

Novel developments in the mechanisms of immune tolerance to allergens

Thomas Eiwegger,^{1,2} Saskia Gruber,¹ Zsolt Szépfalusy¹ and Cezmi A. Akdis^{2,*}

¹Department of Pediatrics and Adolescent Medicine; Medical University of Vienna; Vienna, Austria; ²Swiss Institute of Allergy and Asthma Research (SIAF); University of Zurich; Davos, Switzerland

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Allergy is the result of a disbalanced immune response to environmental innocuous antigens. Despite of accumulating data to define the pathomechanisms that take place in case of allergic diseases a detailed understanding of sequence of events that lead to the “normal” scenario of tolerance development are still under debate. Allergen-specific immunotherapy is the only causal treatment of allergic diseases. It modifies the immune response to a particular antigen to achieve tolerance against the symptom-causing allergen. This process is considered to mirror physiological peripheral tolerance induction. A number of immunological changes have been described to occur under allergen immunotherapy, including the generation of allergen-specific regulatory T cells, the induction of allergen-specific IgG4, an increase in the Th1/Th2 cytokine ratio and decreased activation and function of effector cells such as mast cells, basophils and eosinophils.

Introduction

The prevalence of allergic disease has increased dramatically over the past decades, representing a serious health problem that causes considerable morbidity worldwide. The reasons why some individuals generate an inflammatory response toward non-pathogenic innocuous environmental antigens are not entirely understood. To date specific immunotherapy represents the only causal treatment of allergic disease via restoration of peripheral tolerance to the symptom causing allergen.

The concept of allergen-specific immunotherapy (SIT) was first introduced 100 years ago by Noon and Freeman.¹ Since then, a wide range of treatment modalities and recommendations has evolved. The main underlying principle is the application of gradual increasing doses of allergen via subcutaneous (SCIT), or the sublingual route (SLIT). New routes of application have been recently proposed such as the intralymphatic route (ILIT) or epicutaneous route. In contrast to other anti-allergic treatments, SIT is an immunomodulatory approach, which establishes long-term tolerance to the symptom causing allergen and restores the immune balance. The present review will focus on the known mechanisms that take place during SIT while allergen specific tolerance is established.

*Correspondence to: Cezmi A. Akdis; Email: akdisac@siaf.uzh.ch
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Pathomechanisms of Allergic Disease

The type of immune response elicited by allergen exposure depends on various factors, including individual genetic susceptibility, route and dose of application and co-exposure of other immune-stimulating antigens. In allergic individuals, first contact with allergen is suggested to lead to IL4- and IL13-dependent production of allergen-specific IgE, with subsequent binding of these antibodies to the high affinity receptor for IgE (FcεRI) on the surface of basophils and mast cells (“sensitization phase”).

Allergen re-exposure (“effector phase”) results in cross linkage of membrane bound IgE on mast cells and basophils and subsequent mediator release that induce typical immediate type hypersensitivity reactions. In addition, the release of cytokines like IL4, IL5, IL9 and IL13 by Th2 type cells that promote the recruitment of inflammatory cells to sites of inflammation, eosinophilia, mucus secretion and activation of eosinophils and mast cells take place. Aside from Th2 type cells different T-cells subsets evolve from naïve T-cell subsets into other T-cell subsets, depending on the microenvironment. Th1-cells secrete IFN-γ which induces apoptosis in concert with TNFα in epithelial cells, smooth muscle cells in the lungs of asthmatic patients and in keratinocytes of patients with atopic dermatitis²⁻⁴ and in activated Th1, but not Th2-cells. This may contribute to the limitation of inflammatory responses via elimination of cytokine and chemokine producing cells.⁵ In addition IFN-γ and TNF-α induces IL32. IL32 is upregulated in atopic skin lesions, in sinus tissue from CRS tissue with polyposis and correlates with disease severity in serum of patients with asthma.^{3,6-8} Recently a “new” Th2 type cytokine IL33 was described. It is able to act on precursor populations and dendritic cells that induce Th2 type responses and Th2 effector cell populations ultimately inducing Th2 type inflammation in the lung and the gut.^{3,9-11} In analogy to IL32, IL33 is released via epithelial damage.³

