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

Mechanisms of allergen-specific immunotherapy

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

INFORMATION FOR CATEGORY 1 CME CREDIT

Credit can now be obtained, free for a limited time, by reading the review articles in this issue. Please note the following instructions.

Method of Physician Participation in Learning Process: The core material for these activities can be read in this issue of the Journal or online at the JACI Web site: www.jacionline.org. The accompanying tests may only be submitted online at www.jacionline.org. Fax or other copies will not be accepted.

Date of Original Release: January 2011. Credit may be obtained for these courses until December 31, 2012.

Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

Accreditation/Provider Statements and Credit Designation: The American Academy of Allergy, Asthma & Immunology (AAAAI) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The AAAAI designates these educational activities for a maximum of 1 *AMA PRA Category 1 Credit*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

List of Design Committee Members: Cezmi A. Akdis, MD, and Mübeccel Akdis, MD, PhD

Activity Objectives

1. To understand the early cellular changes after initiation of specific immunotherapy (SIT).
2. To understand mechanisms by which regulatory T cells induce tolerance against the TH2 immune response.
3. To understand the role of IL-10.
4. To understand the changes that take place in allergen-specific IgE and IgG subclasses.

Recognition of Commercial Support: This CME activity has not received external commercial support.

Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations: C. Akdis receives research support from Novartis, Stallergenes, the Swiss National Science Foundation, the Global Allergy and Asthma European Network, and the Christine Kuhne Center for Allergy Research; has consultant arrangements with Actellion, Aventis, and Allergopharma; is a fellow and interest group member of the American Academy of Allergy, Asthma, and Immunology; is vice president of EAACI; and is a former committee member WP leader of GA^{2}LEN. M. Akdis receives research support from the Swiss National Foundation, Invision GmbH, and the European Union.

Allergen-specific immunotherapy has been used for 100 years as a desensitizing therapy for allergic diseases and represents the potentially curative and specific method of treatment. The mechanisms of action of allergen-specific immunotherapy include the very early desensitization effects, modulation of T-and B-cell responses and related antibody isotypes, and migration of eosinophils, basophils, and mast cells to tissues, as well as release of their mediators. Regulatory T (Treg) cells have been identified as key regulators of immunologic processes in peripheral tolerance to allergens. Skewing of allergen-specific effector T cells to a regulatory phenotype appears as a key event in the development of healthy immune response to allergens and successful outcome in patients undergoing allergen-specific immunotherapy. Naturally occurring forkhead box protein 3–positive CD4$^+$CD25$^+$ Treg cells and inducible T$_R$1 cells contribute to the control of allergen-specific immune responses in several major ways, which can be summarized as suppression of dendritic cells that support the generation of effector T cells; suppression of effector T$_H$1, T$_H$2, and T$_H$17 cells; suppression of allergen-specific IgE and induction of IgG4; suppression of mast cells, basophils, and eosinophils; and suppression of effector T-cell migration to tissues. New strategies for immune intervention will likely include targeting of the molecular mechanisms of allergen tolerance and reciprocal regulation of effector and Treg cell subsets. (J Allergy Clin Immunol 2011;127:18-27.)

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

The immune system forms an interactive network with tissues and makes its decisions on the basis of signals coming from resident tissue cells, infectious agents, commensal bacteria, and almost any environmental agents.[1,2] In recent years, induction of immune ***tolerance*** has become a prime target for prevention and treatment strategies for many diseases in which dysregulation of the immune system plays an important role. Immune tolerance to

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

Supported by the Swiss National Foundation grants 320030_125249/1 and 320030_132899 and the Christine Kühne Center for Allergy Research and Education (CK-CARE).

Received for publication September 23, 2010; revised November 22, 2010; accepted for publication November 22, 2010.

Reprint requests: Cezmi A. Akdis, MD, Swiss Institute of Allergy and Asthma Research (SIAF), Obere Strasse 22, CH7270 Davos, Switzerland. E-mail: akdisac@siaf.unizh.ch.

0091-6749/$36.00

doi:10.1016/j.jaci.2010.11.030

Terms in boldface and italics are defined in the glossary on page 19.

Abbreviations used
DC: Dendritic cell
FOXP3: Forkhead box protein 3
HR: Histamine receptor
ICOS: Inducible costimulator
SHP: Src homology 2 domain-containing tyrosine phosphatase
SIT: Specific immunotherapy
SLIT: Sublingual immunotherapy
Treg: Regulatory T
TLR: Toll-like receptor

allergens can be defined as establishment of a long-term clinical tolerance against allergens, which immunologically implies changes in memory-type, allergen-specific T- and B-cell responses, as well as mast cell and basophil activation thresholds that do not cause allergic symptoms.[3-6] In addition, prevention of new allergen sensitizations[7] and progression to more severe disease, such as development of asthma[8] after allergic rhinitis or development of systemic anaphylaxis, are essential clinical implications of immune tolerance.

