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Mechanisms of Subcutaneous and Sublingual Aeroallergen Immunotherapy: What Is New?

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KEYWORDS

• Allergy • Aeroallergen • Allergen immunotherapy
• Subcutaneous allergen immunotherapy • Sublingual allergen immunotherapy
• Treg cells • Breg cells • Innate lymphoid cells

KEY POINTS

- The two most widely used forms of allergen immunotherapy (AIT) for treatment of aeroallergen-induced allergic diseases are the subcutaneous and the sublingual AIT. Substantial developments in AIT took place in the last decade to overcome problems in standardization, efficacy, safety, long duration of treatment, and costs.
- However, accurate descriptions of AIT-responsive disease endotypes with well-defined biomarkers continues to be an unmet need.
- AIT uses general mechanisms of immune tolerance to allergens with changes in allergen-specific memory T- and B-cell responses, regulation of allergen-specific IgE and IgG production, and modification of mast cell and basophil activation thresholds or dendritic cell phenotypes.
- The main highlights of recent years are advances in the understanding of innate lymphoid cells (ILC) including involvement of type 2 ILCs in development of allergic airway inflammation, and contribution of type 3 ILCs to B_{reg} cell–mediated immune tolerance.
- There is a need for further investigation of AIT mechanisms and biomarkers to identify the best candidates for AIT, the long-term responders.

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INTRODUCTION

The two biggest success stories in allergy treatment are topical glucocorticosteroids and allergen immunotherapy (AIT). Glucocorticosteroids, the principal pharmaceuticals of allergy treatment, efficiently treat inflammation without curing the disease. AIT is the only therapeutic approach exerting profound effect on basic mechanisms of the disease, and is able to modify the disease course. Although glucocorticosteroid treatment also exerts some tolerance-inducing effects via its regulatory T (T_{reg}) cell–inducing capacity,[1,2] AIT is considered to be the only treatment option with the promise of healing and induction of long-lasting tolerance, persisting even after discontinuation of therapy. Glucocorticosteroid treatment is efficient, but not effective enough, whereas AIT is effective but not efficient enough.[3] Consequently, aiming for the ideal therapy to overcome the global burden of allergy, a disease-modifying treatment, such as AIT, is the best choice. Substantial progress has been made in the last decade to overcome problems in standardization, efficacy, safety, long treatment duration, patient adherence, and costs of AIT, and as a result, new guidelines have been implemented.[4–12] Here, we summarize some major advancements of the last years in the understanding of AIT mechanisms. This review is focused on recent findings and better understanding of the mechanisms of subcutaneous and sublingual aeroallergen immunotherapy (SCIT and SLIT, respectively).

Difficulties in the management of allergic disease originate from its heterogeneity in patient population. Phenotyping allergic diseases helps identify clinical and morphologic characteristics and unique responses to treatment. However, phenotypes do not seem to be directly linked to molecular mechanisms of the disease. Endotypes of allergy and asthma describe certain mechanisms of the subgroups of the diseases, such as metabolic, inflammatory, immunologic, and remodeling pathways involved in the pathogenesis.[13–15] The relationship of the endotypes to AIT responsiveness is unclear thus far. It is hoped that detailed description of allergy endotypes and identification of their biomarkers will enable more successful, endotype-driven AIT strategies in the future. In particular, AIT-responsive endotypes should be identified and biomarkers of these endotypes should be discovered and become available at the point of care. These biomarkers should define AIT-responsive patients as early as possible.

ROLE OF VARIOUS T CELLS IN ALLERGEN TOLERANCE AND IMMUNOTHERAPY

Regulatory T Cells

Immune tolerance to allergens implies changes in memory-type allergen-specific T- and B-cell responses, regulation of allergen-specific IgE and IgG production, and modification of mast cell and basophil activation thresholds or dendritic cell (DC) phenotypes (**Fig. 1**). Different levels of evidence from direct in vivo data and in vitro experiments demonstrate the role of allergen-specific T_{reg} cells in allergen tolerance in humans.