Antigen-Presenting Cells

Both SCIT and SLIT affect the regional antigen-presenting dendritic cells (DC) in local lymph nodes (SCIT) or directly at the application site (SLIT). DCs play a key role in defining the type of immune response. Whether they engage an immune triggering or regulatory function depends on their maturation stage. Both myeloid DCs (mDCs) and plasmacytoid dendritic cells (pDCs) are known to induce regulatory T-cells depending on their state

of activation. Moreover, the induction of new populations of Treg cells by DCs after stimulation with various other exogenous factors, such as pathogen-derived molecules, Vitamin D3 metabolites or retinoid acid has been described.¹²⁻¹⁴ In the lung, partially mature DCs were shown to produce IL-10 and thereby promote the generation of type 1 T-regulatory (Treg) (Tr1) cells upon respiratory antigen exposure.¹⁵ On the other hand maturing pDCs have the ability to generate T-regulatory cells and are able to suppress allergic inflammation in the lung upon adoptive transfer.^{16,17} Recently, tonsils have been linked to tolerance development that harbor functional allergen specific Treg populations, which are in close proximity to pDC populations and expand *in vivo*. Moreover, pDCs from palatine and lingual tonsils have the ability to generate functional CD4⁺CD25⁺CD127⁺FOXP3⁺ Treg cells from naïve, tonsil derived, T-cells.¹⁸ This mechanism may play a role in the immunological action of SLIT.

Mucosal Langerhans cells, a specialized subset of dendritic cells present in the mucosa, epidermis and lymph nodes are also suggested to have a distinct effect contributing to tolerance. The strategic location of Langerhans cells at the suprabasal epithelial layer may facilitate binding and processing of allergens in SLIT. Moreover oral Langerhans cells express the high affinity IgE receptor and seem to lack upregulation of CD83 and CCR7 upon allergen uptake.¹⁹ This in turn may leave them in a more immature state and reduce their ability to migrate to the lymph nodes. Thus, direct contact with T-cells in the tissue instead of conventional contact in the lymph nodes may take place.^{19,20} Taken together, oral Langerhans cells might also play a role in sublingual SIT by the above described mechanism of tolerance induction via FcεRI dependent allergen binding and production of IL-10 and TGF-β.²¹ However, *in vivo* data monitoring functional changes of local DC subsets at the side of application over time of SIT are missing.

Allergen-Specific Regulatory Cells

The generation of allergen-specific suppressor T cells in the immunomodulatory process induced by SIT was first described by Rocklin et al. in 1980²² and appears to be the most decisive step in the successful development of tolerance. CD4⁺CD25⁺ cells are a subgroup of T cells that inhibit polyclonal and antigenic activation of CD4⁺CD25⁻ cells *in vitro*.²³ These mechanisms are involved in the regulation of T-cell mediated immune responses, such as reactions to infectious agents, autoimmune diseases, allergic diseases and allograft rejections.²⁴

Various studies demonstrated the role of Treg cells in the prevention of allergic diseases. Adoptive transfer of Treg cells reversed asthmatic lung inflammation in a mouse model.²⁵ In humans, peripheral CD4⁺CD25⁺ Tregs from atopic donors seem to have a reduced capacity to suppress allergen-induced T-cell proliferation and cytokine production.²⁶ Also, CD4⁺CD25⁺ cells are reduced in number and function in the lesional skin of patients with atopic dermatitis.²⁷ A negative correlation of peripheral CD4⁺FoxP3⁺ number to IgE levels and eosinophilia was demonstrated in symptomatic atopic individuals.²⁸ On the other hand, children who outgrow their cow's milk allergy

have a higher frequency of circulating CD4⁺CD25⁺ T cells and a decreased allergen-induced proliferative T cell response compared with children that remain allergic.²⁹

