Mechanisms of allergic diseases

Mechanisms of allergen-specific immunotherapy: Multiple suppressor factors at work in immune tolerance to allergens

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Allergen-specific immunotherapy (AIT) has been used for more than 100 years as a desensitizing therapy for IgE-mediated allergic diseases and represents a potentially curative way of treatment. The mechanisms of action of AIT include the induction of very early desensitization of mast cells and basophils; generation of regulatory T and regulatory B (Breg) cell responses; regulation of IgE and IgG4; decreases in numbers and activity of eosinophils and mast cells in mucosal allergic tissues; and decreases in the activity of basophils in circulation. Skewing of allergen-specific effector T and effector B cells to a regulatory phenotype appears as a key event in the course of AIT and normal immune response to allergens. Recently, inducible IL-10–secreting Breg cells were also demonstrated to contribute to allergen tolerance through suppression of effector T cells and selective induction of IgG4 isotype antibodies. Allergen-specific regulatory T and Breg cells orchestrate a general immunoregulatory activity, which can be summarized as suppression of cytokines from inflammatory dendritic cells; suppression of effector Th1, Th2, and Th17 cells; suppression of allergen-specific IgE and induction of IgG4; and suppression of migration of mast cells, basophils, eosinophils, and effector T cells to tissues. A detailed knowledge of the mechanisms of AIT is not only important in designing the prevention and treatment of allergic diseases but might also find applications in the treatment of autoimmune diseases, organ transplantation, chronic infection, and cancer. (J Allergy Clin Immunol 2014;133:621-31)

Key words: Regulatory T cells, immunotherapy, IgE, T cells, IL-10, TGF-β, allergen immunotherapy, T helper cells, immune tolerance, IgE, IgG, T cells, B cells, mast cells, basophils, eosinophils

Allergen-specific immunotherapy (AIT) is effective in reducing symptoms of allergic asthma and rhinitis, as well as venom-induced anaphylaxis. A key feature of AIT is to change the course of disease by altering the underlying pathology. Currently, 2 types of AIT are in clinical practice, subcutaneous immunotherapy and sublingual immunotherapy (SLIT), and several novel AIT approaches are being evaluated in clinical trials.1,2 There is moderate-level evidence for the efficacy of specific immunotherapy against atopic dermatitis3 and SLIT for the treatment of allergic rhinitis and asthma provided by recent meta-analyses.4 Dysregulated immune function plays an essential role in many IgE-mediated diseases, including asthma, atopic dermatitis, allergic rhinitis, food allergy, and venom allergy, as well as autoimmune diseases, organ transplantation, tumors, chronic infections, and successful pregnancy.5-8 Multiple mechanisms of immune regulation take place depending on the type, place, intensity, and chronicity of the immune response, as well as antigens/allergens, adjuvants, cytokines, or small molecules in the micromilieu. In addition, the type of tissue response plays an essential role in the thresholds for inflammation versus tolerance.

The physiopathology of allergic diseases is complex and influenced by many factors, including genetic susceptibility, route of exposure, antigen/allergen dose, time of exposure, structural characteristics of the allergen/antigen, and coexposure with stimulators of innate immune response, such as infections or commensal bacteria. Allergens enter the body through the respiratory tract, gut, conjunctiva, injured skin, or insect stings, and most of the time, the result is induction of tolerance as a natural mechanism.5-8 Immune tolerance to allergens is characterized by establishment of long-term clinical tolerance.9,10 The mechanisms by which allergen tolerance is established in human subjects have been studied through various modes of AIT, as have the processes by which a healthy immune response develops during high dose of allergen exposure in beekeepers and cat owners.10,11-13 Although many mechanisms are not fully elucidated, they include changes in the characteristics of allergen-specific memory T- and B-cell responses and the production of specific antibody isotypes to skew the immune response toward no inflammation, as well as decreased activation, tissue migration, and mediator release of mast cells, basophils, and eosinophils. After the discovery of Th1 and Th2 cell subsets in 1986, during the last 27 years, it is well understood that there is
T (Treg) cells play a major role in the suppression of effector mechanisms. Although there are several clinically relevant allergic diseases are complex disorders with several disease mechanisms, such as those seen in patients with psoriasis, and produced by keratinocytes and monocytes. IL-22 is produced by activated T cells, and increases the production of IL-17 and TNF-α production and are involved in autoimmune diseases, such as inflammatory bowel disease. IL-23 increases IL-17 production and activates the transcription factor signal transducer and activator of transcription 3 to maintain a T17 phenotype of CD4+ T cells. IL-17 in turn induces IL-1β and IL-6.