Strong in vivo evidence has been obtained by investigation of biopsies of affected tissue and skin late-phase responses. A decrease in T helper cell type 2 (T_H2) and eosinophils and a parallel increase in T_{reg} cells and their cytokines in the tissue is observed after AIT.[16] In the last two decades, the pivotal role of T_{reg} cells in induction and maintenance of immune tolerance has been demonstrated. This is further supported by the fact that adoptive transfer of T_{reg} cells, depending on the timing of administration, can play a role in the prevention or treatment of several T-cell-mediated diseases, including allergic airway inflammation and hyperresponsiveness,[17] a large spectrum of autoimmune diseases (eg, diabetes mellitus, experimental autoimmune encephalomyelitis, inflammatory bowel disease,

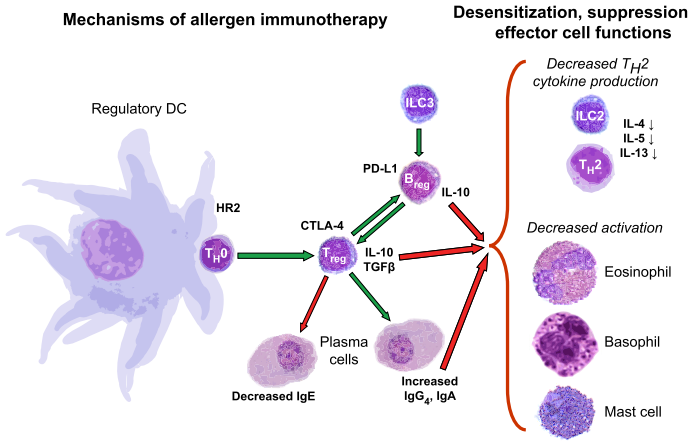

Fig. 1. Overview of the basic mechanisms of allergen immunotherapy. Regulatory T and B cells play a central role in the suppression of allergic inflammation, mainly by means of their interleukin-10 and transforming growth factor-β production. *Green arrows* indicate induction; *red arrows* indicate inhibition. B_{reg}, regulatory B cell; IL, interleukin; TGF, transforming growth factor.

systemic lupus erythematosus, rheumatoid arthritis), and graft-versus-host disease after allogeneic hematopoietic stem cell transplantation; and inhibit rejection of the graft after allogeneic solid organ transplantation in mouse models of these conditions. The human application of adoptive T_{reg} cell transfer therapy is currently being pursued in many studies.[18,19] In the clinical setting, SCIT and SLIT have been shown to induce allergen-specific T_{reg} cells in humans. Peripheral T-cell tolerance is characterized mainly by generation of allergen-specific T_{reg} cells and decrease of T_H2 cells.[20–24]

Direct ex vivo analysis of human peripheral blood cells without any in vitro culture also provided evidence to support the role of T_{reg} cells in maintenance of immune tolerance. Samples were collected from patients allergic to bee venom during AIT and from healthy beekeepers, who were not allergic to bee venom, on acquiring natural tolerance to high-dose allergen exposure during beekeeping season. Allergen-specific $CD4^+$ T cells, identified using major histocompatibility complex class II tetramers, and interleukin (IL)-4, interferon (IFN)- γ, and IL-10 cytokine-secreting cells have been analyzed in these studies. These data demonstrate that allergen-specific T_{reg} cells increase and allergen-specific and nonspecific T_H2 cells decrease in parallel with the induction of natural or clinical immune tolerance in beekeepers during beekeeping season and AIT-treated patients with allergy, respectively.[24,25] AIT also promotes allergen-specific IL-22 and IFN-γ producing T_H cells.[24,26] It is now well understood that allergen-specific T_{reg} cells and allergen-specific and nonspecific effector T cells contain several distinct phenotypic compartments.[27] AIT involves upregulation of the activated T_{reg} and downregulation of allergen-specific immunoglobulin-like transcript 3 (ILT3)-expressing, dysfunctional T_{reg} cells.[24] $ILT3^+$ T_{reg} cells were identified as dysfunctional T_{reg} cells, because they have impaired suppressive potency and they are unable to control the maturation of T_H2-inducing DCs.[28]