The immunologic basis of allergic diseases is observed in 2 phases: sensitization and development of memory T- and B-cell responses and IgE (early phase) and effector functions related to tissue inflammation and injury (late phase).[5] The differentiation and ***clonal expansion*** of allergen-specific $CD4^+$ T_H2 cells producing IL-4 and IL-13 are essential to induce class switching to the ϵ immunoglobulin heavy chain in B cells and the production of allergen-specific IgE antibodies during the sensitization phase. Allergen-specific IgE binds to the FcϵRI on the surface of mast cells and basophils, thus leading to the patient's sensitization.[9] When a new encounter with the allergen causes cross-linking of the IgE-FcϵRI complexes on sensitized basophils and mast cells, they are activated and subsequently release anaphylactogenic mediators responsible for the classical symptoms of the immediate phase (type 1 hypersensitivity).

Depending on the innate immune response activating capacity of the substances coexposed with the antigen, cosignals for cell differentiation, and status of the cells and ***cytokines*** in the microenvironment, $CD4^+$ naive T cells can differentiate into T_H1-, T_H2-, T_H9-, T_H17-, or T_H22-type memory and effector cells. Based on their respective cytokine profiles, responses to ***chemokines***, and interactions with other cells, these T-cell subsets can promote different types of inflammatory responses. During the development of allergic disease, effector T_H2 cells produce IL-4, IL-5, IL-9, and IL-13,[3-5,10,11] and probably other recently identified cytokines, such as IL-25, IL-31, and IL-33, contribute to T_H2 responses.[12-18] These cytokines play a role in the production of allergen-specific IgE, eosinophilia, permissiveness of endothelium for the recruitment of inflammatory cells to inflamed tissues, production of ***mucus***, and decreased threshold of

GLOSSARY

ADJUVANTS: Substances that need to be administered together with protein antigens to elicit maximal T cell–dependent immune responses.

CD25: CD25 is the IL-2 receptor α chain. IL-2 plays a role in the generation and survival of Treg cells.

CHEMOKINES: Cytokines that induce the migration of cells, chemokines act by binding to G protein–coupled receptors. Their function in the immune system is to coordinate leukocyte trafficking and activation.

CLONAL EXPANSION: An increase in the number of cells that express identical receptors for the antigen.

CYTOKINES: Proteins secreted by the cells of the innate and adaptive immune system. Cytokines can stimulate the growth and differentiation of lymphocytes, activate different effector cells to eliminate microbes, and stimulate the development of hematopoietic cells. Cytokines are important as targets for therapeutic agents.

CYTOTOXIC T LYMPHOCYTE–ASSOCIATED ANTIGEN 4 (CTLA-4): CTLA-4 is structurally homologous to CD28, but CTLA-4 is expressed on recently activated $CD4^+$ and $CD8^+$ T cells, and its function is to inhibit T-cell activation.

BOVINE β-LACTOGLOBULIN: Bovine β-lactoglobulin is a whey protein allergen (Bos d 5). Sequential (linear) allergenic (IgE) epitopes have been mapped on the caseins β-lactoglobulin and α-lactalbumin and have been correlated with the persistence of cow's milk allergy.

DENDRITIC CELLS: Hematopoietic cells that function as antigen-presenting cells for naive lymphocytes. Their name is derived from their multiple, thin membranous projections.

EPITOPE: A molecular region on the surface of an antigen capable of eliciting an immune response and for binding of the specific antibody produced by such a response.

IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIF (ITIM): Located in the cytoplasmic tails of inhibitory receptors, ITIMs are essential for the signaling functions of these molecules. ITIMs recruit phosphatase enzymes that counteract the effect of kinases in the signaling cascades initiated by activating receptors.

INDUCIBLE COSTIMULATOR (ICOS): ICOS is a member of the CD28 family of costimulatory receptors on T cells. ICOS binds to ICOS ligand on antigen-presenting cells.

ISOTYPE: Isotypes are a class of antibody. There are 5 different antibody isotypes (IgM, IgD, IgG, IgA, and IgE) that are determined by the type of heavy chain present.

LANGERHANS CELLS: A subset of dendritic cells found in the epithelia and skin-draining lymph nodes. Because of their long cytoplasmic processes, Langerhans cells occupy as much as 25% of the surface area of the epidermis, even though they constitute less than 1% of the cell population.

MUCUS: A substance lining mucous membranes that functions to preserve the membranes, to act as a barrier, and to transport trapped material (in conjunction with cilia). Normal airway mucus is 90% water, and the remaining 10% is composed of protein, carbohydrate, and lipid.

PEPTIDE IMMUNOTHERAPY: Immunotherapy with allergen-derived peptides representing T cell–activating epitopes, which do not react with IgE antibodies.