MECHANISMS OF AIT

Cellular and molecular events that take place during the course of AIT can be classified into 4 groups (Fig 1). Although there is significant variation between donors and protocols, decreases in mast cell and basophil activity and degranulation and the tendency for systemic anaphylaxis start to take place within hours when natural allergens are used. The second group of events are generation of allergen-specific Treg and regulatory B (Breg) cells and suppression of allergen-specific effector T-cell subsets. The third group of events include regulation of antibody isotypes demonstrating an early increase in specific IgE levels, which later decrease, and an early and continuous increase in specific IgG4.

phenotypes for rhinitis, asthma, atopic dermatitis, and even urticaria, these phenotypes do not necessarily provide any insight into the pathomechanisms that underpin the diseases. An important unmet need in patients with AIT is the identification and validation of biomarkers that are predictive of clinical response. It is now thought that some clinical trials might have been unsuccessful in the past because they were performed without attempting to classify patients with AIT into subgroups that are defined by a distinct pathophysiology, namely endotypes. It seems essential to select AIT responder cases from the big pool of patients with asthma, allergic rhinitis, and even atopic dermatitis. The definition of an AIT-responsive endotype of allergic diseases and relevant biomarkers is urgently needed for patient selection and maybe also even for the selection of the type of vaccine or route of application.

GLOSSARY

CYTOTOXIC T LYMPHOCYTE ANTIGEN 4 (CTLA-4): Also known as CD152, CTLA-4 is expressed on activated T cells, is a member of the immunoglobulin superfamily, and contains an immunoreceptor tyrosine-based inhibition motif. CTLA-4 binds to B7 and limits T-cell activation. CTLA-4-deficient mice have lymphoproliferative disease. ENDOTYPES: A definition of a disease subtype that is defined by the underlying pathobiology, as opposed to a phenotype, which is defined by the clinical characteristics. An example of an asthmatic endotype would be aspirin-exacerbated respiratory disease. GM-CSF: GM-CSF stimulates stem cells to produce granulocytes and monocytes. IgG4: IgG4 has been associated with the development of immune tolerance to antigens, including foods, and the ratio of specific IgE to IgG4 might be useful in the context of desensitization. IgG4 does not bind complement and blocks IgE binding to allergens. IL-5: IL-5 promotes the survival, activation, and chemotaxis of eosinophils. Its receptor shares a common β chain with the IL-3 receptor. IL-6: IL-6 is released by dendritic cells, primes for Th2 effector cells, and inhibits the suppressive functions of CD4+ CD25+ Treg cells. IL-19, IL-20, IL-22, IL-24, IL-26: All are members of the IL-10 family. IL-19 is produced by B cells and monocytes in response to GM-CSF and increases the production of IL-4 and IL-13. IL-20 is involved in cutaneous inflammation, such as that seen in patients with psoriasis, and produced by keratinocytes and monocytes. IL-22 is produced by activated T cells, as well as mast cells, and largely targets hepatocytes to induce acute-phase reactants. IL-24 is produced by monocytes, macrophages, and TH2 cells. It controls cell survival and proliferation through signal transducer and activator of transcription (STAT) 1 and STAT3. IL-24 plays important roles in wound healing, psoriasis, and cancer. IL-26 is expressed in certain herpesvirus-transformed T cells but not in primary stimulated T cells. IL-26 signals through IL-20 receptor 1 and IL-10 receptor 2.

PROGRAMMED DEATH 1 (PD-1): A member of the CD28 family, PD-1 binds to its ligands, PD-L1 and PD-L2, to limit immune response development. PD-1 blockade with an mAb has recently been used in patients with B-cell lymphoma.

RUNT-RELATED TRANSCRIPTION FACTOR (RUNX): A family of transcription factors that cause epigenetic changes for gene silencing or activation. For example, Runx3 inhibits IL-4 production in conjunction with T-box transcription factor (T-bet) in TH2 cells and increases IFN-γ production in TH1 cells.

TOLL-LIKE RECEPTOR (TLR): Essential members of the innate immune system, TLRs are pattern recognition receptors that bind both endogenous and exogenous ligands. TLR4 binds LPS from gram-negative bacteria, heat shock protein 6, and respiratory syncytial virus protein F. TLR7 and TLR8 bind single-stranded RNA and are important for antiviral defense, whereas TLR3 binds double-stranded RNA. TLR9 binds Cpg.

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levels. The fourth group of events take place after several months, with decreases in tissue mast cells and eosinophils and release of their mediators. It is accompanied by a decrease in type I skin test reactivity. Multiple cell types in the blood and affected organs show changes and contribute to allergen-specific immune tolerance development (Table I). All of these events are discussed below, with a special focus on Treg and Breg cells and their suppressive functions. AIT represents one of the most forward promising areas for better understanding of antigen-specific immune responses and immune tolerance development in human subjects. However, there still remains much to be investigated (see Box 1).

RAPID DESENSITIZATION OF MAST CELLS AND BASOPHILS BY ALLERGENS

Several mechanisms have been proposed to explain why mast cells and basophils become unresponsive to environmental proteins, even in the presence of specific IgE. Notably, after the first injection of AIT, very early decreases in the susceptibility of mast cells and basophils to degranulation and in systemic anaphylaxis can be observed, even though all the treated subjects have high quantities of specific IgE. This effect occurs when 3-dimensional structure-intact allergens are used. Although the underlying molecular pathways remain to be elucidated, this effect seems similar to the one observed when these 2 immune cell types are rapidly desensitized during anaphylactic reactions to drugs. Anaphylaxis is associated with the release of inflammatory mediators from both mast cells and basophils, and successful hyposensitization alters the magnitude of mediator release. The release of these inflammatory mediators in low quantities, less than the required dose for systemic anaphylaxis, might affect the subsequent threshold of activation of mast cells and basophils.