Basically, Treg cells can be divided into two main subsets, naturally occurring and inducible Tregs. Naturally occurring forkhead box P3 (FoxP3) expressing CD4⁺CD25⁺ regulatory T cells, which develop in the thymus by positive selection. The transcription factor FOXP3 is associated with the suppressive phenotype and is known to be a master regulator for Treg cell differentiation and function.²⁴

Inducible regulatory T cells are generated in the periphery in several tolerogenic settings. There are various different subsets, of which IL10-producing T regulatory type 1 (Tr1) cells play the most important role in maintenance of tolerance toward non-pathogenic environmental antigens, such as house dust mite, cat dander or birch pollens, in healthy individuals.³⁰ Moreover, they are decisive for the development of tolerance to allergens under SIT. Tr1 cells operate by multiple suppressive mechanisms, including the soluble mediators IL-10 and TGF-β and the surface molecules cytotoxic T lymphocyte antigen 4 (CTLA-4), glucocorticoid-induced TNF receptor and programmed cell death1.^{24,31}

Both subsets, thymic selected CD4⁺CD25⁺Tregs and inducible Tr1 cells, seem to play together in the development of tolerance under subcutaneous and sublingual SIT.³²⁻³⁴ Studies demonstrated an increase of CD4⁺CD25⁺FOXP3⁺ and IL-10-producing Tr1 cells in nasal and oral mucosa and in the periphery during SIT as well as an association of CD4⁺CD25⁺ with clinical efficiency.³⁵⁻³⁸ Hypo-proliferation of PBMCs to allergens after SIT has been mainly attributed to the induction of T-regulatory cells.

Treg cells exert their beneficial effects in the prevention of allergen-specific immune response and in tolerance development under SIT via interference with several major pathways^{39,40} (Fig. 1). They antagonize the inflammatory properties of effector Th1 cells (epithelial cell activation and apoptosis), Th2 cells (epithelial inflammation, mucous secretion, mast cell, eosinophil and basophil activation, IgE production) and Th17 cells (epithelial inflammation, chronic neutrophilic inflammation).²⁶ Moreover, they contribute to the generation of IgG4 and the suppression of specific IgE,^{35,41} induce tolerogenic DCs, while suppressing inflammatory DCs^{42,43} and display, directly and indirectly, suppressive effects on mast cells, eosinophils, basophils⁴⁴ and resident tissue cells which mediate chronic tissue remodeling.^{25,45}

The key player in suppression of allergic reactions seems to be IL-10, produced by Tr1 as well as by DC and antigen-presenting B cells. IL-10 production is observed under venom immunotherapy *in vitro* and *in vivo*,^{35,46} under house dust mite immunotherapy,³³ under grass pollen immunotherapy⁴⁷ as well as in healthy non-atopic patients upon allergen stimulation.^{33,35} Thus, the increase of IL10 by SIT seems to restore a tolerance that is constitutionally present in healthy individuals.

IL-10 inhibits T cell activation by blocking the co-stimulatory molecules CD2, CD28 and inducible T cell costimulator (ICOS) through dephosphorylation by Src homology 2 domain-containing tyrosine phosphatase (SHP-1).^{32,48} In monocytes, it inhibits

cytokine synthesis and antigen-presenting capacity,⁴⁹ in mast cells, it decreases IgE-dependent degranulation.⁵⁰ Moreover, IL10 suppresses total and allergen-specific IgE while increasing IgG4 production.³⁵ The other cytokine produced by T-regulatory cells, TGF- β induces the conversion of CD4⁺CD25⁻ into CD4⁺CD25⁺ cells via the expression of FOXP3 and is necessary for the expansion and function of CD4⁺CD25⁺ Treg cells.⁵¹ In conclusion T-regulatory cells are key players in the network of events that are observed in case of tolerance induction during SIT.

