic rhinitis
Breg:	Regulatory B
CCL:	C-C chemokine
CCR:	C-C chemokine receptor
CD:	Cluster of differentiation
CXCR:	C-X-C chemokine receptor
DC:	Dendritic cell
HDM:	House dust mite
ILC:	Innate lymphoid cell
PD-1:	Programmed cell death protein-1
SAR:	Seasonal allergic rhinitis
SCIT:	Subcutaneous immunotherapy
SLIT:	Sublingual immunotherapy
STAT:	Signal transducer and activator of transcription
T_{FH}:	T follicular helper
cT_{FH}:	Circulating T follicular helper
T_{FR}:	Follicular regulatory T
Treg:	Regulatory T
nTreg:	Natural regulatory T
iTreg:	Inducible regulatory T
TR1:	Type-1 regulatory T
iT_R35:	IL-35–induced regulatory T
TLR:	Toll-like receptor

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ALLERGIC RHINITIS: CLINICAL FEATURES AND PATHOPHYSIOLOGY

Allergic rhinitis (AR) is an IgE-mediated inflammatory disease of the nasal mucosa elicited by aeroallergens, such as grass pollens, trees, weed pollen molds, house dust mites (HDM), and animal dander. AR is characterized by symptoms such as rhinorrhea, paroxysmal sneezing, nasal itching, nasal congestion, and watery eyes.[1] AR can be classified according to the etiology into perennial AR and seasonal AR (SAR). SAR can easily be identified because of the onset of seasonal symptoms following exposure to seasonal allergens including pollens and trees, which peaked during spring, summer, and fall months.[2]

AR affects 10% to 30% of the population worldwide. Its prevalence is still increasing, not only in developed countries but also in developing countries of which around 67% cases consist of grass pollen and HDM allergy.[3] In western Europe, AR affects

25% of the population[4] while in the United States and the United Kingdom, SAR affects 20% of adults and 40% of children.[2,4-7] SAR symptoms pose as a major clinical problem and socioeconomic burden that impair quality of life of the patients due to sleep disturbance, poorer work performance and school grades.[8,9]

Allergic inflammation can be classified into 2 phases, early and late phase. The early-phase allergic responses occur almost immediately, within minutes of allergen exposure, and last for about 2 to 3 hours in sensitized individuals. Following re-exposure of the allergens, the cross-linking of allergens to IgE results in the degranulation of mast cells and basophils and the release of various proinflammatory mediators (ie, histamines, leukotrienes, and prostaglandins),[10] promoting symptoms of immediate type I hypersensitivity such as rhinorrhea.[11]

The early-phase response is then followed by the late-phase allergic response, which occurs within 4 to 12 hours following allergen exposure.[11] The late-phase allergic response is characterized by the recruitment of eosinophils, basophils, T cells, and monocytes into the lining of the nasal mucosa. It is associated with prolonged tissue edema and persistent symptoms, particularly nasal congestion. TNF produced by mast cells induces nuclear factor-kappa B and mitogen-activated protein kinase signaling pathways,[12] and subsequently upregulates the endothelial expression of leukocytes adhesion molecules, such as E-selectin and intracellular adhesion molecule-1 and vascular cell adhesion molecule-1.[13] In addition to driving allergen-specific IgE production, IL-4 secreted by T_H2 cells promotes the expression of vascular cell adhesion molecule-1, but not intracellular adhesion molecule-1 and E-selectin.[14] The expression of endothelial adhesion molecules facilitates the infiltration of eosinophils into the lining of the nasal mucosa[15] and promotes further production of mucus. Furthermore, IL-5 secreted by T_H2 cells induces eosinophil precursor proliferation and differentiation into eosinophils, and IL-9 secreted by T_H9 drives differentiation and recruitment of mast cells.[16] Moreover, epithelial cells secrete chemokines including Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES; C-C chemokine [CCL]5), eotaxin, and thymus and activation-regulated chemokine/CCL17), which promote recruitment and infiltration of eosinophils, basophils, and T cells.[17-19] Collectively, this results in sustained inflammation, which is the hallmark of late-phase allergic response.

MANAGEMENT OF SAR

Although allergen avoidance is the most effective way to improve clinical symptoms in patients with SAR, this is not always feasible.[20] Exposure to seasonal allergens could be limited by keeping windows closed, using an air conditioner, and limiting outside activities during spring, summer, and fall seasons, where seasonal allergens are at their peak.[21] Because this is not always possible, patients are encouraged to use multiple measures for optimal management of symptoms. The nonsedating second-generation oral antihistamines are the first-line pharmacological treatments for SAR. They are effective in relieving sneezing, itching, and rhinorrhea when taken before exposure to the allergen or when symptoms are at peak.[21] The sedating first-generation oral antihistamines are equally effective; however, they are not regularly prescribed for treatment of SAR as they have been reported to have anticholinergic effects and to impair cognitive performance and functioning.[22] Intranasal corticosteroids are the first-line treatment for patients with mild persistent or moderate to severe symptoms. They are effective in reducing inflammation of the nasal mucosa and have been demonstrated to have higher efficacy in reducing nasal congestion and rhinorrhea compared with antihistamines and leukotriene receptor antagonists.[23] Furthermore, intranasal corticosteroids are effective in reducing ocular symptoms and improving the quality of life of patients with SAR,[21,24] especially when administered before exposure to the sensitizing allergens.