The investigation of histamine receptor (HR) expression on basophils of patients undergoing venom immunotherapy (VIT) demonstrated that selective suppression of basophils mediated by H2R might be highly relevant for the very early induction of allergen tolerance and the so-called desensitization effect of VIT. Rapid upregulation of H2R within the first 6 hours of the build-up phase of VIT was observed. H2R strongly suppressed FceRI-induced activation and mediator release of basophils, including histamine and sulfidoleukotrienes, as well as cytokine production in vitro.

TREG AND BREG CELLS IN AIT

It is now generally appreciated that peripheral T-cell tolerance is essential for a normal immune response and successful immunotherapy of allergic disorders (Fig 2). Although multiple factors contribute, the tolerant state of specific cells essentially results from increased IL-10 secretion. Suppressor capacity for


**TABLE I. Roles of different cells in the development of allergen tolerance**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Role in Allergen Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>Decreased allergen-induced proliferation (PBMCs)</td>
</tr>
<tr>
<td></td>
<td>Increased FOXP3 expression (PBMCs and T cells)</td>
</tr>
<tr>
<td></td>
<td>Suppression of T&lt;sub&gt;1&lt;/sub&gt; cells and cytokines (PBMCs)</td>
</tr>
<tr>
<td></td>
<td>Increased FOXP3 expression (nasal biopsies in allergic rhinitis)</td>
</tr>
</tbody>
</table>

**Box 1. What is unknown in the mechanisms of AIT**

- Molecular mechanisms of Treg and Breg cell generation *in vivo*
- Adjuvants that promote Treg and Breg cells *in vivo*
- Lifespan of AIT-induced Treg and Breg cells *in vivo*
- Relationship of resident tissue cells with AIT-induced immune tolerance
- Early biomarkers and predictors for the success of immunotherapy
- Local events in the microenvironment during SLIT
- Identifying the optimal allergen dose and mechanisms of high-dose and low-dose AIT
- Mechanisms of long-term maintenance of allergen tolerance
- Is boosting needed for long-term effect? What should be the optimum time?
- Mechanisms of inducing high-affinity IgG<sub>4</sub> and low-affinity IgE antibodies

Allergen/antigen-stimulated T cells is particularly confined to IL-10 but not its other family members, such as IL-19, IL-20, IL-22, IL-24, and IL-26. IL-10 particularly originates from activated and antigen-specific Treg and Breg cell populations and increases during AIT and natural allergen exposure. High IL-10–producing Treg and Breg cell subsets are called IL-10–secreting regulatory T (T<sub>R1</sub>) cells and IL-10–secreting regulatory B (B<sub>R1</sub>) cells, respectively. Allergen-specific CD4<sup>+</sup> T cells that predominantly produce IFN-γ, IL-4, and IL-10 represent T<sub>R1</sub>1-, T<sub>R1</sub>2-, and T<sub>R1</sub>3-like cells, respectively. Healthy and allergic subjects exhibit all 3 subsets, although in different proportions. In healthy subjects IL-10–secreting T<sub>R1</sub>1 or IL-10–Treg cells are the dominant subset against common environmental allergens, whereas in allergic subjects allergen-specific IL-4–secreting T cells (T<sub>R1</sub>2-like) exist in high frequency. Therefore a change in the dominant subset might lead to either the development of allergy or its reversal. Peripheral tolerance to allergens involves multiple suppressive factors, such as IL-10, TGF-β, cytotoxic T lymphocyte antigen 4 (CTLA-4), and programmed death 1 (PD-1).

Similar to T<sub>R1</sub> cells, B cells can be classified into subsets according to the cytokines that they produce. One functional B-cell subset, Breg cells, has recently been shown to contribute to the maintenance of the fine equilibrium required for tolerance. Breg cells control excessive inflammatory responses through IL-10, which inhibits proinflammatory cytokines and supports Treg cell differentiation. As observed in T<sub>R1</sub> cells, recently, highly purified IL-10–secreting Breg cells (B<sub>R1</sub>) cells were phenotypically and functionally characterized. B cells specific for the major bee venom allergen phospholipase A<sub>2</sub> (PLA) were isolated from beekeepers, who displayed tolerance to bee venom antigens and allergic patients before and after AIT. Human IL-10<sup>+</sup> B<sub>R1</sub> cells expressed high surface CD25 and CD71 levels and low CD73 levels. Sorting of CD73<sup>-</sup>CD25<sup>+</sup>CD71<sup>+</sup> B cells allowed enrichment of human B<sub>R1</sub> cells, which produced high levels of IL-10 and potently suppressed antigen-specific CD4<sup>+</sup> T-cell proliferation. Apparently, T- and B-cell subsets, which become predominant during AIT and natural antigen exposure, represent the T<sub>R1</sub> or IL-10–Treg cells and B<sub>R1</sub> or IL-10–Breg cells in human subjects. Although there are limited data on the recently demonstrated B<sub>R1</sub> cells, there is substantial evidence on the role of T<sub>R1</sub> cells and allergen tolerance.