***PHLEUM* SPECIES ALLERGEN MIXTURES:** A genera of grasses that contains timothy grass.

PIECEMEAL RELEASE: An induced vesicular transport of granular content that does not involve direct granule extrusion.

SMOOTH MUSCLE: Muscle tissue that lacks cross-striations and is found especially in vertebrate hollow organs and structures (eg, the bronchus, intestine, and bladder) as thin sheets performing functions not subject to direct voluntary control.

TOLERANCE: Unresponsiveness, noninflammatory response, or non-anaphylactogenic response to an antigen that is induced by immunologic mechanisms that develop because of previous exposure to that antigen.

The Editors wish to acknowledge Daniel Searing, MD, for preparing this glossary.

BOX 1: Components of the immune response to allergens in healthy and allergic subjects and the response to allergen SIT

Allergen-specific T-cell response	
Healthy nonallergic subjects	No or very low-dose exposure: no T-cell proliferation or cytokine response, no sensitization
	Low-dose exposure: T_H0 response in PBMCs and specific T-cell clones with low frequency
	High-dose exposure: T_R1, particularly IL-10–dominating response with relatively high frequency
Allergic subjects	T_H2 response with varying quantities of IL-4, IL-5, and IL-13 in the presence of relatively low but detectable IL-10 and IFN-γ levels
Allergen SIT	Decreased allergen-induced T-cell proliferation and T_H1 and T_H2 cytokines, induction of Treg cells, increased suppression by Treg cells
Specific antibodies in serum	
Healthy nonallergic subjects	No or very low-dose exposure: not detectable, no sensitization
	Low-dose exposure: detectable IgG1, IgG4, and IgA
	High-dose exposure: high amounts of IgG4, detectable IgG1, IgA, and IgE
Allergic subjects	Relatively high amounts of IgE together with low or high amounts of IgG1, IgG4, and IgA
Allergen SIT	Induction of specific IgG4, very late decrease in specific IgE levels

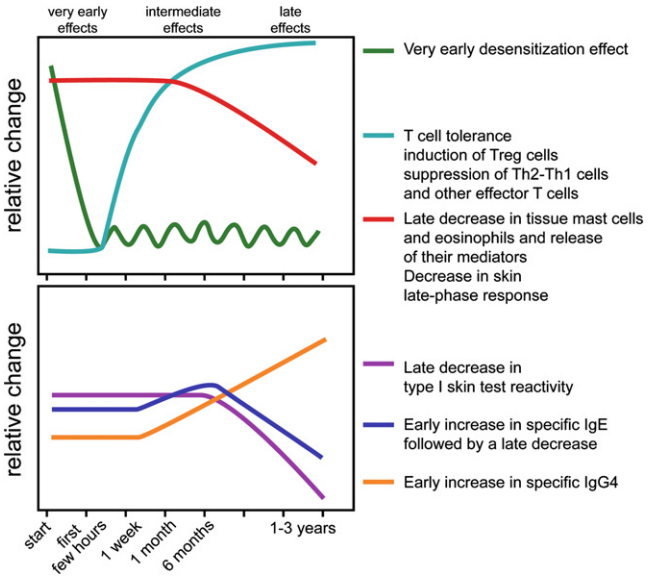

FIG 1. Immunologic changes during the course of allergen SIT. Although there is significant variation between subjects and protocols, right after the first administration of allergens with native-like structures, an early decrease in mast cell and basophil degranulation and a decreased tendency for systemic anaphylaxis are observed. This is followed by generation of allergen-specific Treg cells and suppression of both allergen-specific T_H1, T_H2, and maybe other effector cells. An early increase and a very late decrease in specific IgE levels is observed. In particular, the IgG4 level shows a relatively early increase that is dose dependent. In some studies allergen-specific IgG1 and IgA levels also increase. A significant decrease in the allergen-specific IgE/IgG4 ratio occurs after several months. A significant decrease in type I skin test reactivity is also observed relatively late in the course of SIT. A decrease in tissue mast cell and eosinophil numbers and a release of their mediators and decrease in the late-phase response is observed after a few months. These effects are partially demonstrated in SLIT and are rather weak compared with those seen in injection SIT. Novel allergen SIT approaches might or might not show these effects, although they still can be effective.

contraction of ***smooth muscles***.[19] The commonly observed T_H2 profile in patients with atopic diseases might be a result of (1) increased differentiation and clonal expansion of T_H2 cells[20] or (2) increased tendency to activation-induced cell death of high IFN-γ–producing T_H1 cells.[21] T_H1 cells also efficiently contribute to the effector phase of allergic diseases with their role in apoptosis of the epithelium in patients with asthma and atopic dermatitis[22-24] and apoptosis of smooth muscle cells in patients with fatal asthma.[25]