ALLERGEN-SPECIFIC IMMUNOTHERAPY

Although pharmacotherapy interventions are effective in reducing symptoms and improving the quality of life of most patients with SAR, a small proportion of patients do not respond to even high-dose pharmacotherapy treatments. Allergen-specific immunotherapy (AIT) is beneficial for the treatment of SAR in these patients who typically exhibit moderate to severe symptoms.[8] AIT was first reported in 1911 by Noon[25] and Freeman,[26] where prophylactic inoculation with grass pollen extracts in patients with SAR, before the pollen season, resulted in desensitization and improved ocular symptoms following allergen exposure. AIT involves the repeated administration of high-dose allergens, either subcutaneously (subcutaneous immunotherapy [SCIT]) or sublingually (sublingual immunotherapy [SLIT]),[8] for at least 3 years to confer clinical benefits.[27] SCIT has been shown to be highly effective in the treatment of SAR[28,29] and is considered as a criterion standard while SLIT has emerged as a safer alternative while maintaining efficacy.[30,31] Following cessation of the treatment, both SCIT and SLIT are known to confer long-term clinical benefit and tolerance in patients[30-33] as well as prevention of disease progression.[34,35] It is noteworthy that most studies of AIT have been conducted using allergen extracts other than those derived from fungi such as major allergen Alt a 1 of the fungus *Alternaria alternata*. In a randomized, double-blind placebo-controlled dose-response study of Alt a 1–specific SCIT in patients with *A alternata*–induced allergic rhinoconjunctivitis, there was a dose-dependent reduction in the combined symptoms and the need for rescue medication scores at 12 months in those who received high dose of Alt a 1–specific SCIT compared with placebo. Dose-dependent Alt a 1–specific IgG_4 antibodies were also induced in the actively treated group compared to placebo.[36]

AIT is currently the only disease-modifying therapy for IgE-mediated allergies[32,37] though it is not without limitations, which include associated side effects, poor compliance, and lack of efficacy in nonresponders. Novel approaches of AIT to improve safety while maintaining or enhancing efficacy are currently under investigation. The efficacy of AIT has been attributed to the induction of allergen-neutralizing antibodies.[38,39] These can capture allergens at the mucosal surfaces, inhibit FcεRI mast cell activation, prevent FcεRII (cluster of differentiation [CD]23)-mediated facilitated allergen presentation, and promote allergic inflammation. In a randomized, double-blind, placebo-controlled, proof-of-concept study, 2 fully human IgG_4 mAbs directed against 2 distinct, nonoverlapping epitopes on Fel d 1 were passively administered in individuals with allergy to cat. A single subcutaneous dose of the combined mAbs (600 mg, 1:1 ratio) reduced total nasal symptom scores and visual analogue scale score, and improved peak nasal inspiratory flow. Moreover, suppression of FcεRI-, FcεRII-, and T_H2-mediated responses was

also observed. This passive immunotherapy approach was recently reproduced in relation to birch pollen allergy. A single dose (900 mg) consisting of 3 fully human IgG_4 mAbs against Bet v 1, the major birch tree pollen allergen, was also well-tolerated. It reduced total nasal symptoms scores as early as 8 weeks, with a sustained effect of up to 2 months.[40] The reduction of clinical symptoms following the intranasal birch pollen challenge was associated with lower allergen-induced basophil responsiveness.[40] Another approach of AIT, in particular, intralymphatic allergen immunotherapy, was shown to alleviate grass pollen–induced symptoms and improve rescue medication intake. In a 3-year follow-up double-blind, randomized placebo-controlled trial, 2-month intralymphatic allergen immunotherapy significantly reduced symptoms and use of rescue medication in patients with grass pollen–induced allergic conjunctivitis in the first season after treatment. However, a booster intralymphatic allergen injection did not maintain the clinical benefit.

IMMUNOLOGIC MECHANISMS OF IMMUNE TOLERANCE FOLLOWING SCIT AND SLIT

Immune tolerance induction following AIT occurs in multiple phases and involves the complex interplay of various immune cells of the innate and adaptive immune response.[41,42] Within the innate compartment, AIT has been shown to modulate effector cells (mast cells and basophils),[43-45] dendritic cells (DCs),[46,47] and innate lymphoid cells (ILCs)[48-50] while also extending its effect on the adaptive immune cells and inducing T and B regulatory cells[41] (Fig 1).

Modulation of innate immune compartment by SCIT and SLIT

Initial AIT dose in both SCIT and SLIT results in an early reduction in the degranulation of mast cells and basophils, as well as the release of histamine. However, the mechanism is yet to be fully elucidated.[42,51] Moreover, reduction in proinflammatory cytokines (IL-4, IL-5, IL-13, and IL-9), eosinophil proliferation, and infiltration into inflammatory sites have been reported. Exposure to high-dose allergen administration in AIT induces expression of regulatory DC markers, complement component 1, and stabilin-1.[46] Moreover, AIT promotes DCs to secrete IL-12, IL-27, and IL-10 and downregulates the expression of costimulatory receptor CD86.[52] In an allergic murine model, CD86 blockade, but not CD80, was shown to dampen airway eosinophilia and hyperresponsiveness.[53] These findings have revealed that CD86 may be more relevant in the allergy pathomechanism. Moreover, IL-10 can downregulate CD86[54,55] and induce T-cell anergy.[56]

AIT has also been demonstrated to modulate type 2 innate lymphoid cells (ILC2s) and its regulatory counterpart, as shown in multiple recent studies. ILC2s play key roles in the pathophysiology of SAR. Epithelial-derived mediators such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 can promote ILC2s to produce type 2 cytokines such as IL-5 and IL-13. ILC2s have been thoroughly investigated in recent years as targets of AIT. SCIT treatment has been shown to be effective in suppressing the level of ILC2s. This was demonstrated in both grass pollen SCIT[49,57] and in HDM SCIT.[58] More recently, AIT has also been shown to induce a novel regulatory ILC counterpart with the capacity to produce immunomodulatory cytokine IL-10 following grass pollen SCIT and SLIT[48] and HDM SCIT.[59] Both grass pollen SCIT and SLIT were shown to result induce ILC2s that can produce IL-10 *in vitro* following stimulation with IL-2, IL-7, IL-33, and retinoic acid. Furthermore, the levels of $IL\text{-}10^+$ ILC2s strongly correlated with improvement in clinical symptoms (Fig 2). Single-cell CITE-seq analysis was used to investigate the molecular mechanisms underlying the induction of $IL\text{-}10^+$ ILC2s following AIT, which revealed that genes implicated in the retinol metabolism pathway (*RDH10, DHRS3, ALDH1A2, RARA*, and *RARG*), cytokine-cytokine receptor pathway, and Janus kinase-signal transducer and activator of transcription (STAT) pathway modified following AIT.[48] In addition to the regulatory counterpart of ILCs, a durable increase in circulating type 1 ILCs with marked expression of CD25 was reported during the course of AIT in a recent study.[50] Moreover, the study demonstrated changes in the dynamic and heterogeneity of type 1 ILCs. This observation highlights the important role that ILCs play and provides early evidence of trained immunity during AIT within the innate compartment.