The investigation of human high-dose allergen exposure models has also provided important insights into the nature of Treg cell responses in tolerance. In nonallergic bee keepers and cat owners, T<sub>R1</sub> cells specific for the major allergens present in bee venom and cat saliva represent the major T-cell subset in healthy subjects. These Treg cells use numerous suppressive mechanisms, including the involvement of IL-10, TGF-β, CTLA-4, and PD-1.

**AIT AND TREG AND BREG CELLS INFLUENCE ALLERGEN-SPECIFIC ANTIBODY RESPONSES**

Natural exposure to a relevant allergen is often associated with an increase in IgE synthesis. Similarly, AIT often induces a transient increase in serum specific IgE levels, followed by a gradual decrease over months or years of continued treatment. In pollen-sensitive patients desensitization prevents increases in serum specific IgE titers during the pollen season. However, the changes in IgE levels cannot account for the diminished responsiveness to specific allergen caused by AIT because the decrease in serum IgE levels is late, relatively small, and poorly correlated with clinical improvement after AIT.

Research focused on the subclasses of IgG antibodies, especially IgG<sub>4</sub>, suggests that the allergen can be captured before reaching the effector cell–bound IgE, thus preventing activation of mast cells and basophils. Data from several studies indicated that increases in specific IgG<sub>4</sub> levels accompanied clinical improvement. With venom allergy, the increased anti-venom IgG<sub>4</sub> levels correlate, at least at the onset of desensitization, with protection achieved by the treatment. Blocking antibodies seem to inhibit allergen-induced release of
inflammatory mediators from basophils and mast cells, IgE-facilitated allergen presentation to T cells, and allergen-induced boost of memory IgE production during high allergen exposure in the pollen season. Grass pollen immunotherapy induces allergen-specific, IL-10–associated “protective” IgG4 responses. These studies demonstrated an association between IgG4-dependent blocking of IgE binding to B cells. However, IgG4 antibodies can be viewed as having the ability to modulate the immune response to allergen and thus the potential to influence the clinical response to allergen. In a study using well-defined recombinant allergen mixtures, all treated subjects had strong allergen-specific IgG1 and IgG4 antibody responses. Some patients were not showing IgE and IgG4 against Phl p 5 at the start of AIT but had strong IgG4 antibody responses to that allergen without induction of any IgE, supporting the immune tolerance–inducing role of AIT.

IL-10, which is induced in T<sub>H</sub>2 and T<sub>reg</sub> cells and increasingly secreted during AIT, appears to counterregulate antigen-specific IgE and IgG4 antibody synthesis. Recently, it was demonstrated in a bee venom model that IgG4 production was selectively confined to human B<sub>reg</sub> cells. B cells specific for the major bee venom allergen PLA isolated from nonallergic beekeepers show increased expression of IL-10 and IgG4. Furthermore, the frequency of IL-10<sup>+</sup> PLA-specific B cells increased in allergic patients receiving AIT. Apparently, IL-10 potently suppresses both total and allergen-specific IgE and simultaneously increases IgG4 production. Thus IL-10 not only generates tolerance in T cells; it also regulates specific isotype formation and skews the specific response from an IgE- to an IgG4-dominated phenotype.

**SUPPRESSION OF LATE-PHASE RESPONSES OF EFFECTOR CELLS DURING AIT**

Long-term AIT is associated with a significant reduction in the immediate response to allergen provocation and the late-phase response (LPR) in the nasal and bronchial mucosa or the skin. The mechanism of LPRs is different from that of mast cell–mediated immediate reactions and involves the recruitment, activation, and persistence of eosinophils and activation of T cells at sites of allergen exposure. The immunopathologic changes seen in mucosal tissues of subjects chronically exposed to inhalant allergens resemble those seen during the LPR. Because the LPR is associated with increased bronchial and nasal hyperresponsiveness and mimics the pathologic condition of chronic allergic inflammation, it has been postulated that the effect of AIT on the LPR is relevant to its clinical efficacy.

