Series editors: Joshua A. Boyce, MD, Fred Finkelman, MD, and William T. Shearer, MD, PhD

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

Mübeccel Akdis, MD, PhD, and Cezmi A. Akdis, MD *Davos, Switzerland*

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 IgG_4; 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 IgG_4 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 T_H1, T_H2, and T_H17 cells; suppression of allergen-specific IgE and induction of IgG_4; 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 dermatitis[3] 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.[5,6] 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.[1,2,11-13] 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 T_H1 and T_H2 cell subsets in 1986, during the last 27 years, it is well understood that there is

From the Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich.

The authors' laboratories are supported by the Swiss National Foundation grants 320030_140772 and 320030_132899 and the Christine Kühne Center for Allergy Research and Education (CK-CARE), European 7th frame work projects MeDALL: Mechanisms of the Development of Allergy (no. 261357), and PREDICTA: Post-Infectious Immune Reprogramming and Its Association with Persistence and Chronicity of Respiratory Allergic Diseases (no. 260895).

Disclosure of potential conflict of interest: M. Akdis has received grants from the Swiss National Science Foundation, EF7 MeDALL, and EF7 Predicta. C. A. Akdis has consultant arrangements with Actellion, Aventis, Stallergenes, Allergopharma, and Circacia; is employed by the Swiss Institute of Allergy and Asthma Research, University of Zurich; and has received grants from Novartis, PREDICTA, the Swiss National Science Foundation, MeDALL, and the Christine Kühne Center for Allergy Research and Education Research.

Received for publication November 14, 2013; revised December 18, 2013; accepted for publication December 24, 2013.

Corresponding author: Mübeccel Akdis, MD, PhD, or Cezmi A. Akdis, MD, Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, CH7270 Davos, Switzerland. E-mail: akdism@siaf.uzh.ch. Or: akdisac@siaf.uzh.ch.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.12.1088

Terms in boldface and italics are defined in the glossary on page 622.

Abbreviations used
AIT: Allergen-specific immunotherapy
B_R1: IL-10–secreting regulatory B
Breg: Regulatory B
CTLA-4: Cytotoxic T lymphocyte antigen 4
DC: Dendritic cell
FOXP3: Forkhead box protein 3
HR: Histamine receptor
ICOS: Inducible costimulator
LPR: Late-phase response
PD-1: Programmed death 1
pDC: Plasmacytoid dendritic cell
PLA: Phospholipase A_2
SHP-1: Src homology domain 2–containing protein tyrosine phosphatase 1
SLIT: Sublingual immunotherapy
TLR: Toll-like receptor
T_R1: IL-10–secreting regulatory T
Treg: Regulatory T
VIT: Venom immunotherapy

reciprocal regulation between individual T_H cell subsets, such as T_H1, T_H2, ***T_H9***, ***T_H17***, and T_H22[14-16]; however, regulatory T (Treg) cells play a major role in the suppression of effector T-cell responses in different diseases.[6,10]

Allergic diseases are complex disorders with several disease variants caused by different underlying cellular and molecular mechanisms. Although there are several clinically relevant 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***.[17,18] 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.

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 ***IgG_4***

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.

IgG_4: IgG_4 has been associated with the development of immune tolerance to antigens, including foods, and the ratio of specific IgE to IgG_4 might be useful in the context of desensitization. IgG_4 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 T_H2 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 T_H2 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 T_H2 cells and increases IFN-γ production in T_H1 cells.

T_H9: A T-cell subset that is defined by the production of IL-9 and promoted in the presence of IL-4 and TGF-β1. IL-9 has a number of functions, including increased mucus production, in asthmatic patients.

T_H17: T_H17 cells are defined by IL-17A, IL-17F, IL-6, IL-21, IL-22, 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 T_H17 phenotype of $CD4^+$ T cells. IL-17 in turn induces IL-1β and IL-6.

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.

The Editors wish to acknowledge Seema S. Aceves, PhD, for preparing this glossary.