T-cell subsets in immune tolerance induction following SCIT and SLIT

Immune tolerance induction following SCIT and SLIT is associated with the deletion of T_H2 and T_H2A cells, immune deviation from a T_H2- to a T_H1-cell response, and induction of regulatory T (Treg) cells.[41,42]

Deletion and suppression of T_H2 cells. T_H2 cells express the C-C chemokine receptor (CCR) 3, CCR4, and CCR8, which aids their migration into sites of inflammation and infection.[60] Although initial studies suggested CCR3 as the main chemokine expressed by T_H2 cells, polarization of naive T cells into T_H2 cells results in the upregulation of CCR4 expression, suggesting that CCR4 is the predominantly expressed chemokine of T_H2 cells.[60] The ligands for the chemokine receptors expressed on T_H2 cells are highly expressed at sites of helminthic infections or allergic reactions, particularly in mucosal tissues, thus resulting in increased migration of T_H2 cells to these tissues.

Differentiation of T_H2 cells from naive $CD4^+$ T cells is mediated by the cytokine IL-4. Following the persistent and repeated exposure to the antigen, the level of IL-4 is elevated, thus promoting polarization of naive T cells into T_H2 cells. The binding of IL-4 and IL-13 to IL-13 receptor present on cells such as B cells, DCs, and basophils results in signaling through the Janus kinase-STAT pathway.[61,62] This subsequently results in the upregulation of STAT6 expression, promoting T_H2 and B-cell differentiation. Moreover, IL-4 produced by T_H2 cells functions to stimulate B-cell immunoglobulin class-switching to IgE, which is key in eosinophil-mediated responses against helminthic infection and hypersensitivity reaction and the development of allergies.[62]

T_H2-cell responses are inhibited following AIT. Previous studies have reported a reduction in *IL4* mRNA and T_H2 cytokines in the nasal mucosa and following a nasal allergen challenge.[27] A short-course SCIT treatment in patients with ragweed allergy was associated with lower $IL\text{-}4^+$ cells in the nasal biopsies compared with placebo, following allergen challenge after the pollen season, but not before the pollen season, suggesting that the priming effect of natural allergen exposure following SCIT treatment may be required to achieve allergen desensitization.[63]