B-cells are the central cell population to mount humoral immune responses. Aside from this function they induce a paramount of properties ranging from antigen presentation and initiation of immune responses to suppression of immune responses.⁵² B-cells express co-stimulatory molecules and consequently are able to induce allergen specific T-cell responses. In contrast to these immunostimulatory properties, B-cells produce significant amounts of IL-10. These, so called regulatory B-cells or Bregs, have the ability to suppress the development, expansion and cytokine production of effector T-cell responses⁵³ and monocyte cytokine production in an IL-10 dependent manner.⁵⁴ Several regulatory B-cell subsets have been described in humans (reviewed by Hussaarts et al.⁵⁵).

Regulation of Allergen-Specific IgE and IgG4

In contrast to T-cell tolerance, which occurs relatively at an early stage in the course of SIT, B-cell changes seem to develop at relatively later phase. Allergen-specific IgE transiently increases within the first weeks of SIT,⁵⁶ whereas later on changes seem to be modest. A mitigation of the season-associated rise of pollen-specific IgE was demonstrated in patients with seasonal rhinoconjunctivitis under SIT.⁵⁷ Very late in the course and after termination of SIT, some authors describe a decrease of allergen-specific IgE, occurring 1 to 3 years after implementation of therapy.^{58,59} The decline of specific IgE does not correlate with clinical improvement⁵⁷ and rather occurs at a later time point. Consequently, it is not an appropriate marker to assess SIT efficacy. The reason for the persistence of IgE despite clinical improvement may relate to long-lived bone-marrow-resident IgE-producing plasma cells.⁶⁰

Relatively early in SIT (weeks to months after start of treatment) an allergen dose dependent (10- to 100-fold) increase of IgG4^{58,61} is observed. This increase occurs relatively early and is more pronounced compared with the small rise in allergen-specific IgE.⁵⁶ Some studies also show an elevation of IgG1 and IgA.^{33,38} The mechanism of IgG4 in SIT as a 'blocking effect' was first described in 1982.⁶² Allergen-specific IgG4 is thought to bind the allergen before it is linked to cell-bound IgE and therefore competes with specific IgE. However, this theory has some drawbacks. IgG4 is a marker of applied allergen dose,⁵⁶ but studies show contradictory correlation of IgG4 with clinical improvement.^{63,64} Moreover, clinical effects of SIT seem to precede the increase of IgG, especially in rush protocols.^{65,66} On

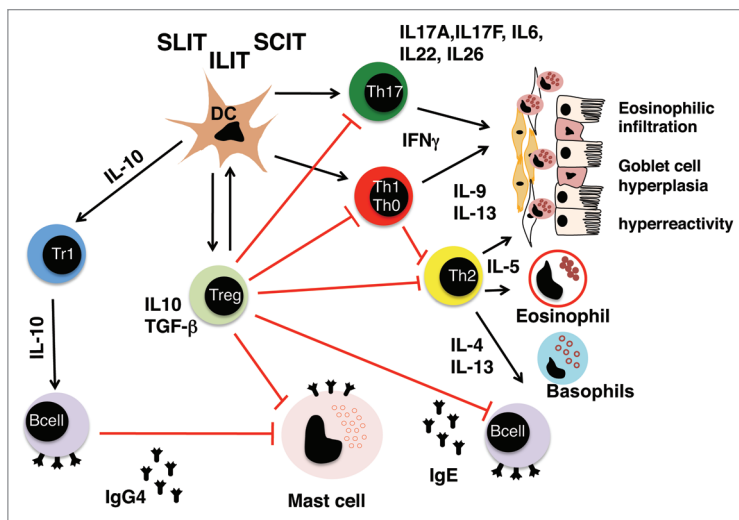


Figure 1. Mechanisms of allergen-specific immunotherapy: Subcutaneous and sublingual immunotherapy initially affects antigen-presenting cells at the side of administration and in the draining lymph nodes. This leads to the induction of Treg cells via so far not completely understood mechanisms. Treg cells in turn exert potent inhibitory effects by using multiple mechanisms. They antagonize the inflammatory properties of effector Th1 cells (epithelial cell activation and apoptosis), Th2 cells (epithelial inflammation, mucous secretion, mast cell, eosinophil and basophil activation, IgE production) and Th17 cells (epithelial inflammation, chronic neutrophilic inflammation). Moreover, they contribute to the generation of IgG4 and the suppression of specific IgE, induce tolerogenic DCs while suppressing inflammatory DCs and display, directly and indirectly, suppressive effects on mast cells, eosinophils, basophils and resident tissue cells mediating chronic tissue remodeling.