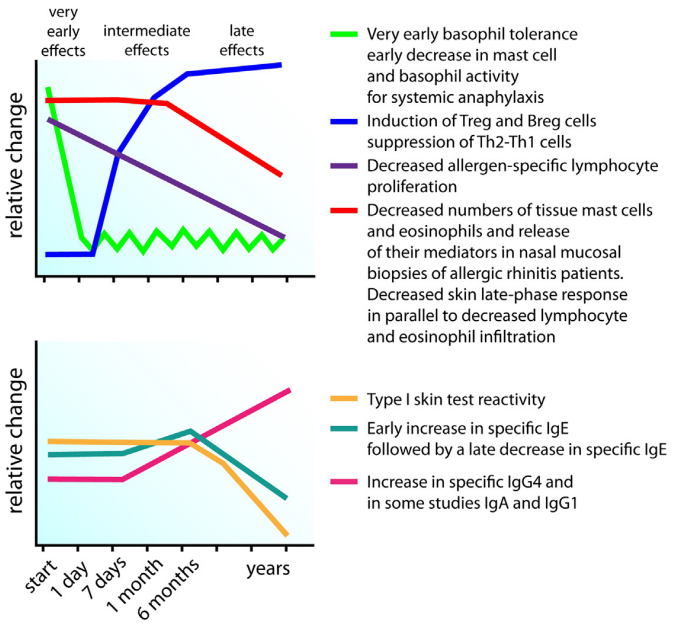

FIG 1. Immunologic changes during the course of AIT. Although it shows differences between protocols and routes of administration, a similar profile is observed. Changes in ultrarush protocols of venom AIT appear relatively early and show more intense effects compared with SLIT for pollen allergens. Starting with the first injection, decreases in mast cell and basophil activity, degranulation, and tendency for systemic anaphylaxis degranulation take place within the first hours. This is followed by generation of allergen-specific Treg and Breg cells and suppression of allergen-specific T_H1 and T_H2 cells. Specific IgE levels show an early increase and relatively late decreases. These events are in parallel to increases in IgG_4 levels, which continuously increase as long as the treatment continues. After several months, the allergen-specific IgE/IgG_4 ratio decreases. After a few months, decreases in tissue mast cell and eosinophil counts and release of their mediators and skin LPRs occur. A significant decrease in type I skin test reactivity is also observed relatively late in the course.

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.[19] Anaphylaxis is associated with the release of inflammatory mediators from both mast cells and basophils, and successful hyposensitization alters the magnitude of mediator release.[20] 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.[20,21]

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 FcεRI-induced activation and mediator release of basophils, including histamine and sulfidoleukotrienes, as well as cytokine production *in vitro*.[22]

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).[23] Although multiple factors contribute, the tolerant state of specific cells essentially results from increased IL-10 secretion.[24-26] Suppressor capacity for

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

T cells	Decreased allergen-induced proliferation (PBMCs)
	Induction of allergen-specific T_R1 cells (PBMCs and nasal biopsies in allergic rhinitis)
	Increased FOXP3 expression (PBMCs and T cells)
	Increased secretion of IL-10 and TGF-β (PBMCs and nasal biopsies in allergic rhinitis)
	Suppression of T_H2 cells and cytokines (PBMCs)
	Decreased T-cell numbers in LPRs (skin LPRs)
	Increased FOXP3 expression (nasal biopsies in allergic rhinitis)
B cells	Induction of allergen-specific IL-10–secreting B_R1 cells
	Early increased and late decreased specific IgE production (serum)
	Increased specific IgG_4 production (serum)
	Increased specific IgA production (serum)
	Suppressed IgE-facilitated antigen presentation (blood and cell lines)
DCs	Suppressed IgE-facilitated antigen presentation (blood)
Eosinophils	Reduction of tissue numbers (allergic rhinitis)
	Decrease in mediator release (nose and blood)
Mast cells	Reduction of tissue numbers (allergic rhinitis)
	Decrease in mediator release (allergic rhinitis)
	Decrease in proinflammatory cytokine production (allergic rhinitis)
Basophils	Decrease in mediator release (blood)
	Decrease in proinflammatory cytokine production (blood)
	Increased HR2 levels with suppressive effects on degranulation and cytokine production (blood)

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_4 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***.[27] IL-10 particularly originates from activated and antigen-specific Treg and Breg cell populations and increases during AIT and natural allergen exposure.[28-32] High IL-10–producing Treg and Breg cell subsets are called IL-10–secreting regulatory T (T_R1) cells and IL-10–secreting regulatory B (B_R1) cells, respectively. Allergen-specific $CD4^+$ T cells that predominantly produce IFN-γ, IL-4, and IL-10 represent T_H1-, T_H2-, and T_R1-like cells, respectively. Healthy and allergic subjects exhibit all 3 subsets, although in different proportions. In healthy subjects IL-10–secreting T_R1 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_H2-like) exist in high frequency.[31] 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)***.[31]

Similar to T_H 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.[33] As observed in T_R1 cells, recently, highly purified IL-10–secreting Breg cells (B_R1) cells were phenotypically and functionally characterized.[28] B cells specific for the major bee venom allergen phospholipase A_2 (PLA) were isolated from beekeepers, who displayed tolerance to bee venom antigens and allergic patients before and after AIT. Human $IL\text{-}10^+$ B_R1 cells expressed high surface CD25 and CD71 levels and low CD73 levels. Sorting of $CD73^-CD25^+CD71^+$ B cells allowed enrichment of human B_R1 cells, which produced high levels of IL-10 and potently suppressed antigen-specific $CD4^+$ T-cell proliferation. Apparently, T- and B-cell subsets, which become predominant during AIT and natural antigen exposure, represent the T_R1 or IL-10–Treg cells and B_R1 or IL-10–Breg cells in human subjects. Although there are limited data on the recently demonstrated B_R1 cells, there is substantial evidence on the role of T_R1 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,[11,12] Treg 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.[11,31]