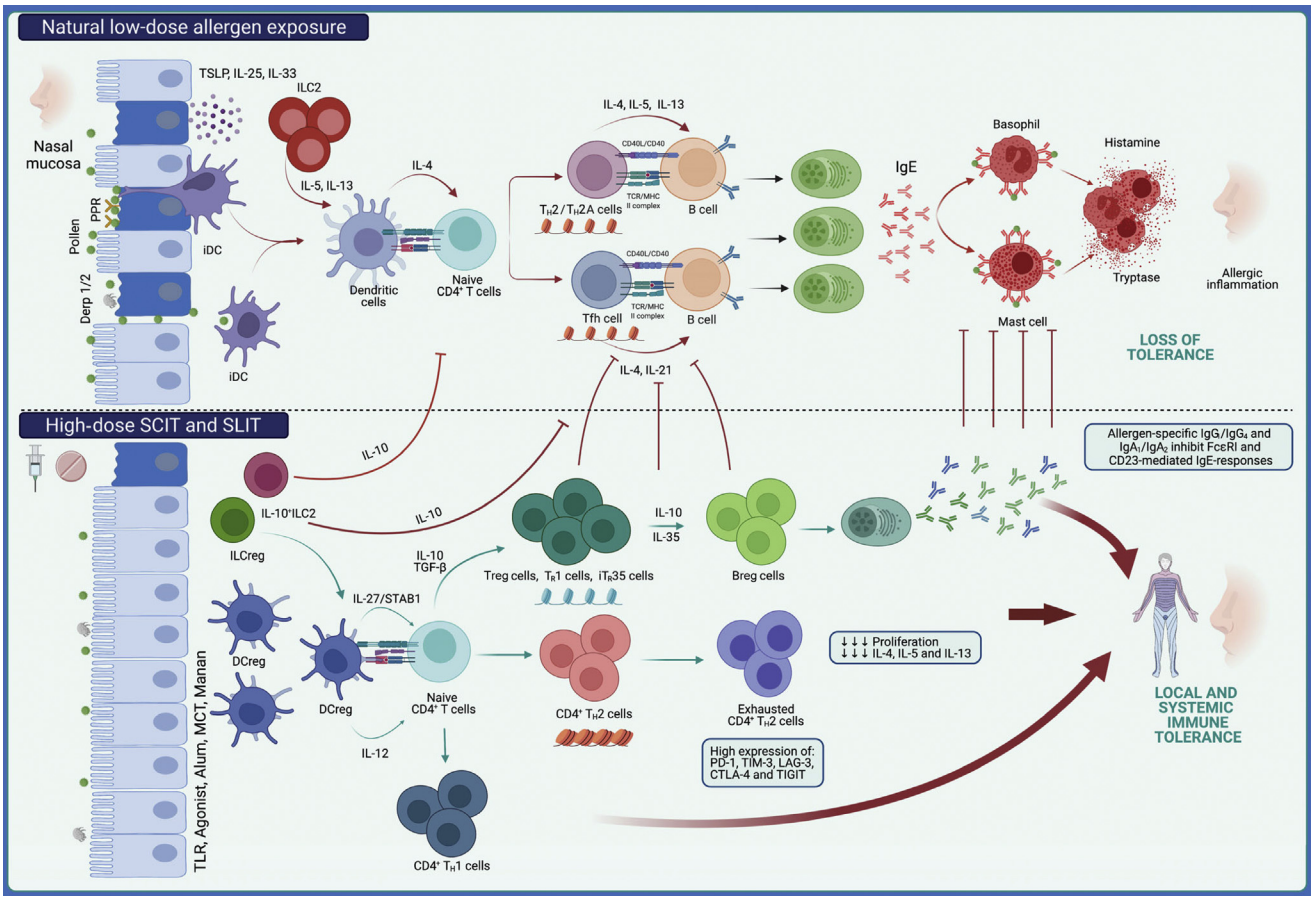

FIG 1. Diverse immune mechanism of AIT. In a susceptible individual with allergy, natural low-dose allergen exposure on damaged nasal epithelium promotes a T_H2, T_H2A, and T_{FH}-cell–dependent chronic IgE-mediated allergic inflammation. Subcutaneous and sublingual immunotherapy stimulate the generation of ILCreg, IL-10^+ILC2s, and DCreg. IL-12 and IL-27 secretion by DCregs induce proliferation of Treg cells. Treg-cell subsets suppress T_H2, T_H2A, and T_{FH}-cell responses, inducing a delayed shift toward a T_H1 response. IL-10 and IL-35 Treg cells support the induction of Breg cells and the production of nasal allergen-neutralizing and systemic IgG_1, IgG_4, and $IgA_{1/2}$ antibodies. These antibodies compete with IgE for allergen binding to IgE, thus inhibiting the formation of allergen-IgE complexes. This prevents the cross-linking of allergen-IgE complexes to FcεRI on mast cells and basophils and to CD23 on B cells, subsequently inhibiting IgE-facilitated allergen presentation to T cells. *CTLA-4*, Cytotoxic T-lymphocyte–associated protein 4; *DCreg*, regulatory dendritic cells; *iDC*, immature dendritic cells; *ILCreg*, regulatory innate lymphoid cells; *LAG-3*, lymphocyte-activation gene 3; *MCT*, microcrystalline tyrosine; *PPR*, pattern recognition rceptors; *TCR*, T cell receptors; *TIGIT*, T-cell immunoreceptor with immunoglobulin and ITIM; *TIM-3*, T-cell immunoglobulin domain and mucin domain 3; *TSLP*, thymic stromal lymphopoietin.

Another study reported similar findings, whereby timothy grass pollen SCIT- and SLIT-treated patients demonstrated lower nasal fluid T_H2-cell cytokines, including IL-4, IL-5, and IL-13, compared with untreated patients with allergies, following nasal allergen challenge.[64] More recently, allergen-specific T_H2A cells that were identified using tetramer analysis have been described.[65-67] These allergen-specific T_H2A cells are characterized by the expression of chemoattractant receptor-homologous molecule expressed on T_H2 cells, CD161, and CD49d and lack the expression of CD27.[65] They secrete IL-4, IL-5, and IL-13 and have been shown to be elevated in patients with allergy to grass pollen during the pollen season compared with nonatopic controls.[65] The phenotype of these T_H2A cells was also consistent across different allergic diseases, including those caused by timothy grass, alder pollen, HDM, and peanut.[68] In the Gauging Responses in Allergic Rhinitis to SCIT (GRASS) versus SLIT trial, SCIT and SLIT were associated with clinical improvement and lower frequency of these tetramer-positive chemoattractant receptor-homologous molecule expressed on T_H2 cells $(CRTH2)^+CCR4^+CD27^-CD161^+$ T_H2A cells following 2 years of treatment.[27,68] Moreover, in the same study, the nasal IL-4, IL-5, and IL-13 were also lower following nasal allergen challenge in SCIT- and SLIT-treated groups compared with placebo, which was lost at year 3 (1-year posttreatment). These findings suggest that SCIT and SLIT need to be administered for at least 3 years for long-term clinical benefit, which is associated with the reduction in T_H2 and T_H2A-cell responses.

Immune deviation toward T_H1-cell response. Unlike T_H2 cells, T_H1 cells are identified by the expression of C-X-C chemokine receptor (CXCR) 3 and CCR5, which aids their recruitment and migration to sites of infections.[60] CXCR3 and CCR5 bind to their ligands C-X-C chemokine 9, C-X-C