Successful AIT results in an increase in allergen concentration necessary to induce immediate responses or LPRs in the target tissue and decreased responses to nonspecific stimulation.
Bronchial, nasal, and conjunctival hyperreactivity to nonspecific stimuli, which seem to reflect underlying mucosal inflammation, decrease after AIT and correlate with clinical improvement.\textsuperscript{56,57} During birch pollen AIT, reduced plasma levels of eosinophil cationic protein, a marker of eosinophil activation, and chemotactic factors for eosinophils and neutrophils correlate with decreased bronchial hyperreactivity and clinical improvement.\textsuperscript{50} AIT also inhibits the seasonal increase in eosinophil priming.\textsuperscript{58} After grass pollen AIT, decreased eosinophil and mast cell infiltration in nasal and bronchial mucosa correlates with an anti-inflammatory effect. In addition, plasma concentrations and \textit{in vitro} production of endothelin-1 (a bronchoconstrictor and proinflammatory peptide) are significantly decreased in asthmatic children after 2 years of immunotherapy with mite extract.\textsuperscript{39,50} In addition, mast cell and basophil suppression require T-cell cytokines for priming, survival, and activity, which are not efficiently provided by suppressed T\textsubscript{H}2 cells and activated Treg cells.\textsuperscript{51,52} Peripheral T-cell tolerance to allergens, which is characterized by functional inactivation of the cell to antigen encounter, can overcome both acute and chronic events in allergic reactions. AIT efficiently modulates the thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release.\textsuperscript{53,54} In addition, IL-10 reduces proinflammatory cytokine release from mast cells.\textsuperscript{55} IL-10 downregulates eosinophil function and activity and suppresses IL-5 production by human resting T\textsubscript{H}2 and T\textsubscript{H}3 cells.\textsuperscript{56} Moreover, IL-10 inhibits endogenous GM-CSF production and CD40 expression by activated eosinophils and enhances eosinophil cell death.\textsuperscript{57}

**TREG AND BREG CELLS AND OTHER CELLS OF IMMUNE REGULATION**

More than 30 years ago, it was postulated that CD8\textsuperscript{+} suppressor cells limit ongoing immune responses and might prevent autoimmune disease.\textsuperscript{58} The recent phenotypic and functional characterization of suppressive Treg cells has led to a renaissance of interest in their therapeutic application in a number of immune-mediated diseases. Two broad subsets of CD3\textsuperscript{+} CD4\textsuperscript{+} Treg cells have been described: constitutive or naturally occurring Treg cells and adaptive or inducible Treg cells. It has been recently proposed that Treg cells generated in the thymus appear sufficient for control of systemic and tissue-specific autoimmunity and that extrathymic differentiation of inducible Treg cells affects commensal microbiota composition and serve a distinct and essential function in restraint of allergic-type inflammation at mucosal interfaces.\textsuperscript{59} In human subjects there is strong evidence that these subsets overlap substantially. Other Treg cell populations, including CD8\textsuperscript{+} Treg cells and double-negative (CD4\textsuperscript{+} CD8\textsuperscript{+}) Treg cells, mediate tolerance in several experimental autoimmune diseases.\textsuperscript{60} The Breg cell subset, particularly IL-10–secreting B cells, have strong regulatory-suppressor properties.\textsuperscript{61,62} In addition, natural killer cells, epithelial cells, macrophages, and glial cells express suppressor cytokines, such as IL-10 and TGF-\textbeta.\textsuperscript{52} These cell types might efficiently contribute to generating and maintaining a regulatory-suppressor type of immune response.

Human inducible IL-10–secreting B regulatory 1 (Breg1) cells can produce high levels of IL-10 and potently suppress antigen-specific CD4\textsuperscript{+} T-cell proliferation.\textsuperscript{63} It has been demonstrated that IgG\textsubscript{2a} has been selectively confined to human Breg1 cells. In nonallergic beekeepers increased expression of IL-10 and IgG\textsubscript{2a} has been shown in B cells specific for the major bee venom allergen PLA. Also, an increase in the frequency of IL-10\textsuperscript{+} PLA-specific B cells has been reported in patients with bee venom allergy receiving AIT. These data point out 2 essential features of allergen tolerance: suppressive B cells and IgG\textsubscript{2a}-expressing B cells that are confined to IL-10\textsuperscript{+} Breg1 cells.\textsuperscript{28} B cells also require specific Toll-like receptor (TLR) stimulation and T-cell and plasmacytoid dendritic cell (DC) help for distinct activation of immunoglobulin production profiles. In response to TLR3, TLR7, and TLR9 triggering, human B cells proliferate and turn into antibody-secreting cells. This response cannot be influenced by stimulation with TLR2, TLR4, TLR5, and TLR8 ligands.\textsuperscript{61}

Although the ultimate goal of AIT is to modify the immune response toward allergens so that immune tolerance lasts after discontinuation of therapy, it is not clear whether this actually occurs with all therapies because natural exposure to environmental allergens can vary. For example, many patients who receive grass pollen AIT continue to have environmental exposure to the allergen, even after therapy is discontinued. This sustained exposure likely contributes to maintaining tolerance.

**Forkhead box protein 3–positive Treg cells**

Naturally occurring Treg cells constitute less than 5% of the CD3\textsuperscript{+} CD4\textsuperscript{+} population in healthy mice and human subjects.\textsuperscript{64-66} Natural Treg cells emerge from the thymus as a distinct subset of mature T cells with defined functions.\textsuperscript{67,68} and inducible Treg cells differentiate from naive T cells in the periphery.\textsuperscript{69,70} Several studies suggest that thymic differentiation accounts for Treg cells that are specific for self-peptides, whereas peripheral differentiation might be required for environmental antigen/allergen–specific T cells. The relative contribution of the 2 pathways \textit{in vivo} in human subjects is still unclear.