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.[34-36] In pollen-sensitive patients desensitization prevents increases in serum specific IgE titers during the pollen season.[37,38] 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_4, 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_4 levels accompanied clinical improvement.[39,40] With venom allergy, the increased anti-venom IgG levels correlate, at least at the onset of desensitization, with protection achieved by the treatment.[41,42] Blocking antibodies seem to inhibit allergen-induced release of

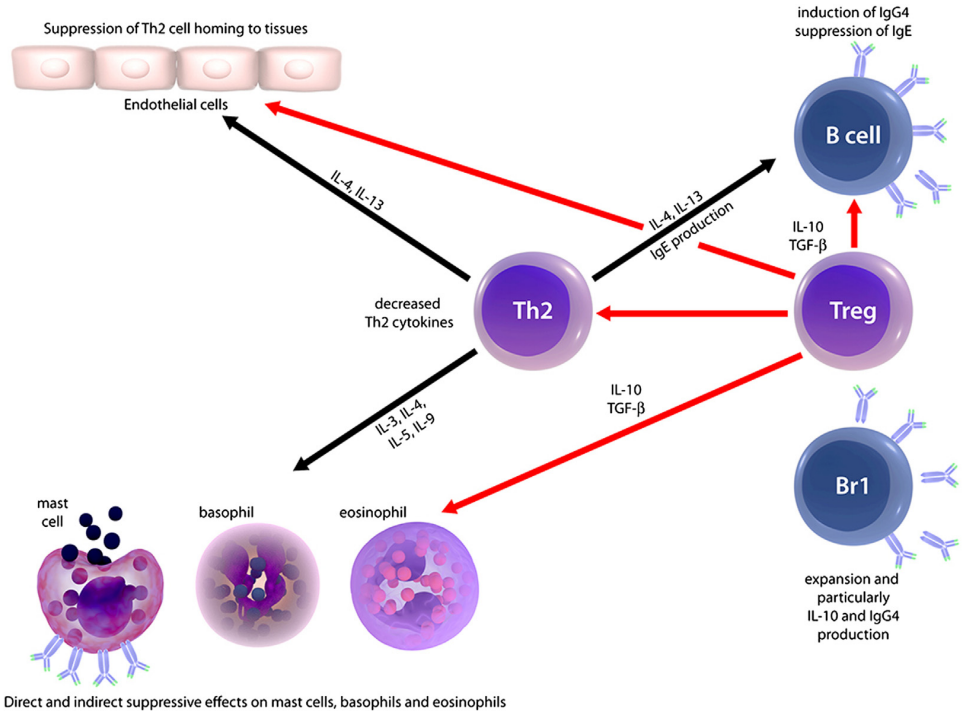

FIG 2. Role of Treg and Breg cells in the suppression of allergic inflammation. The balance between T_H2 cells and Treg cells is decisive for the development or suppression of allergic inflammation. Treg cells and their cytokines suppress T_H2-type immune responses and contribute to the control of allergic diseases in several major ways. *Red arrows* indicate the regulatory and suppressive effects of Treg cells, which exert their regulatory functions directly or indirectly on B cells by inducing IgG_4 and IgA and suppressing IgE; on vascular endothelium by suppressing T_H2 cell homing to tissues; on mast cells, basophils, and eosinophils through direct and indirect suppressive effects; and on direct and indirect suppression of epithelial cell activation and proinflammatory properties. In addition, Breg cells also suppress effector T cells and contribute to IgG_4 synthesis.

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" IgG_4 responses.[43]

These studies demonstrated an association between IgG_4-dependent blocking of IgE binding to B cells. However, IgG_4 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 IgG_1 and IgG_4 antibody responses.[44] Some patients were not showing IgE and IgG_4 against Phl p 5 at the start of AIT but had strong IgG_4 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_R1 and B_R1 cells and increasingly secreted during AIT, appears to counterregulate antigen-specific IgE and IgG_4 antibody synthesis.[24-26,28] Recently, it was demonstrated in a bee venom model that IgG_4 production was selectively confined to human B_R1 cells. B cells specific for the major bee venom allergen PLA isolated from nonallergic beekeepers show increased expression of IL-10 and IgG_4. Furthermore, the frequency of $IL\text{-}10^+$ PLA-specific B cells increased in allergic patients receiving AIT. Apparently, IL-10 potently suppresses both total and allergen-specific IgE and simultaneously increases IgG_4 production.[26] 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 IgG_4-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.[45]