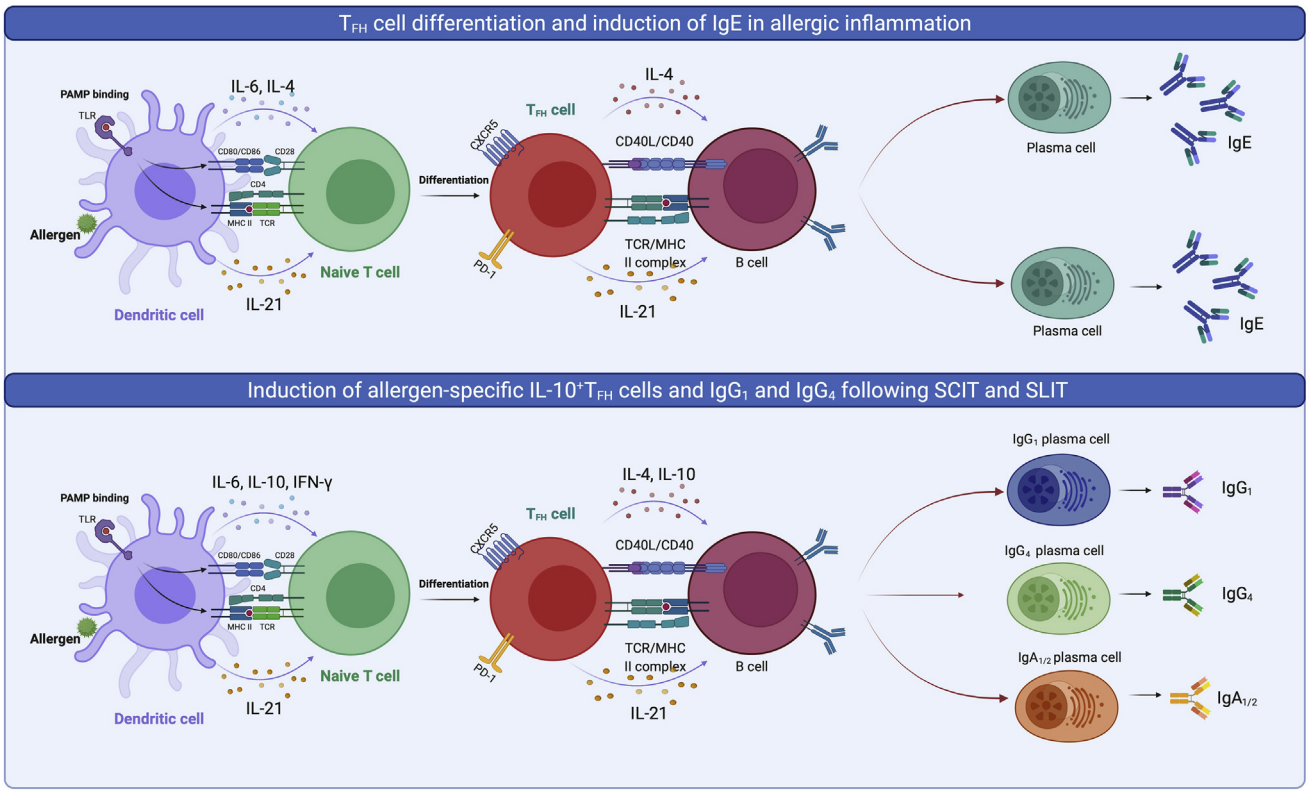

FIG 2. Role of T_{FH} cells in allergic inflammation and immunotherapy. Cognate interaction between allergen-primed dendritic cells and a naive T cell leads to the differentiation of IL-4^{+}IL-21^{+}T_{FH} cells under the influence of IL-4, IL-6, and IL-21. IL-4^{+}T_{FH} cells help B cells to differentiate, proliferate, and class switch to IgE-producing B cells and promote allergic inflammation. SCIT favors the induction of T_{FH} cells that promote IgG_1, and IgG_4 , whereas SLIT induces $IgA_{1/2}$ antibodies. *CD40L*, CD40 ligand; *PAMP*, pathogen-associated molecular patterns; *TCR*, T cell receptor. Open/close chromatins represent their ability to bind to transcription factors and promote/downregulate gene expression.

chemokine 10, and C-X-C chemokine 11 for CXCR3, and CCL3, CCL4, and CCL5 for CCR5,[60] mediating the infiltrations of T_H1 cells into infection and inflammatory sites.[69] On differentiation, T_H1 cells produce IFN-γ, further promoting T_H1-cell development.[70] Activated T cells may help to increase the cytokine production by DCs, through the binding of CD40 ligand on activated T cells and CD40 on the DCs, which can result in the secretion of IL-12.

Following AIT, T_H2-cell responses were inhibited and resulted in an immune deviation toward T_H1 cells. Previous studies have reported higher levels of nasal IFN-γ and a reduction in the *IL5*:*IFNG* mRNA ratio on exposure to naturally occurring allergens following grass pollen SCIT, which negatively correlated with the clinical response.[71,72] Another study also demonstrated the time-course induction of T_H1-cell cytokine, IFN-γ, from a T_H2-cell (IL-4) phenotype, thus confirming the immune deviation following AIT.[73] This finding was supported by another study that reported an increase in IL-12 mRNA^{+} cells in skin biopsies from SCIT-treated patients, but not in untreated patients with allergies.[74] In this study, the frequency of IL-12 mRNA^{+} cells positively correlated with the frequency of IFN-γ mRNA^{+} cells, and negatively correlated with IL-4 mRNA^{+} cells.[74] A study performed on patients with allergy to birch showed an attenuated circulating Bet v 1–specific T_H2 to IFN-γ^{+} T_H1 cell ratio following 1 year of SCIT treatment.[75] Several studies have suggested that the immune deviation could be due to the apoptosis of T_H2 cells. Cells obtained from AIT-treated patients with allergy to grass pollen were shown to have an increased proportion of IL-4^{+} cells undergoing apoptosis compared with untreated patients with allergy.[76] Similarly, T_H1 cells were shown to have increased expression of the antiapoptotic protein Bcl-2 following 1 year of SLIT treatment compared with baseline, thus suggesting a potentially persistent T_H1-cell population that was induced following SLIT.[77]

Suppression of T follicular helper cells by AIT. More recently, a distinct subset of T cells located in the germinal center in secondary lymphoid organs and tissues, known as T follicular helper (T_{FH}) cells, was identified. T_{FH} cells were shown to produce IL-21[78] and were identified as the main producers of IL-4 cytokine in secondary lymphoid tissues. In addition to CXCR5, programmed cell death protein 1 (PD-1) has been identified as a marker for T_{FH} cells and potentially regulates the interaction between T_{FH} and B cells.[79] The expression of BCL6 is crucial for the initial cognate interaction of T_{FH} and B cells in the T-:B-cell border.[80] Following their discovery in the secondary lymphoid organs, a circulating counterpart of T_{FH} cells has been identified in human peripheral blood and has been studied in various diseases. These circulating T_{FH} cells also express CXCR5 and PD-1 though they lack the expression of BCL6.[81] Circulating T_{FH} cells have been proposed to represent the memory counterpart of germinal

center T_{FH} cells. Moreover, they can be further identified into T_H1-, T_H2-, and T_H17-like T_{FH} cells by the expression of chemokine receptors CXCR3 and CCR6, which is predominantly expressed by T_H1 and T_H17 cells.