The transcription factor forkhead box protein 3 (FOXP3) is required for the development and function of naturally occurring Treg cells,\textsuperscript{71} and its expression is sufficient to convert nonregulatory CD4\textsuperscript{+} CD25\textsuperscript{+} T cells into T cells with regulatory activity. Conversion of peripheral CD4\textsuperscript{+} CD25\textsuperscript{−} naive T cells to FOXP3\textsuperscript{+} CD4\textsuperscript{+} CD25\textsuperscript{+} Treg cells can be induced by TGF-\textbeta.\textsuperscript{72} In natural Treg cells FOXP3 can directly interact with Runx-related transcription factor (RUNX) 1, which impairs the expression of IL-2 and IFN-\gamma and exerts suppressive activity.\textsuperscript{74} Induction of RUNX1 and RUNX3 by TGF-\beta plays an essential role in the generation and suppressive function of induced Treg cells.\textsuperscript{75,76} RUNX1 and RUNX3 bind to the FOXP3 promoter and activate the induction of FOXP3-expressing functional Treg cells. In both human subjects and mice mutations diminishing the function of FOXP3 result in loss of the naturally occurring Treg cell compartment. Human subjects affected by the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome or gus regulation, polyendocrinopathy, enteropathy, X-linked syndrome have severe atopy, including eczema, food allergy, and eosinophilic inflammation and autoimmunity in infancy.\textsuperscript{77,78} FOXP3 expression negatively correlates with levels of IgE, eosinophilia, and IFN-\gamma, and the ratio of FOXP3\textsuperscript{+} T cells to total CD4\textsuperscript{+} T cells is significantly lower in patients with asthma or atopic dermatitis compared with that seen in healthy subjects.\textsuperscript{77} In addition, the
expression of FOXP3 correlates with the suppressive capacity of Treg cells.74

**Molecular mechanisms of inducible Treg cell generation**

The immune system has the ability to induce peripheral mechanisms of immune tolerance to allergens. The consequences of Treg cell development are the maintenance of immune tolerance to tumor antigens, microbial persistence caused by defective clearance, and limitation of collateral tissue damage. The upper respiratory and gastrointestinal submucosa, starting from the tonsils and adenoids, is constantly exposed to a high antigenic pressure (commensal bacteria, food-derived antigens, aeroallergens, and pathogens) and is thus a suitable microenvironment for the generation of Treg cells that contribute to homeostasis.78 It has been generally accepted that immature or partially mature DCs have the ability to induce peripheral tolerance through the generation of Treg cells.79 It is generally accepted that myeloid DCs and plasmacytoid dendritic cells (pDCs) are different functional subsets that play distinct and complementary roles in innate and adaptive immunity.80 In human subjects maturing pDCs have the ability to generate Treg cells, which indicates that pDCs constitute a unique DC subset exhibiting intrinsic tolerogenic capacity.80,81 Loss of allergen-specific peripheral T-cell tolerance in response to IL-1, **IL-6**, TLR4, and TLR8 stimulation, but not to TLR7 or TLR9 stimulation, underlines the importance of a proinflammatory microenvironment in breaking allergen tolerance.82 In addition, the role of TLR8, but not TLR7 and TLR9, stimulations in breaking allergen tolerance supports the contribution of pDCs to tolerance induction,83 which is in line with findings supporting the important roles of pDCs in the induction and maintenance of peripheral tolerance to food and inhalant allergens in human tonsils.84

A novel mechanism for the inhibition of tolerance induction by a T<sub>H</sub>2-type immune response has been reported, whereby GATA3 directly binds to the FOXP3 promoter region, thus inhibiting its expression.85 A dichotomy in the generation of pathogenic T<sub>H</sub>17 and Treg cells prevents the development of autoimmune diseases. TGF-β contributes to the generation of both T<sub>H</sub>17 and Treg cells.86 TGF-β directs the peripheral conversion of effector T cells into FOXP3<sup>+</sup> Treg cells.87 However, in the presence of IL-6, TGF-β promotes the generation of T<sub>H</sub>17 from naive T cells.88 Recently, the analysis of DC signature in PBMCs during grass pollen AIT in a double-blind, placebo-controlled clinical trial with sublingual tablets provided some candidate biomarkers related to DC activity.89 Levels of complement component 1Q and stabilin 1 were increased in PBMCs from clinical responders compared with nonresponders or placebo-treated patients, suggesting candidate biomarkers of early efficacy of AIT.