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.[46,47] 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.[46] AIT also inhibits the seasonal increase in eosinophil priming.[48] 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 *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.[49,50] In addition, mast cell and basophil suppression require T-cell cytokines for priming, survival, and activity, which are not efficiently provided by suppressed T_H2 cells and activated Treg cells.[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.[53,54] In addition, IL-10 reduces proinflammatory cytokine release from mast cells.[55] IL-10 downregulates eosinophil function and activity and suppresses ***IL-5*** production by human resting T_H0 and T_H2 cells.[56] Moreover, IL-10 inhibits endogenous ***GM-CSF*** production and CD40 expression by activated eosinophils and enhances eosinophil cell death.[57]

TREG AND BREG CELLS AND OTHER CELLS OF IMMUNE REGULATION

More than 30 years ago, it was postulated that $CD8^+$ suppressor cells limit ongoing immune responses and might prevent autoimmune disease.[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 $CD3^+CD4^+$ 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.[59] In human subjects there is strong evidence that these subsets overlap substantially. Other Treg cell populations, including $CD8^+$ Treg cells and double-negative ($CD4^-CD8^-$) Treg cells, mediate tolerance in several experimental autoimmune diseases.[60] The Breg cell subset, particularly IL-10–secreting B cells, have strong regulatory/suppressor properties.[28,61] In addition, natural killer cells, epithelial cells, macrophages, and glial cells express suppressor cytokines, such as IL-10 and TGF-β.[62] 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 $CD4^+$ T-cell proliferation.[28] It has been demonstrated that IgG_4 has been selectively confined to human Breg1 cells. In nonallergic beekeepers increased expression of IL-10 and IgG_4 has been shown in B cells specific for the major bee venom allergen PLA. Also, an increase in the frequency of $IL\text{-}10^+$ 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_4-expressing B cells that are confined to $IL\text{-}10^+$ Breg1 cells.[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.[63]

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 $CD3^+CD4^+$ population in healthy mice and human subjects.[64-66] Natural Treg cells emerge from the thymus as a distinct subset of mature T cells with defined functions,[67,68] and inducible Treg cells differentiate from naive T cells in the periphery.[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 *in vivo* in human subjects is still unclear.[71,72]

The transcription factor forkhead box protein 3 (FOXP3) is required for the development and function of naturally occurring Treg cells,[73] and its expression is sufficient to convert nonregulatory $CD4^+CD25^-$ T cells into T cells with regulatory activity. Conversion of peripheral $CD4^+CD25^-$ naive T cells to $FOXP3^+CD4^+CD25^+$ Treg cells can be induced by TGF-β.[69] In natural Treg cells FOXP3 can directly interact with ***Runt-related transcription factor (RUNX)*** 1, which impairs the expression of IL-2 and IFN-γ and exerts suppressive activity.[74] Induction of RUNX1 and RUNX3 by TGF-β plays an essential role in the generation and suppressive function of induced Treg cells. 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 X-linked autoimmune and allergic dysregulation syndrome have severe atopy, including eczema, food allergy, and eosinophilic inflammation and autoimmunity in infancy.[75,76] FOXP3 expression negatively correlates with levels of IgE, eosinophilia, and IFN-γ, and the ratio of $FOXP3^+$ T cells to total $CD4^+$ T cells is significantly lower in patients with asthma or atopic dermatitis compared with that seen in healthy subjects.[77] In addition, the

TABLE II. Functions of IL-10 and TGF-β

Cell type	IL-10	TGF-β
Immature DCs	Inhibits DC maturation, leading to reduced MHC class II and costimulatory ligand expression Inhibits antigen presentation for stimulation of T-cell proliferation and cytokine production Inhibits proinflammatory cytokine secretion	Promotes Langerhans cell development Inhibits DC maturation and antigen presentation Downregulates FcεRI expression on Langerhans cells
T cells	Suppresses allergen-specific effector T-cell subsets Blocks B7/CD28, ICOS, and CD2 costimulatory pathways on T cells	Inhibits proliferation, differentiation, and effector function of T_H1 and T_H2 cells Promotes T_H17 and Treg cells
B cells and immunoglobulins	Enhances survival Promotes IgG production, particularly IgG_4	Inhibits B-cell proliferation Induces apoptosis of immature or naive B cells Inhibits most immunoglobulin class-switching
IgE	Suppresses allergen-specific IgE	Suppresses allergen-specific IgE
$CD25^+FOXP3^+$ Treg cells	Indirect effect on generation	Upregulates FOXP3 Promotes inducible FOXP3 Treg cell generation in the periphery
IL-10–T_R1 cells	Promotes IL-10–Treg induction	Can promote IL-10 synthesis
Monocytes/macrophages	Inhibits proinflammatory cytokine production and antigen presentation	Inhibits scavenger and effector functions, including proinflammatory cytokine production and antigen presentation Promotes chemotaxis
Eosinophils	Inhibits survival and cytokine production	Chemoattractant for eosinophils
Mast cells	Inhibits mast cell activation, including cytokine production	Promotes chemotaxis Variable effects on other functions Might inhibit expression of FcεR
Neutrophils	Inhibits chemokine and proinflammatory cytokine production	Chemoattractant for neutrophils