the other hand, changes in the fine specificity in the epitope-binding regions of the T cell receptor have been documented by some authors within the first hours of rush immunotherapy.⁶⁷ In addition to the increase of specific IgG4 levels, SIT increases the blocking activity of IgG on the allergen capture and cross-linking of mast cell and basophil Fc ϵ -receptor bound IgE.⁶⁸

Moreover, IgG4 does not activate the complement system, has a lower affinity for Fc γ receptors, interferes with basophil histamine release and reduces antigen-specific T cell proliferation by prevention of IgE-facilitated allergen binding to B cells and subsequent antigen-presentation.^{65,68} Formation of multivalent IgE-IgG-allergen complexes has been suggested to result in mast cell Fc ϵ RI autoinhibition. After binding of the allergen-IgG4 complex to the inhibitory Fc γ RIIb, this is phosphorylated by Fc ϵ RI-associated kinases. Phosphorylated Fc γ RIIb then mediates a reciprocal inhibition of Fc ϵ RI signaling.^{69,70}

The relevance of newly developing antigen-specific IgA in the development of allergen tolerance under SIT has to be elucidated. Allergen-specific secretory IgA was found to protect sensitized children from allergic symptoms in the early years of life, suggesting a possible protective role of IgA.

Several studies show that IgG4 is a better marker than IgE. However, instead of being an independent tolerance inducing factor, the increase of allergen-specific IgG4 during successful SIT might only be a surrogate marker for Treg activity since Tr1-produced IL-10 was shown to decrease total and allergen-specific

IgE and to increase IgG production.⁴¹ Moreover, it might be more important to measure the blocking activity via in vitro assays at an allergen-specific level.^{65,68}

Shift of the Th1 / Th2 Cytokine Ratio

The balance between distinct T-cell subsets such as Th1, Th2, Th9, Th17, Th22, Tr1, CD4⁺CD25^{high}FoxP3⁺ T cells, and their cytokines plays a pivotal role in directing the immune response to different kinds of antigens. The pro-allergenic T helper 2 (Th2) cells produce IL-4, IL-5 and IL-13, whereas the counter-regulatory Th1 cells produce IFN- γ , which are important in the defense against microbes.

Measurement of Th1/Th2 ratio in peripheral blood of patients undergoing SIT showed controversial results. Both, a shift toward Th1,^{71,72} as well as an unchanged Th1/Th2-ratio,⁷³⁻⁷⁵ was observed. However, cultures of PBMCs do not provide accurate insights into immune responses of affected organs and associated lymphoid tissue. Indeed, in tissue, results on the effect of SIT on cytokine expressions are more consistent. An increased Th1/Th2 ratio, with mostly unchanged Th2 but increased Th1 cytokines (IL-2, IFN- γ and IL-12) was demonstrated in skin biopsy upon intradermal challenge after grass pollen SIT,⁷⁶ in mucosal biopsy upon intranasal provocation after pollen SIT,⁷⁷ and in nasal biopsies and nasal fluids in response to natural pollen exposure in patients after pollen SIT.^{73,75} However, these studies demonstrated the change of whole T cells in the tissues and due to technical problems could not focus on allergen-specific T cell subsets.