The production of IgE is key in the pathophysiology of allergic diseases. Although IgE production has been associated with T_H2 cells through the production of IL-4, T_{FH} cells have now emerged as another IL-4–producing cell subset. Interestingly, deletion of a conserved noncoding sequence 2, an essential enhancer element for IL-4 expression, in mice abrogated IL-4 production, and subsequently antigen-specific IgE antibodies by T_{FH} cells, but not T_H2 cells.[82,83] This highlights the critical role of T_{FH} cells in IgE production. Several murine studies reported that exposure to HDM allergen or ovalbumin promotes the development of T_{FH} cells and IgE production.[84,85] In humans, an "impaired" high T_{FH} to follicular regulatory T (T_{FR}) cell ratio was observed in patients with AR.[86,87] Furthermore, $T_{FH}2$ cells have been reported to be higher in patients with AR with and without asthma,[88] and in patients who developed food allergy following liver transplantation.[89] Patients allergic to HDM demonstrated higher levels of T_{FH} cells and attenuated T_{FR}-cell proportion compared with healthy controls.[86,90] The T_{FH} cells from patients with allergy to HDM have enhanced capacity to induce IgE production compared with healthy controls,[88] whereas the T_{FR} cells have diminished suppressive capacity,[86] further highlighting the role of T_{FH} and T_{FR} cells in the development of allergic diseases. A recent study assessed the functional role of circulating T follicular helper (cT_{FH}) cells after grass pollen SCIT and SLIT.[91] The authors evaluated cT_{FH} and cT_{FR} in patients with allergy to grass pollen and nonatopic controls by flow cytometry and mathematical algorithms developed to manage high-dimensional data. cT_{FH} cells were defined as a distinct subset of T cells from T_H2 and T_H2A cells, efficient in secreting both IL-4 and IL-21. cT_{FH} cells were elevated in patients with allergy to grass pollen compared with nonatopic controls and were lower following both SCIT and SLIT. In contrast, cT_{FR} and IL-10^+ cT_{FH} cells were induced following SCIT and SLIT. Moreover, ATAC-seq analyses revealed differentially accessible chromatin regions in all patient groups[91] (Fig 3).

T-cell exhaustion in AIT. Patients undergoing AIT receive either SCIT or SLIT of increasing dose of allergen extracts for a period of 3 to 5 years to induce immunologic tolerance. The exposure to the allergen during AIT is substantially higher than natural exposure to the allergen. For example, patients would be exposed to a cumulative dosage of 5.4 μg of soluble Phl p 5 during the timothy pollen season.[92,93] However, when they receive AIT, the soluble Phl p 5 dose is 5 to 20 μg/injection for subcutaneous AIT and 15 to 25 μg daily for sublingual AIT.[2,94] Chronic stimulation with persistent antigen/allergens is the precondition of T-cell exhaustion.

Few studies have explored the effect of AIT on T cell exhaustion. However, suppressive mediators (such as IL-10 and TGF-β), which are reportedly induced following AIT, have been demonstrated to facilitate T-cell exhaustion.[95,96] In a study involving patients with Japanese cedar pollinosis, the proportion of PD-1–expressing $CD4^+$ T cells, but not $CD8^+$ T cells or $CD19^+$ B cells, was found to be increased following AIT treatment.[97] Another AIT study that interrogated the transcriptional profile of $CD4^+$ T cells following the treatment demonstrated that the regulatory $CD4^+$ T-cell populations express MAF transcription factor, nuclear factor, IL-3 regulated, and costimulatory molecules lymphocyte-activation gene 3 (LAG-3), T-cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), PD-1, and T cell immunoglobulin and mucin-domain containing-3 (TIM-3).[98] In a recent study, allergen-specific $CD4^+$ T cells showed strong upregulation of PD-1, LAG-3, and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) on *in vitro* stimulation with the sensitizing allergen. Blocking antibody-targeting PD-1 enhanced proliferation and cytokine production (IL-10, IFN-γ, TNF-α, IL-5, and IL-13) of the allergen-specific $CD4^+$ T cells in both allergic and nonallergic individuals. Meanwhile, blocking LAG-3 did not affect T-cell response, whereas blocking CTLA4 reduced proliferation and cytokine production.[99] This highlights the unique role of the PD-1 pathway in dampening allergen-specific human T cells.[99] Finally, a study revealed that AIT resulted in the deletion of allergen-specific $CD4^+CD27^-$ T cells.[100] The authors speculated that T-cell exhaustion might be induced in terminally differentiated $CD27^-$ T_H2 cells if exposure to a high dose of allergen persisted following the desensitization phase.[101] In a very recent study by Wang et al,[102] CTLA4 and PD-1 were identified to be increased on T_H2 cells following allergen exposure in patients with AR and even more so in those with asthma. The study also revealed late-differentiated T_H2 population expressing both exhaustion markers was reduced during the updosing of AIT but persisted long-term during the maintenance phase of treatment.[102] Although further studies are warranted to confirm T-cell exhaustion in AIT, these recent findings may explain the need for long duration of AIT treatment to effectively reduce symptoms.