Additional evidence has been obtained from cell cultures. IL-10 and transforming growth factor-β are produced by antigen-specific T_{reg} cells. T_{reg} cells from donors

with atopy have a reduced capability to suppress T-cell proliferation. Immune tolerance induced by SLIT is associated with an increase in T_{reg} cell numbers; elevated IL-10 production in sublingual FOXP3-expressing T_{reg} cells; and elevated allergen-specific IgG_4, IgA levels and serum blocking activity for IgE-facilitated allergen binding (IgE-FAB) to B cells.[23,29,30] IL-10-producing T_{reg} cells suppress T_H2-type immune response and IL-17-producing T_H cells.[31,32]

It has been recently demonstrated that a significant increase in the numbers of allergen-specific $FOXP3^+Helios^+CD25^+CD127^-$ T_{reg} cells took place at the end of up-dosing phase (10 weeks), which only slightly decreased at the end of the maintenance phase (3 years) of house dust mite (HDM) SCIT.[24] In contrast, $ILT3^+$ dysfunctional T_{reg} cells[28] decreased substantially after 3 years of SCIT.[24] Additionally, $IL\text{-}10^+$ allergen-specific T_{reg} cells are still present at high frequency at the end of SCIT. Increased number of $FOXP3^+Helios^+$ and $IL\text{-}10^+$ T_{reg} cell and decreased $ILT3^+$ T_{reg} cell responses correlated with improved allergic symptoms. These data further confirm that the induction of allergen-specific functional T_{reg} cells is one of the key events of AIT.[24] In all of these mechanisms, the H_2 receptor plays an important role. It has been shown that it can suppress DCs, T cells, natural killer T cells, and basophils. This results in antagonizing the proallergic effects of histamine via the H_1 receptor.[33–35] Histamine signaling is a newly determined link between microbiome and immune tolerance. It has been recently shown that histamine-secreting commensal bacteria exist in human gut and are linked to severity of asthma[36] and have pleiotropic effects on tolerance in the lungs.[37,38]

Taken together, the critical role for allergen-specific T_{reg} cell–mediated immune tolerance in successful AIT is well established. However, this is not the only mechanism operational in the AIT-induced tolerance.

The equally important immunologic phenomenon that warrants the favorable clinical outcome of AIT is the eradication of allergen-specific T_H2 cells (**Fig. 2**). It has been recently demonstrated that there are subsets of allergen-specific T_H2 cells, such as $CD27^-CD45RB^-CRTH2^+CD49d^+CD161^+$ T_H2 memory cells, $CRTH2^+CCR4^+CD27^-CD4^+$ T_H2 cells,[32] or $ST2^+CD45RO^+CD4^+$ cells,[39] of special importance in the pathogenesis of allergic diseases. These so-called T_H2A cells or pathogenic T_H2 cells are specifically targeted during AIT, and their eradication is indicative of favorable clinical response.[3,39,40] These cells are characterized by high

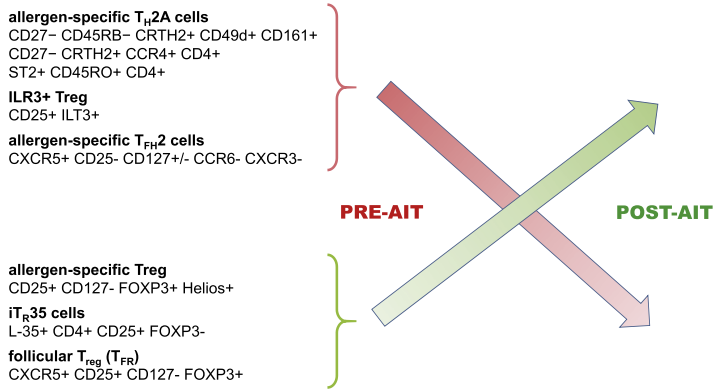

Fig. 2. Direction of change in some important T-cell subsets in response to AIT. Allergen-specific pathogenic T cells decrease and regulatory T cells increase during a successful AIT.