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,[82] 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.[83]

A novel mechanism for the inhibition of tolerance induction by a T_H2-type immune response has been reported, whereby GATA3 directly binds to the FOXP3 promoter region, thus inhibiting its expression.[84] A dichotomy in the generation of pathogenic T_H17 and Treg cells prevents the development of autoimmune diseases. TGF-β contributes to the generation of both T_H17 and Treg cells.[13] TGF-β directs the peripheral conversion of effector T cells into $FOXP3^+$ Treg cells.[85] However, in the presence of IL-6, TGF-β promotes the generation of T_H17 from naive T cells.[86] 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.[87] 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).[88] 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 III. Differences and similarities in mechanisms of SCIT and SLIT

Mechanisms	SLIT	SCIT
Very early desensitization	Not known	+
T-cell tolerance	+	+
Treg cell generation	+	+
Breg cell generation	Not known	+
Role of IL-10	+	+
Role of TGF-β	+	+
Decreased tissue mast cell functions	+	+
Decreased tissue eosinophils and mediators	+	+
Decreased IgE	+/−	++
Increased IgG_4	+/−	++++

SCIT, Subcutaneous immunotherapy.

and (3) induction of fibrosis to exaggerate disease development in human subjects.[89]

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 T_H1 and T_H2 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 II). 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) and then tyrosine phosphorylates SHP-1 on IL-10 binding.[90-92] SHP-1 rapidly binds to the CD28 and inducible costimulator (ICOS) costimulatory receptors and dephosphorylates them within minutes, and downstream signaling is inhibited. Accordingly, T cells from SHP-1–deficient 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 IgG_4 synthesis, and the induction of IL-10 after AIT might account for the favorable change in IgG_4/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.[70,93]

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.[94] 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.[95] During AIT with high allergen doses, histamine released from mast cells and basophils interferes with the peripheral tolerance induced in several pathways.[22] Histamine enhances T_H1-type responses by triggering the histamine receptor HR1. HR2 negatively regulates both T_H1- and T_H2-type responses. Human $CD4^+$ T_H1 cells predominantly express HR1, and $CD4^+$ T_H2 cells predominantly express HR2, which results in their differential regulation by histamine.[96] Histamine induces the production of IL-10 by DCs.[97] In addition, histamine induces IL-10 production by T_H2 cells[98] and enhances the suppressive activity of TGF-β on T cells.[99] All 3 of these effects are mediated through HR2, which is relatively highly expressed on T_H2 cells and suppresses IL-4 and IL-13 production and T-cell proliferation.[96] 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.[96] A high amount of allergen-specific IgE is induced in HR1-deleted 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.[100,101] The immunologic mechanisms of SLIT are less well established than those for subcutaneous immunotherapy (Table III). Meta-analyses concluded that IgG_4 levels increase but IgE levels remain stable in adults.[100,101] In addition, allergen-specific IgA is induced.[102] There are conflicting data concerning lymphoproliferative responses.[103,104] 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.[103] In a different study of SLIT, IL-10 mRNA increased, and TGFB mRNA positively correlated with IL-10 and negatively correlated with IL-5.[105] After 6 months of SLIT, eosinophil cationic protein and serum IL-13 levels are decreased.[106] Nasal tryptase secretion after nasal allergen challenge tests also decreases.[107] 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.[104]

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 T_R1 cells, B-cell response to IL-10–secreting B_R1 cells, increased IgG_4 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

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 immunomodulation of allergic diseases.