Induction of natural Treg and inducible Treg cells. Treg cells are considered a critical subpopulation of T cells that can suppress effector T-cell responses and other immune cells such as DCs and B cells. Treg cells can be broadly categorized into 2 main populations: thymus-derived natural Treg (nTreg) cells and periphery-derived inducible Treg (iTreg) cells. Both have comparable suppressive function and similar phenotype, which includes low expression of IL-7R (CD127) and high expression of CD25. nTreg cells are classically identified as cells that express their master transcription regulator FoxP3, whereas iTreg-cell (such as type-1 regulatory T [T_R1] and IL-35–induced regulatory T [iT_R35]) subsets are characterized by other markers that include CD49b and LAG-3 in T_R1 cells and CCR7 in iT_R35 cells.[103] T_R1 cells are known to have the capacity to produce IL-10, TGF-β, and granzyme B, whereas iT_R35 cells predominantly secrete IL-35.

The induction of nTreg and iT_{reg} cell subsets has been associated with immune tolerance following immunotherapy treatment in patients with allergic diseases.[104] Previous studies have demonstrated higher proportions of $FoxP3^+$ and $CD25^+FoxP3^+$ cells in the nasal mucosa in SCIT-treated groups compared with untreated patients with grass pollen allergy. Moreover, the same study reported a seasonal increase in $FoxP3^+$ and $CD25^+FoxP3^+$ cells in the SCIT-treated group but not placebo.[105] Another study demonstrated higher FoxP3-expressing cells in the sublingual mucosal biopsies following SLIT compared with placebo.[106] Epigenetic changes in the Treg-cell population were previously observed in patients with grass and HDM allergy and following SLIT treatment. The study showed induction of $CD25^{hi}CD127^{lo}FoxP3^+$ Treg cells with suppressive capacity following SCIT, but not placebo.[107] Furthermore, a reduction in the CpG methylation within the *forkhead box P3* regions was observed following SLIT

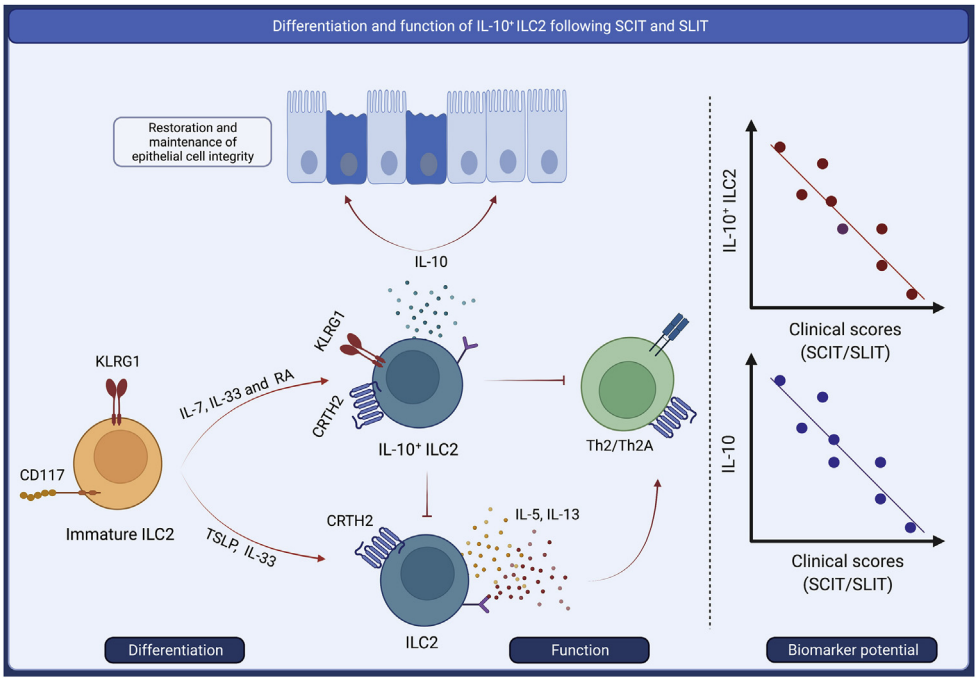

FIG 3. Differentiation and function of IL-10–producing ILC2s. Immature ILC2s can differentiate into a proallergic ILC2 or IL-10$^+$ ILC2. Natural or allergen-induced tolerance through SCIT or SLIT induces the induction of IL-10$^+$ILC2s with modulatory characteristics. IL-10$^+$ILC2s can restore epithelial cell integrity and suppress proallergic T_H2 responses. Their induction following SCIT and SLIT is associated with a reduction in clinical symptoms. IL-10–producing ILC2s contribute to immune tolerance induction to aeroallergens. *CRTH2,* Chemoattractant receptor-homologous molecule expressed on T_H2 cells; *KLRG1,* killer cell lectin like receptor G1; *RA,* retinoic acid; *TSLP,* thymic stromal lymphopoietin.

treatment, which was accompanied by an increase in the FoxP3 transcript.[107] *In situ* hybridization study of nasal mucosal biopsies following 2 years of AIT demonstrated an increase in the frequency of IL-10 mRNA$^+$ cells, which correlated with the frequency of IL-10$^+$ cells.[108] A 2-year SCIT treatment was shown to have higher TGF-β mRNA$^+$ cells in nasal biopsies compared with placebo during the pollen season, which positively correlates to the levels of serum IgA_2.[109] Similarly, the HDM SLIT study reported an induction of FoxP3$^+$ Treg cells that have the capacity to suppress the proliferation of T-effector cells and cytokine production.[110] A time-course SCIT study reported the early induction of IL-10 that paralleled suppression of late-phase responses in the skin that was observed at 2 to 4 weeks. Following this, induction of serum-blocking IgG_4 and IgA antibodies was observed at 6 to 12 weeks and associated with a reduction in basophil histamine release and IgE-facilitated allergen binding to B cells.[111]