expression of *IL17RB* (gene encoding IL-25 receptor), *IL1RL1* (gene encoding ST2, a receptor for IL-33), and *CRLF2* (gene encoding TSLP receptor), and they highly express *IL13* and *IL5* genes.[40,41]

Interleukin-35

IL-35 is a newly identified inhibitory cytokine produced by T_{reg} cells and is essential for their immunosuppressive function.[42] IL-35-inducible regulatory T (iT_R35) cell is a newly recognized subset of induced T_{reg} cells with IL-35$^+$CD4$^+$CD25$^+$FOXP3$^-$ phenotype and potent immune regulatory properties.[43] The role of IL-35 and IL-35-producing iT_R35 cells in SLIT has recently been described (see **Fig. 2**). Proliferation and cytokine production of allergen-specific T_H2 effector cells, IL-25- or IL-33-induced IL-5 and IL-13 production of innate lymphoid cells (ILC) 2 cells, and CD40L-, IL-4-, and IL-21-mediated IgE production of B cells can all be inhibited by IL-35, whereas the production of IFN-γ and IL-10 is induced by this cytokine. Moreover, iT_R35 cells reduce memory T-cell proliferation and T_H2 cytokine production in an IL-10- and IL-35-dependent manner. Of note, the number of iT_R35 cells and allergen-induced IL-35 production is higher in the peripheral blood of patients with allergic rhinitis after successful SLIT than in the blood of untreated patients, and similar to that of healthy control subjects. Both the percentage and the IL-35-producing capacity of the iT_R35 cells inversely correlate with rhinitis symptom score.[44] Taken together, IL-35 and its source (iT_R35 cell) may represent a new target for biomarker development and for future therapies.[45]

Follicular Helper T Cells

Increasing evidence indicates that the regulation of follicular helper T (T_{FH}) cells is also of great importance in the success of AIT (see **Fig. 2**). Circulating T_{FH} cells are divided into subgroups corresponding to T_H1, T_H2, and T_H17 helper cells. IgE antibody production by B cells is supported by type 2 T_{FH} ($T_{FH}2$) cells.[46] $T_{FH}2$ cell numbers are increased in the peripheral blood of patients with allergic rhinitis, and are able to induce IgE production on an IL-4-dependent manner. Moreover, allergen-specific IgE levels correlate only with allergen-specific $T_{FH}2$ cell frequencies, but not with non-follicular T_H2 cells frequencies in patients allergic to HDM. The numbers of $T_{FH}2$ cells reduced more sharply than T_H2 cells in response to AIT. Of note, the combined symptom and medication score improvement in response to SCIT correlated only with the percent reduction of allergen-specific $T_{FH}2$ cell numbers, and not with that of T_H2 cells.[47] T_{FH} activities are modulated by follicular T_{reg} (T_{FR}) cells[48] and by regulatory B (B_{reg}) cells.[49] T_{FR} cells were first described in human tonsils and differentiate from natural T_{reg} cells.[50] T_{FR} cell frequencies are decreased in tonsils of patients allergic to HDM suffering from allergic rhinitis. Tonsillar and blood T_{FR} cell phenotypes and numbers correlate with each other. Circulating T_{FR} cells of patients with allergic rhinitis fail to suppress IgE production of B cells in vitro. Circulating T_{FR} cell frequencies are increased and their IgE-suppressing function is improved after AIT.[51] Therefore, therapeutic targeting of T_{FR} and T_{FH} cells and their use as a biomarker has been proposed.