REFERENCES

1. Burks AW, Calderon MA, Casale T, Cox L, Demoly P, Jutel M, et al. Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. J Allergy Clin Immunol 2013;131:1288-96.e3.
2. Calderon MA, Casale T, Cox L, Akdis CA, Burks AW, Nelson HS, et al. Allergen immunotherapy: a new semantic framework from the European Academy of Allergy and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL consensus report. Allergy 2013;68:825-8.
3. Bae JM, Choi YY, Park CO, Chung KY, Lee KH. Efficacy of allergen-specific immunotherapy for atopic dermatitis: a systematic review and meta-analysis of randomized controlled trials. J Allergy Clin Immunol 2013;132:110-7.
4. Lin SY, Erekosima N, Kim JM, Ramanathan M, Suarez-Cuervo C, Chelladurai Y, et al. Sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. JAMA 2013;309:1278-88.
5. Berin MC, Mayer L. Can we produce true tolerance in patients with food allergy? J Allergy Clin Immunol 2013;131:14-22.
6. Akdis CA. Therapies for allergic inflammation: refining strategies to induce tolerance. Nat Med 2012;18:736-49.
7. Akdis CA. Allergy and hypersensitivity: mechanisms of allergic disease. Curr Opin Immunol 2006;18:718-26.
8. Akdis M, Akdis AC. Immune tolerance. In: Adkinson NF Jr, Bochner BS, Burks AW, Busse WW, Holgate ST, Lemanske RF Jr, editors. Middleton's allergy: principles and practice. 8th ed. Amsterdam: Elsevier; 2013.
9. Durham SR, Emminger W, Kapp A, Colombo G, de Monchy JG, Rak S, et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. J Allergy Clin Immunol 2010;125:131-8, e1-7.
10. Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. Nat Rev Drug Discov 2009;8:645-60.
11. Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. J Exp Med 2008;205:2887-98.
12. Platts-Mills TA, Woodfolk JA. Allergens and their role in the allergic immune response. Immunol Rev 2011;242:51-68.
13. Jutel M, Van de Veen W, Agache I, Azkur KA, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy and novel ways for vaccine development. Allergol Int 2013;62:425-33.
14. Akdis M, Palomares O, van de Veen W, van Splunter M, Akdis CA. TH17 and TH22 cells: a confusion of antimicrobial response with tissue inflammation versus protection. J Allergy Clin Immunol 2012;129:1438-51.
15. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. J Allergy Clin Immunol 2011;127:701-21, e1-70.
16. Smarr CB, Bryce PJ, Miller SD. Antigen-specific tolerance in immunotherapy of Th2-associated allergic diseases. Crit Rev Immunol 2013;33:389-414.
17. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 2011;127:355-60.
18. Akdis CA, Bachert C, Cingi C, Dykewicz MS, Hellings PW, Naclerio RM, et al. Endotypes and phenotypes of chronic rhinosinusitis: a PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol 2013;131:1479-90.
19. Romano A, Torres MJ, Castells M, Sanz ML, Blanca M. Diagnosis and management of drug hypersensitivity reactions. J Allergy Clin Immunol 2011;127(Suppl):S67-73.
20. Eberlein-Konig B, Ullmann S, Thomas P, Przybilla B. Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitization. Clin Exp Allergy 1995;25:704-12.
21. Plewako H, Wosinska K, Arvidsson M, Bjorkander J, Skov PS, Hakansson L, et al. Basophil interleukin 4 and interleukin 13 production is suppressed during the early phase of rush immunotherapy. Int Arch Allergy Immunol 2006;141:346-53.
22. Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. J Allergy Clin Immunol 2012;130:1153-8.
23. James LK, Shamji MH, Walker SM, Wilson DR, Wachholz PA, Francis JN, et al. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. J Allergy Clin Immunol 2011;127:509-16, e1-5.
24. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998;102:98-106.
25. Walker SM, Durham SR, Till SJ, Roberts G, Corrigan CJ, Leech SC, et al. Immunotherapy for allergic rhinitis. Clin Exp Allergy 2011;41:1177-200.
26. Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. Allergy 2008;63:1455-63.
27. Oral HB, Kotenko SV, Yilmaz M, Mani O, Zumkehr J, Blaser K, et al. Regulation of T cells and cytokines by the interleukin-10 (IL-10)-family cytokines IL-19, IL-20, IL-22, IL-24 and IL-26. Eur J Immunol 2006;36:380-8.
28. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. J Allergy Clin Immunol 2013;131:1204-12.
29. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Müller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-g secretion in specific allergen stimulated T cell cultures. J Immunol 1995;154:4178-94.
30. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, et al. IL-10 and TGF-b cooperate in regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003;33:1205-14.
31. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic Individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med 2004;199:1567-75.
32. Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. J Allergy Clin Immunol 2003;111:1255-61.
33. Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol 2012;30:221-41.
34. Van Ree R, Van Leeuwen WA, Dieges PH, Van Wijk RG, De Jong N, Brewczyski PZ, et al. Measurement of IgE antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. Clin Exp Allergy 1997;27:68-74.
35. Bousquet J, Braquemond P, Feinberg J, Guerin B, Maasch HJ, Michel FB. Specific IgE response with a standardized allergen or allergoid in grass pollen allergy. Ann Allergy 1986;56:456-9.
36. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. J Allergy Clin Immunol 1982;70:261-71.
37. Lichtenstein LM, Ishizaka K, Norman P, Sobotka A, Hill B. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. J Clin Invest 1973;52:472-82.
38. Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. J Allergy Clin Immunol 1988;82:439-46.
39. Flicker S, Valenta R. Renaissance of the blocking antibody concept in type I allergy. Int Arch Allergy Immunol 2003;132:13-24.
40. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol 2004;4:313-8.
41. Golden DB, Meyers DA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. J Allergy Clin Immunol 1982;69:489-93.
42. Müller UR, Helbling A, Bischof M. Predictive value of venom-specific IgE, IgG and IgG subclass antibodies in patients on immunotherapy with honey bee venom. Allergy 1989;44:412-8.

43. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 2004;172:3252-9.
44. Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005;116:608-13.
45. Van Bever HP, Stevens WJ. Suppression of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite *(Dermatophagoides pteronyssinus)*. Clin Exp Allergy 1989;19:399-404.
46. Rak S, Rowhagen O, Venge P. The effect of immunotherapy on bronchial hyper-responsiveness and eosinophil cationic protein in pollen allergic patients. J Allergy Clin Immunol 1988;82:470-80.
47. Varney VA, Edwards J, Tabbah K, Brewster H, Mavroleon G, Frew AJ. Clinical efficacy of specific immunotherapy to cat dander: a double-blind placebo-controlled trial. Clin Exp Allergy 1997;27:860-7.
48. Hakansson L, Heinrich C, Rak S, Venge P. Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: effect of immunotherapy. J Allergy Clin Immunol 1997;99:551-62.
49. Creticos PS, Adkinson NF Jr, Kagey-Sobotka A, Proud D, Meier HL, Naclerio RM, et al. Nasal challenge with ragweed pollen in hay fever patients. Effect of immunotherapy. J Clin Invest 1985;76:2247-53.
50. Chen WY, Yu J, Wang JY. Decreased production of endothelin-1 in asthmatic children after immunotherapy. J Asthma 1995;32:29-35.
51. Walker C, Virchow J-C, Bruijnzeel PLB, Blaser K. T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. J Immunol 1991;146:1829-35.
52. Schleimer RP, Derse CP, Friedman B, Gillis S, Plaut M, Lichtenstein LM, et al. Regulation of human basophil mediator release by cytokines. I. Interaction with anti-inflammatory steroids. J Immunol 1989;143:1310-27.
53. Treter S, Luqman M. Antigen-specific T cell tolerance down-regulates mast cell responses in vivo. Cell Immunol 2000;206:116-24.
54. Shim YK, Kim BS, Cho SH, Min KU, Hong SJ. Allergen-specific conventional immunotherapy decreases immunoglobulin E-mediated basophil histamine releasability. Clin Exp Allergy 2003;33:52-7.
55. Marshall JS, Leal-Berumen I, Nielsen L, Glibetic M, Jordana M. Interleukin (IL)-10 Inhibits long-term IL-6 production but not preformed mediator release from rat peritoneal mast cells. J Clin Invest 1996;97:1122-8.
56. Schandane L, Alonso-Vega C, Willems F, Gerard C, Delvaux A, Velu T, et al. B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. J Immunol 1994;152:4368-74.
57. Ohkawara Y, Lim KG, Glibetic M, Nakano K, Dolovich J, Croitoru K, et al. CD40 expression by human peripheral blood eosinophils. J Clin Invest 1996;97:1761-6.
58. Gershon RK. A disquisition on suppressor T cells. Transplant Rev 1975;26:170-85.
59. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature 2012;482:395-9.
60. Strober S, Cheng L, Zeng D, Palathumpat R, Dejbakhsh-Jones S, Huie P, et al. Double negative (CD4-CD8- alpha beta+) T cells which promote tolerance induction and regulate autoimmunity. Immunol Rev 1996;149:217-30.
61. Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. J Exp Med 2003;197:489-501.
62. Deniz G, van de Veen W, Akdis M. Natural killer cells in patients with allergic diseases. J Allergy Clin Immunol 2013;132:527-35.
63. Sackesen C, van de Veen W, Akdis M, Soyer O, Zumkehr J, Ruckert B, et al. Suppression of B-cell activation and IgE, IgA, IgG1 and IgG4 production by mammalian telomeric oligonucleotides. Allergy 2013;68:593-603.
64. Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. Nat Immunol 2001;2:816-22.
65. Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. Nat Rev Immunol 2003;3:253-7.
66. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801-5.
67. Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Holenbeck AE, Lerman MA, et al. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. Nat Immunol 2001;2:301-6.
68. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004;22:531-62.
69. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 2003;198:1875-86.
70. Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. Nat Rev Immunol 2005;5:271-83.
71. Sakaguchi S. The origin of FOXP3-expressing CD4+ regulatory T cells: thymus or periphery. J Clin Invest 2003;112:1310-2.
72. Sakaguchi S, Vignali DA, Rudensky AY, Niec RE, Waldmann H. The plasticity and stability of regulatory T cells. Nat Rev Immunol 2013;13:461-7.
73. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 2005;22:329-41.
74. Klunker S, Chong MMW, Mantel PY, Palomares O, Bassin C, Ziegler M, et al. Transcription factors RUNX1 and RUNX3 in the induction and suppressive function of Foxp3+ inducible regulatory T cells. J Exp Med 2009;206:2701-15.
75. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. J Clin Invest 2000;106:R75-81.
76. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001;27:20-1.
77. Orihara K, Narita M, Tobe T, Akasawa A, Ohya Y, Matsumoto K, et al. Circulating Foxp3+CD4+ cell numbers in atopic patients and healthy control subjects. J Allergy Clin Immunol 2007;120:960-2.
78. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med 2007;204:1757-64.
79. Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. J Exp Med 2000;192:1213-22.
80. Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA, et al. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J Exp Med 2007;204:105-15.
81. Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. J Immunol 2008;181:5396-404.
82. Kucuksezer UC, Palomares O, Ruckert B, Jartti T, Puhakka T, Nandy A, et al. Triggering of specific Toll-like receptors and proinflammatory cytokines breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood. J Allergy Clin Immunol 2013;131:875-85.e9.
83. Palomares O, Rückert B, Jartti T, Kücüksezer UC, Puhakka T, Gomez E, et al. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. J Allergy Clin Immunol 2012;129:510-20.
84. Mantel PY, Kuipers H, Boyman O, Rhyner C, Ouaked N, Ruckert B, et al. GATA3-driven Th2 responses inhibit TGF-beta1-induced FOXP3 expression and the formation of regulatory T cells. PLoS Biol 2007;5:e329.
85. Zhang L, Zhao Y. The regulation of Foxp3 expression in regulatory CD4(+)CD25(+)T cells: multiple pathways on the road. J Cell Physiol 2007;211:590-7.
86. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 2006;441:231-4.
87. Zimmer A, Bouley J, Le Mignon M, Pliquet E, Horiot S, Turfkruyer M, et al. A regulatory dendritic cell signature correlates with the clinical efficacy of allergen-specific sublingual immunotherapy. J Allergy Clin Immunol 2012;129:1020-30.
88. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 2006;24:99-146.
89. Branton MH, Kopp JB. TGF-beta and fibrosis. Microbes Infect 1999;1:1349-65.
90. Taylor A, Akdis M, Joss A, Akkoc T, Wenig R, Colonna M, et al. IL-10 inhibits CD28 and ICOS costimulations of T cells via src homology 2 domain-containing protein tyrosine phosphatase 1. J Allergy Clin Immunol 2007;120:76-83.
91. Joss A, Akdis M, Faith A, Blaser K, Akdis CA. IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. Eur J Immunol 2000;30:1683-90.
92. Taylor A, Verhagen J, Akkoc T, Wenig R, Flory E, Blaser K, et al. IL-10 suppresses CD2-mediated T cell activation via SHP-1. Mol Immunol 2009;46:622-9.
93. Urry Z, Xystrakis E, Hawrylowicz CM. Interleukin-10-secreting regulatory T cells in allergy and asthma. Curr Allergy Asthma Rep 2006;6:363-71.
94. O'Mahony L, Akdis M, Akdis CA. Regulation of the immune response and inflammation by histamine and histamine receptors. J Allergy Clin Immunol 2011;128:1153-62.
95. Del Valle J, Gantz I. Novel insights into histamine H2 receptor biology. Am J Physiol 1997;273:G987-96.

96. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OAR, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 2001;413:420-5.
97. Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. J Clin Invest 2001;108:1865-73.
98. Osna N, Elliott K, Khan MM. Regulation of interleukin-10 secretion by histamine in TH2 cells and splenocytes. Int Immunopharmacol 2001;1: 85-96.
99. Kunzmann S, Mantel P-Y, Wohlfahrt JG, Akdis M, Blaser K, Schmidt-Weber CB. Histamine enhances TGF-beta1-mediated suppression of Th2 responses. FASEB J 2003;17:1089-95.
100. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005;60:4-12.
101. Radulovic S, Wilson D, Calderon M, Durham S. Systematic reviews of sublingual immunotherapy (SLIT). Allergy 2011;66:740-52.
102. Bahceciler NN, Arikan C, Taylor A, Akdis M, Blaser K, Barlan IB, et al. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. Int Arch Allergy Immunol 2005;136:287-94.
103. Ciprandi G, Fenoglio D, Cirillo I, Vizzaccaro A, Ferrera A, Tosca MA, et al. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. Ann Allergy Asthma Immunol 2005;95:38-44.
104. Rolinck-Werninghaus C, Kopp M, Liebke C, Lange J, Wahn U, Niggemann B. Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. Int Arch Allergy Immunol 2005;136:134-41.
105. Savolainen J, Jacobsen L, Valovirta E. Sublingual immunotherapy in children modulates allergen-induced in vitro expression of cytokine mRNA in PBMC. Allergy 2006;61:1184-90.
106. Marcucci F, Sensi LG, Migali E, Coniglio G. Eosinophil cationic protein and specific IgE in serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma. Allergy 2001;56:231-6.
107. Marcucci F, Sensi L, Frati F, Bernardini R, Novembre E, Barbato A, et al. Effects on inflammation parameters of a double-blind, placebo controlled one-year course of SLIT in children monosensitized to mites. Allergy 2003;58:657-62.