**TGF-β, IL-10, AND IMMUNE TOLERANCE**

TGF-β is a potent regulatory cytokine produced by a wide range of cell types playing a pivotal role in maintaining tolerance, particularly oral tolerance, within the immune system (Table II).89 TGF-β inhibits the proliferation, differentiation, and survival of both B and T lymphocytes. Given its broad functions, the effects of TGF-β in patients with allergic disease are complex, with evidence of both disease inhibition and promotion. TGF-β is involved in (1) a negative feedback mechanism to control airway inflammation, (2) repair of asthmatic airways,

### Table II. Functions of IL-10 and TGF-β

<table>
<thead>
<tr>
<th>Cell type</th>
<th>IL-10</th>
<th>TGF-β</th>
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<tr>
<td>Immature DCs</td>
<td>Inhibits DC maturation, leading to reduced MHC class II and costimulatory ligand expression</td>
<td>Promotes Langerhans cell development</td>
</tr>
<tr>
<td></td>
<td>Inhibits antigen presentation for stimulation of T-cell proliferation and cytokine production</td>
<td>Inhibits DC maturation and antigen presentation</td>
</tr>
<tr>
<td></td>
<td>Inhibits proinflammatory cytokine secretion</td>
<td>Downregulates FcRRI expression on Langerhans cells</td>
</tr>
<tr>
<td>T cells</td>
<td>Suppresses allergen-specific T-cell subsets Blocks B7/CD28, ICOS, and CD28 costimulatory pathways on T cells</td>
<td>Inhibits proliferation, differentiation, and effector function of T&lt;sub&gt;H&lt;/sub&gt;1 and T&lt;sub&gt;H&lt;/sub&gt;2 cells</td>
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<tr>
<td></td>
<td>Inhibits survival and cytokine production Chemoattractant for eosinophils</td>
<td>Promotes T&lt;sub&gt;H&lt;/sub&gt;17 and Treg cells</td>
</tr>
<tr>
<td>B cells and immunoglobulins</td>
<td>Enhances survival Promotes IgG production, particularly IgG&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Inhibits B-cell proliferation</td>
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<tr>
<td></td>
<td>Inhibits most immunoglobulin class-switching</td>
<td>Induces apoptosis of immature or naive B cells</td>
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<td>IgE</td>
<td>Suppresses allergen-specific IgE</td>
<td>Suppresses allergen-specific IgE</td>
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<td>CD25&lt;sup&gt;+&lt;/sup&gt;FOXP3&lt;sup&gt;+&lt;/sup&gt; Treg cells</td>
<td>Indirect effect on generation</td>
<td>Upregulates FOXP3</td>
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<tr>
<td>IL-10–T&lt;sub&gt;H&lt;/sub&gt;1 cells</td>
<td>Promotes IL-10–Treg induction</td>
<td>Can promote IL-10 synthesis</td>
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<tr>
<td>Monocytes/macrophages</td>
<td>Inhibits proinflammatory cytokine production and antigen presentation</td>
<td>Inhibits scavenger and effector functions, including proinflammatory cytokine production and antigen presentation</td>
</tr>
<tr>
<td></td>
<td>Promotes chemotaxis</td>
<td>Promotes chemotaxis</td>
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<tr>
<td>Eosinophils</td>
<td>Inhibits survival and cytokine production</td>
<td>Chemoattractant for eosinophils</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Inhibits mast cell activation, including cytokine production</td>
<td>Promotes chemotaxis</td>
</tr>
<tr>
<td></td>
<td>Variable effects on other functions Might inhibit expression of FcεR</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Inhibits chemokine and proinflammatory cytokine production</td>
<td>Chemoattractant for neutrophils</td>
</tr>
</tbody>
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and (3) induction of fibrosis to exaggerate disease development in human subjects.99

IL-10 is of interest in the control of allergy and asthma because it inhibits many effector cells and disease processes. IL-10 is synthesized by a wide range of cell types, including B cells, monocytes, DCs, natural killer cells, and T cells. It inhibits proinflammatory cytokine production, as well as TH1 and TH2 cell activation, which is likely attributable to the effects of IL-10 on antigen-presenting cells; in addition, direct effects on T-cell function have also been demonstrated (Table I). In T cells the IL-10 receptor–associated tyrosine kinase Tyk-2 acts as a constitutive reservoir for Src homology domain 2-containing protein tyrosine phosphatase 1 (SHP-1). Tyk-2 dephosphorylates them within minutes, and downstream signaling is inhibited. Accordingly, T cells from SHP-1−/− mice showed increased proliferation with CD28 and ICOS stimulation in comparison with wild-type mice, which was not suppressed by IL-10. IL-10 impairs activation of mast cells and eosinophils, the 2 effector cell types associated with early and allergic LPRs. It promotes IgG1 synthesis, and the induction of IL-10 after AIT might account for the favorable change in IgG1/IgE ratios associated with a successful response. Data from studies in murine models and human subjects have indicated that IL-10 plays a role in maintaining immune homeostasis in lung health.100,101