INNATE LYMPHOID CELLS IN ALLERGY AND ALLERGEN IMMUNOTHERAPY

Exploration of the diversity of ILCs has substantially changed the understanding of tissue inflammation and immune tolerance in the last few years.[52] ILCs are developmentally related to natural killer cells. Novel members of the ILC family (ILC1s, ILC2s, and ILC3s) show similar biologic characteristics, including transcription factor expression and cytokine production, to the key cellular elements of adaptive immune response,

the T_H cell subpopulations: T_H1, T_H2, and T_H17/T_H22, respectively.[53] The whole genome signatures of different ILCs subpopulations and ILC heterogeneity in humans have recently been published.[54,55] Particularly, ILC2s play an important role in allergic airway inflammation.[56] They are dependent on GATA3, and they substantially contribute to IL-5 and IL-13 production in allergic airway responses.[57] ILC3s are RORγt-dependent cells. Their signature-cytokine is IL-22, an important mediator of the mucosal immune defense, tissue repair, and the maintenance of epithelial barrier integrity.[58]

It has recently been demonstrated that seasonal increase in peripheral ILC2s is inhibited by grass pollen extract SCIT.[59] Cross-sectional studies suggest that the relative proportion of circulating ILCs is affected by AIT. The ILC2/ILC1 ratio is increased in patients allergic to HDM suffering from allergic rhinitis[60]; however, it is normalized by a successful AIT. Nonresponders show similar ILC2/ILC1 ratio to untreated patients with allergic rhinitis.[61,62]

Our group demonstrated that IL-10^+ regulatory ILCs (ILC_{reg}s) are induced from ILC2s by retinoic acid.[63] CRTH-2 and ST2 (hallmarks of ILC2s) are downregulated and CD25 and CTLA-4 are upregulated on ILC_{reg}s. These cells are able to suppress T-cell and ILC2 activation. Retinoic acid–producing DCs were found to help peripheral T_{reg} cell differentiation.[64,65] Retinoic acid–induced regulatory DCs have a protective effect on adoptive transfer in a mouse model of food-induced anaphylaxis.[66] Considering the general role of retinoic acid in tolerance-induction, the participation of ILC_{reg}s in the mechanisms of AIT-induced tolerance is also possible.

Moreover, we demonstrated recently that activated, CD40L-expressing ILC3s reside on the border of the T cell–B cell areas in tonsils, and are in close contact with B cells in vivo. Furthermore, we showed that $CD40L^+$ ILC3s and B cells are in a mutually beneficial relationship with each other: ILC3s induce IL-15 production in B cells via BAFF-receptor, whereas IL-15, a potent growth factor for ILC3s, upregulates CD40L expression on ILC3s. IL-15-activated $CD40L^+$ ILC3s help B-cell survival, proliferation and differentiation of IL-10 secreting, and functional B_{reg} cells in a CD40L- and BAFF-receptor-dependent manner. Moreover, ILC3-induced B_{reg} cells dominantly have $CD27^-IgD^+IgM^+CD24^{high}$ $CD38^{high}$ $CD1d^+$ immature transitional (itB_{reg}) phenotype. This mechanism contributes to the maintenance of immune tolerance to innocuous antigens and becomes insufficient in allergic diseases.[67] Tonsils are important mucosal sites of immune tolerance,[68] where generation of functional allergen-specific T_{reg} cells occurs. We showed that ILC3s and B_{reg} cells colocalize in the interfollicular regions of palatine tonsils, together with T_{reg} cells. These data suggest that there are regulatory niches in tonsils where T_{reg} and B_{reg} cells develop next to each other. $CD40L^+$ ILC3s might be involved in maintenance of immune tolerance in tonsils through induction of functional itB_{reg} cells. These cells can contribute to suppression of T-cell responses through cell-cell contact via programmed cell death-ligand 1 and via secreted IL-10.[67] These mechanisms can also play a role in immune tolerance induction during successful SLIT (see **Fig. 1**).[35,69]