Regulatory B-cell responses following AIT

Regulatory B (Breg) cells have been shown to exert a negative immuneregulatory role. They are known to induce their tolerance properties through the production of different cytokines such as IL-10, IL-35, and TGF-β and through cell contact–dependent suppressive mechanisms.[112,113] Immune activation is required for Breg cells to exhibit their suppressive functions, and these signals include the activation of Toll-like receptor (TLR) signaling (ie, TLR2, TLR4, and TLR9), B-cell receptor signaling, and costimulation mediated by CD40/CD40 ligand and CD80/CD86. Moreover, Breg cells can be activated by proinflammatory cytokines such as IL-1β, IL-2, IL-6, IL-21, IL-35, and IFN-α/β via the STAT3 pathway. Breg cells can also be induced by anti-inflammatory cytokines IL-35 through IL-12Rβ2 and IL-27Rα.[114,115]

Breg cells have been found to be involved in the induction and maintenance of allergen tolerance and hence, it is one of the key underlying mechanisms of AIT. Breg cell–mediated allergen tolerance include suppression of T-effector cells (ie, T_H2 responses) by IL-10, induction of Treg cells, inhibition of DC maturation, modulation of T_{FH} cells as well as production of specific IgG_4-blocking antibodies.[116,117] Studies have shown that grass pollen[118,119] and HDM[120] AIT result in elevated frequencies of IgA- and IgG_4-expressing Der p 1–specific B cells, plasmablasts, and IL-10$^+$ Breg cells, which significantly correlated with improvement of clinical symptoms throughout AIT. These roles of Breg cells are critical in AIT and therefore allow new windows for the development of targeted therapies.

Immunoglobulin responses following SCIT and SLIT

A successful AIT involves the modulation of cellular and humoral responses. Although an initial rise in IgE levels is often observed following SCIT and SLIT, patients remain asymptomatic on exposure to the sensitizing allergens.[121] Furthermore, studies have reported that the level of IgE was reduced following long-term SCIT treatment.[122] This is accompanied by an induction of allergen-specific IgG, predominantly IgG_4, and IgA with blocking activity.[106,123] These antibodies have allergen-neutralizing capacity and can compete with IgE for the allergen,

resulting in reduced formation of allergen-IgE complexes and the subsequent allergen presentation to T cells.[124] In 1935, Robert Cooke et al first reported that the passive transfer of serum from SCIT-treated patients was able to suppress ragweed sensitivity in another.[125] Then onwards, numerous studies have been performed and shown induction of IgG_4 following SCIT and SLIT.[126-128] Two years of grass pollen immunotherapy resulted in the blunting of seasonal induction allergen-specific IgE and induction of blocking IgG and IgG_4 antibodies, which correlated with clinical improvement.[108] Similarly, a study on HDM SLIT reported an increased Der p 2–specific IgG_4 following SLIT but not placebo.[110] These IgG_4 antibodies have been shown to be induced according to the duration and dose of the immunotherapy.[123] IgG_4 antibodies consist of a Fab-arm exchange that contributes to its bispecific nature with 2 distinct antigen-binding sites.[129] In AIT, the induction of IgG, IgG_4, and IgA with blocking activity has been well-described.[106,123,128] These blocking antibodies compete with IgE for allergen binding, thus inhibiting the formation of allergen-IgE complexes. This subsequently hinders the cross-linking of FcεRI on mast cells and basophils, and therefore their degranulation and histamine release. Moreover, the binding of allergen-IgE complexes to low-affinity FcεRII on B cells is inhibited, preventing IgE-facilitated antigen presentation to T cells, thus impeding IgE-driven T_H2-cell responses,[43,45,130] which can be evaluated using the IgE-facilitated allergen-binding assay.[131] The IgE-facilitated allergen-binding assay demonstrated that IgG_1 and IgG_4-blocking activity following SCIT was time- and dose-dependent, which peaked between 3 and 6 months following administration of SCIT.[106,123] The blocking activity of IgG antibodies persists for at least 2 years following cessation of SCIT treatment, despite a reduction in the IgG levels.[132] Similarly, SLIT induced IgG_4 antibodies with blocking activity as assessed by IgE-FAB, which persisted for at least 1 year following discontinuation of a 3-year treatment period.[33] This, therefore, suggests the induction of blocking antibodies-secreting memory B cells, which leads to the long-term immune tolerance following discontinuation of SCIT and SLIT. It is noteworthy that findings from the GRASS trial (SCIT vs SLIT study) revealed that SCIT induced IgG-associated blocking antibodies while SLIT induced allergen-specific IgA.[38] More importantly, IgG, IgG_4, and $IgA_{1/2}$ and associated IgE-inhibitory activity following immunotherapy was observed in the nasal fluid. These antibodies induced in the local mucosa exhibited allergen-neutralizing capacity.

CONCLUSIONS

Ongoing mechanistic studies have allowed a more thorough understanding of the underlying mechanisms of tolerance induced by AIT. An efficacious AIT is associated with dampening of various proinflammatory responses within the innate (DC2 and ILC2) and adaptive (T_H2, T_H2A, and T_{FH} cells) compartment while inducing strong regulatory counterparts (DCregs, IL-10–producing ILCs, Treg and Breg cells). Although it is still unclear as to whether there are differences in the cellular and molecular mechanisms underlying SCIT and SLIT, early evidences reveal differential antibody induction in the 2 modes of AIT. More studies are needed to understand the underpinning molecular and epigenetic changes mediated by SCIT and SLIT on innate immune and adaptive immune compartment. The advent of novel pan-omic approach to understand molecular mechanisms will yield potential biomarkers of response and no response as well as novel therapeutic target to induce immune tolerance. This will ultimately lead toward uncovering of the full mechanisms of AIT, which will be instrumental in the development of a more targeted therapy for AR with and without asthma.

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