HR2 AS A MAJOR PLAYER IN PERIPHERAL TOLERANCE

A small-molecular-weight monoamine that binds to 4 different G protein–coupled receptors, histamine regulates several essential events in the immune response.102 HR2 is coupled to adenylate cyclase, and studies in animal models and human cells indicate that HR2 inhibits characteristic features of cells primarily through cyclic AMP formation.103 During AIT with high allergen doses, histamine released from mast cells and basophils interferes with the peripheral tolerance induced in several pathways.104 Histamine enhances TH1-type responses by triggering the histamine receptor HR1. HR2 negatively regulates both TH1- and TH2-type responses. Human CD4+ TH1 cells predominantly express HR1, and CD4+ TH2 cells predominantly express HR2, which results in their differential regulation by histamine. Histamine induces the production of IL-10 by DCs. In addition, histamine induces IL-10 production by TH2 cells and enhances the suppressive activity of TGF-β on T cells.105 All 3 of these effects are mediated through HR2, which is relatively highly expressed on TH2 cells and suppresses IL-4 and IL-13 production and T-cell proliferation. HR2 might represent an essential receptor that participates in peripheral tolerance or active suppression of inflammatory/immune responses. Histamine also regulates antibody isotypes, including IgE.106 A high amount of allergen-specific IgE is induced in HR1−/− mice. In contrast, deletion of HR2 leads to a significant reduction in allergen-specific IgE production, probably through direct effects on B cells and indirect effects through T cells.

IMMUNE TOLERANCE INDUCED IN SLIT

SLIT has a well-established safety profile, with more than several hundred million doses administered to human subjects, and is considered an alternative to subcutaneous AIT.107,108 The immunologic mechanisms of SLIT are less well established than those for subcutaneous immunotherapy (Table III). Meta-analyses concluded that IgG4 levels increase but IgE levels remain stable in adults.109,110 In addition, allergen-specific IgA is induced.111 There are conflicting data concerning lymphoproliferative responses.109,112 The effects of SLIT on T-cell reactivity and cytokine secretion vary between studies. T-cell proliferation was reduced in allergic patients successfully treated with house dust mite SLIT.113 In a different study of SLIT, IL-10 mRNA increased, and TGF-β mRNA positively correlated with IL-10 and negatively correlated with IL-5.114 After 6 months of SLIT, eosinophil cationic protein and serum IL-13 levels are decreased.115 Nasal tryptase secretion after nasal allergen challenge tests also decreases.116 During 2 years of SLIT with grass pollen allergens in children, no significant effects on in vitro T-cell immune responses or immunoglobulin levels were observed, although SLIT reduced the need for rescue medication.117

CONCLUSION

Immune tolerance to allergens is essential to develop a healthy immune response to allergens in highly exposed subjects. Allergen-specific tolerance involves a deviation in T-cell response to TH1 cells, B-cell response to IL-10–secreting B cells, increased IgG4 isotype specific antibody response, and decreased activation of effector cells, such as basophils, mast cells, and eosinophils. Multiple mechanisms and receptors play a role in this, such as IL-10, TGF-β, CTLA-4, PD-1, and HR2. These mechanisms play a role in correcting dysregulated immune responses by inducing peripheral allergen tolerance by AIT. Despite the benefits of AIT for most treated subjects, not everyone’s condition improves, life-threatening side effects can occur, the treatment effect might not be permanent, and the duration of the treatment is long. Therefore the development of advanced vaccines, novel adjuvants, and reliable biomarkers to select patients with a good clinical response are strongly expected. There is a strong rationale to develop novel biomarkers related to the genetic and epigenetic state of the patient, allergen/antigen tolerance capacity, and tissue responses in patients with AIT. Biomarkers should be easily measured in readily accessible body fluids (eg, blood, saliva, nasal secretions, and skin scrapings); they should be cost effective and should fulfill the

<table>
<thead>
<tr>
<th>TABLE III. Differences and similarities in mechanisms of SCIT and SLIT</th>
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<tbody>
<tr>
<td><strong>Mechanisms</strong></td>
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<tr>
<td>Very early desensitization</td>
</tr>
<tr>
<td>T-cell tolerance</td>
</tr>
<tr>
<td>Treg cell generation</td>
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<tr>
<td>Breg cell generation</td>
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<tr>
<td>Role of IL-10</td>
</tr>
<tr>
<td>Role of TGF-β</td>
</tr>
<tr>
<td>Decreased tissue mast cell functions</td>
</tr>
<tr>
<td>Decreased tissue eosinophils and mediators</td>
</tr>
<tr>
<td>Decreased IgE</td>
</tr>
<tr>
<td>Increased IgG4</td>
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unmet needs for prediction and better patient care. Different biomarkers for AIT and different stages of allergic diseases and endotypes are expected to be developed in the near future.

AIT-based curative approaches might also find application for the prevention of allergic disease. The major challenges for prevention include the requirement for very early intervention, safety problems for pediatric use, and missing early biomarkers of who will have allergy. The future should be exciting because advances in immunology and bioengineering are being applied to the development of multiple immune-modifying biological agents. In particular, the combination of immune response modifiers with AIT might provide a way for efficient immune-modulation of allergic diseases.

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