The significant reduction in total and allergen-specific IgE after 3 month of HDM SLIT was not affected by previous tonsillectomy; however, the mitigation of nasal symptoms and wheeze was less pronounced in the tonsillectomy group.[70] In fact, only the palatine tonsils and/or adenoids are removed or reduced during those operations, and the lingual tonsils remained untouched.[69] These data suggest that reduction of the lymphoid tissue mass in Waldeyer ring does not impede systemic tolerance induction by SLIT; however, it may delay the improvement of clinical symptoms. The small-scale study did not reveal reasons for this dichotomy.[70] Neither the cellular infiltration of the affected tissues by T_H2 cells and eosinophils, nor the local IgE levels (entopy) were examined, both of which certainly has an impact on the symptoms and may have been perpetuated in

tonsillectomized patients after SLIT. Larger scale, randomized, controlled studies with appropriate blinded design are needed to be able to appreciate the influence of tonsillectomy on AIT efficacy. In addition, it should be considered that lingual tonsil, which has the same structure and cellular composition as palatine tonsils, is a large lymphoid organ that remains intact in tonsillectomy patients. IgE-mediated allergy was found to be associated with substantially lower risk of complicated appendicitis in a cohort of children who underwent surgery for acute appendicitis.[71] However, the influence of appendectomy on development of allergy, and specifically on the response to immunotherapy, has not been investigated thus far.

REGULATORY B CELLS AND ANTIBODY PRODUCTION IN ALLERGEN TOLERANCE AND IMMUNOTHERAPY

Regulatory B Cells

Deficiency of IL-10-producing B_{reg} cells leads to increased inflammation in mouse models of autoimmunity, transplantation, and chronic infection. Absolute numbers of T_{reg} cells and expression levels of FOXP3 in these cells are dependent on IL-10 production by B cells. Modulation of T_{reg} cell frequencies in vivo is exclusively restricted to transitional 2 marginal zone precursor B_{reg} cells in mice.[72] Of note, itB_{reg} cells can prevent and reverse airway inflammation in allergized mice by inducing recruitment of T_{reg} cells to the lung.[73] Adoptive transfer of itB_{reg} cells can ameliorate allergic airway inflammation and hyperresponsiveness in a mouse model of allergic asthma in an IL-10-dependent manner.[74] Human itB_{reg} cells are also able to induce T_{reg} cells in vitro.[75] The relative proportion of B_{reg} cell subsets within B cells was found to be reduced in patients with allergic diseases, such as allergic rhinitis[76] and allergic asthma,[77] and the frequency of IL-10-producing, antigen-specific B cells increased after AIT.[78,79] In vivo evidence for allergen tolerance has been demonstrated: the suppressive B cells and IgG_4-expressing B cells are developed from IL-10^+ BR1 cells in human subjects after AIT.[80] Supporting the role of IL-10, solely IL-10-overexpressing B cells acquired an immunoregulatory profile comprising upregulation of suppressor of cytokine signaling 3 (SOCS3), glycoprotein A repetitions predominant (GARP), CD25, and programmed cell death-ligand 1.[81] These cells showed a significant reduction in levels of many proinflammatory cytokines, and augmented the production of anti-inflammatory IL-1 receptor antagonist and vascular endothelial growth factor. In addition, IL-10-overexpressing B cells secreted less IgE and showed a general T-cell and DC suppression.[81] Moreover, a novel mechanism of AIT-induced specific immune tolerance is suggested by our recent data. Increased numbers of IgA- and IgG_4-expressing allergen-specific memory B cells, plasmablasts, and IL-10^+ and/or IL-$1RA^+$ B_{reg} cells were found in the peripheral blood over a 2-year period during successful HDM SCIT (see **Fig. 1**).[82]

Modulation of Allergen-Specific IgE and IgG Responses During Allergen Immunotherapy

Specific IgE increases in individuals allergic to pollen during the pollen season and a successful AIT can prevent this increase. AIT itself also induces an early increase in IgE during the up-dosing phase, which rings out in maintenance phase in parallel with the increase in IgA and IgG_4 (see **Fig. 1**).[83]