The mechanism responsible for this shift is not yet defined. IL10 might again be involved through its inhibitory effect on CD28 co-stimulatory signals since IL-5, but not IFN- γ production upon allergic stimulation, seems to be CD28 dependent.^{78,79} However, cytokine changes might also occur unrelated to IL-10. For example, different DC cell subsets are known to elicit different T-helper cytokine responses according to their maturation state.³¹

Role of Allergen Specific Immunotherapy on Mast Cells, Basophils, Eosinophils and Neutrophils

Effects of SIT on inflammatory cells, including mast cells, basophils, eosinophils and neutrophils, have been described both at very early stages and in the late phase, occurring after a few months.

Specific immunotherapy represents the only causative treatment of allergic reactions and confers protection based on typical kinetics. Protection against systemic anaphylactic response to bee stings and inhibition of late phase reactions upon cutaneous provocation is observed already in the first hours of repetitive insect venom or drug rush desensitization protocols.⁶⁶ There is limited information on these early desensitization effects but gradual mediator release might be responsible for the early protection against anaphylaxis. Release of histamine and leukotrienes upon allergen challenge is not abolished during SIT, however, it does not induce a systemic anaphylactic reaction.⁸⁰ The proportion of released to total histamine was shown to be unchanged after

allergen stimulation during desensitization, however, there is a decreased absolute amount of histamine release. Thus, it seems that piecemeal degranulation with release of histamine and leukotrienes below the threshold of anaphylaxis protects from systemic reactions by decreasing the granule contents and increasing the threshold of activation of mast cells and basophils.^{80,81}

SIT reduces mast cell, basophil and neutrophil activation and IgE-mediated release of preformed and newly generated mediators by mast cells and basophils.⁸⁰⁻⁸³ Moreover, SIT inhibits the number and function of eosinophils,⁸⁴ mitigates eosinophil and neutrophil migration to the site of allergic reaction,⁸⁴ and reduces priming of eosinophil adhesion during allergen season.⁸⁵ IL-10 seems to play a key in the anti-inflammatory actions on tissue cells, as it was shown to reduce degranulation of mast cells⁸³ and a reduction of eosinophil chemotactic IL-5 production by Th2 cells.⁷⁹ Moreover, Treg cells were shown to directly inhibit Fc ϵ RI-dependent mast cell degranulation through cell to cell contact.⁴⁴

Recent data demonstrated that early mast cell and basophil suppression might be mediated via IgG binding to Fc γ RIIb on mast cells.⁸⁶ Changes in histamin receptor³¹ expression, enhanced tryptophan degradation and upregulation of human IgG receptor inhibitory transcript expression in monocytes are also discussed to occur at a very early stage of SIT.⁸⁷

In late stages of SIT, the effect of SIT on tissue cells and inflammatory response leads to a suppression of late phase response in nasal mucosa and of skin test reactivity.^{76,88} It was shown, that it also decreases mucosal response to non-specific stimuli, which demonstrates a general reduction of underlying mucosal inflammation.⁸⁹

Conclusions

Allergen-specific immunotherapy is the only available causative treatment of allergic diseases that induces a number of immunological changes at an allergen specific level. In brief, it leads to the generation of allergen-specific regulatory T-cells and blocking antibodies and thereby counter-regulates in a direct or indirect way pathogenic effector cell populations that contribute the clinical features of allergic diseases.

However, established allergy is extremely difficult to treat and even systemic immunosuppressive therapy does not affect clinically manifest allergy.⁹⁰⁻⁹² Despite of the benefits of SIT for most of the treated individuals, not everyone improves, and in some patients, changes are not permanent. Also, the duration of SIT is a controversial issue, with no consistent guideline. Therefore, reliable markers of successful and durable SIT are still a clinical unmet need.

On the other hand, therapeutic approaches that use new routes of application and increase the efficacy of antigen presentation leading to a decrease the number of allergen application are needed.^{93,94} Moreover future immunotherapeutic approaches may combine biological immune response modifiers with allergen-specific immunotherapy to induce long lasting immune tolerance.⁹⁵ Finally, there is also an eminent need for preventive strategies that favor tolerance induction early in life prior to sensitization or in sensitized individuals prior to allergy development.

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