AIT-induced IgG may be directed against the same epitopes as IgE, resulting in direct competition for allergen binding and a blocking effect.[84] Allergen-specific IgG antibodies can inhibit IgE-mediated basophil and mast cell activation and degranulation with two different mechanisms: allergen neutralization and binding to the inhibitory FcγRIIb. It has been demonstrated recently that although only high-affinity IgG

antibodies can have both of these effects, low-affinity IgG antibodies are still able to inhibit mast cell activation via FcγRIIb.[85] IgG_4 antibodies have several features that may play a role in their anti-inflammatory effects. Two arms of IgG_4 have the ability to separate and repair by dynamic Fab arm exchange, which leads to bispecific antibodies that are functionally monomeric.[86] Furthermore, IgG_4 does not fix complement and is capable of inhibiting immune-complex formation by other isotypes.[87] It is likely the decrease in IgE/IgG_4 ratio during AIT is a biomarker of skew from allergen-specific T_H2 to T_{reg} cell and B_{reg} cell predominance. IL-10 is a potent suppressor of total and allergen-specific IgE, whereas it simultaneously increases IgG_4 production.[21,88]

The characteristic changes in the level of IgE and IgG_4 during SCIT is mirrored by the local concentrations of those immunoglobulins in nasal secretions[45] and saliva.[89]

The results of some recent studies confirm that the IgG_4-mediated inhibition of CD23-dependent IgE-FAB,[90] rather than the serum concentration of IgG_4 solely, is a valuable biomarker of the AIT clinical outcome.[29,91] Other investigators evidenced that specific IgG_4 and inhibition of IgE-FAB correlates well with clinical efficacy of AIT.[92] Inhibition of IgE-FAB by nasal secretions correlates more closely with clinical outcome than that of serum samples after SCIT.[45]

Regulation of Mast Cells and Basophils by Allergen Immunotherapy

Clinical desensitization starts as early as after the first injection of SCIT. This is mainly linked to decreased mast cell and basophil degranulation, which has been demonstrated in a mouse model.[93] One of the main soluble factors released from mast cells and basophils following allergen challenge is histamine, which mediates its effects via histamine receptors. Immunosilencing of FcεRI-activated basophils by means of selective suppression mediated by H_2 receptor might be highly relevant for the early desensitization effect, at least in venom AIT.[94] Although there are individual differences and risks for developing systemic anaphylaxis, the suppression of mast cells and basophils is further increased during the course of AIT. Early reduction in basophil sensitivity predicts symptom relief also in grass pollen immunotherapy.[95] Furthermore, basophil expression of diamine oxidase shows a significant increase after AIT and is suggested as a novel biomarker of AIT responsiveness.[96]

OTHER ASPECTS OF IMMUNOTHERAPY

It has been demonstrated in mouse model of allergic asthma in vivo and human bronchial epithelial cell cultures in vitro that AIT contributes to the restoration of the airway epithelial barrier integrity by the reduction of IL-25 production. IL-25 is one of the main inducers of endoplasmic reticulum stress and tight junction damage.[97] It has been recently shown in a 5-year clinical study that grass pollen SLIT significantly reduces the risk of experiencing asthma symptoms and decreases the need for asthma medications.[98] The systematic meta-analyses of contribution of AIT in allergic asthma[99] and allergic rhinoconjunctivitis[100] have been recently published, supporting the previously mentioned study and confirming that AIT has positive effects on various allergic diseases. The effects of AIT for various phenotypes and endotypes of allergic diseases need to be further elucidated in vivo in animal models and directly in human tissue.[101,102]

SUMMARY

AIT offers an efficient treatment of allergic diseases with a possibility of cure. There is strong evidence for clinical safety and efficacy of AIT. Demonstration of allergen-specific T- and B-cell tolerance, mediated, among others, by the immunosuppressive

functions of IL-10, led to a major conceptual change in this area and opened a way to bring AIT from empiricism to a treatment with solid mechanisms of action linked to immune tolerance. The accurate definition of disease endotypes and a correct selection of the responder patients with well-defined biomarkers still remain essential unmet needs in the clinical setting. Therefore, deeper understanding of immuno-pharmaco-metabolo-genomics of allergy and the impact of the environmental exposure and the microbiome on it will probably help improve the AIT to accomplish vaccination strategies that are suitable for whole population-based allergy prevention and cure in the future.

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