The discovery of T_H17 cells fills an essential gap in our understanding of inflammatory processes. T_H17 cells are characterized by IL-17A, IL-17F, IL-6, IL-8, TNF-α, IL-22, and IL-26 expression.[26-32] Neutralization of IL-17 and T_H17-related functions resolves tissue pathology in autoimmunity models, improves joint destruction in experimental arthritis, and reduces neutrophil infiltration in an experimental asthma model while increasing eosinophil infiltration.[33-36] It was shown in 2 recent studies that TGF-β in the presence of IL-4 reprograms T_H2 cell differentiation and leads to the development of a new population of T_H9 cells that produce IL-9 and IL-10.[37,38]

A T-cell subset known as T_H22 cells has been demonstrated in T cells that independently express IL-22 with low expression levels of IL-17, and these cells play a role in atopic dermatitis.[39] All of these T-cell subsets and related events represent targets in the treatment of allergic diseases and the induction of regulatory T (Treg) cells, and allergen tolerance can balance their overactivation.

The pivotal role of Treg cells in inducing and maintaining immune tolerance has been demonstrated during the last 15 years, during which their adoptive transfer was shown to prevent or cure several T cell–mediated disease models, including asthmatic lung inflammation, autoimmune diseases, and allograft rejection.[40] In the clinical setting both injection and sublingual versions of allergen-specific immunotherapy (SIT) have been shown to induce allergen-specific Treg cells in human subjects (Box 1). In addition to Treg cells, several other factors appear to play a mechanistic role in allergen SIT (Fig 1).

SEQUENTIAL EVENTS IN ALLERGEN SIT AND THEIR UNDERLYING MECHANISMS

Very early mast cell and basophil suppression–related desensitization effect

Although decreases in IgE antibody levels and IgE-mediated skin sensitivity normally require years of SIT, most patients are protected against bee stings or tolerate skin late-phase response challenges at early stages of respective venom or grass pollen SIT.[41,42] An important observation starting from the first injection is an early decrease in mast cell and basophil activity for degranulation and systemic anaphylaxis (Fig 1). There is surprisingly little information about the mechanisms by which SIT modifies,

suppresses, or both immune responses of basophils and mast cells, in particular during repetitive administration of increasing doses of allergens within the first hours. Although it seems similar to rapid desensitization for hypersensitivity reactions to drugs, the mechanism of this desensitization effect for SIT is yet unknown. Acute oral desensitization in mice demonstrated that antigen-specific mast cell desensitization is one of the main underlying mechanisms for oral desensitization.[43] It has been shown that mediators of anaphylaxis (histamine and leukotrienes) are released during SIT and sting challenges without inducing a systemic anaphylactic response.[44] Their ***piecemeal release*** below the threshold of systemic anaphylaxis might decrease the granule content of mediators and also might affect the threshold of activation of mast cells and basophils because decreased mediator release in these cells is a well-demonstrated feature a short time after the start of allergen SIT.[44-46] One of the main soluble factors liberated by effector cells after allergen challenge is histamine, which mediates its effects through histamine receptors (HRs). Thus far, 4 different human HR types have been identified as HR1 to HR4.[47] Both the expression pattern of HRs and modifications in the intensity of the expression of a single HR type are decisive for the nature of the developing immune response.[48,49] HR1 has significant proinflammatory and cell-activating properties, whereas HR2 has been shown to be coupled to adenylate cyclase and phosphoinositide second messenger systems and is supposed to be involved primarily in tolerogenic immune responses.[50] Although there are individual differences and risks for systemic anaphylaxis during the course of allergen SIT, the suppression of mast cells and basophils continues to be affected by changes in other immune parameters, such as the generation of allergen-specific Treg cells and decreased specific IgE levels. In a recent study significantly enhanced tryptophan degradation and increased human immunoglobulin receptor inhibitory transcript expression in monocytes were found within a few hours after the first injection on day 1, representing markers of very early changes.[51]

Very early effects related to antigen-presenting cells and adjuvants

Aluminium hydroxide is a commonly used ***adjuvant*** in allergen SIT vaccines. Although generally proved to be efficacious and having a good safety profile, novel adjuvants are needed to overcome current problems in conventional immunotherapy. For example, depending on the type of Toll-like receptor (TLR), different types of antigen-presenting cells can be targeted. TLR-triggering compounds that can control the overexpression of T_H2 cytokines or skew the T_H1-T_H2 balance toward a T_H1 and Treg profile have been effective in murine models of allergy.[52]

The epidermis contains high numbers of potent antigen-presenting ***Langerhans cells***. Accordingly, transcutaneous or epicutaneous allergen SIT was recently introduced as a treatment option for allergies.[53] A few applications of allergens by means of skin patches with a treatment duration of a few weeks were sufficient to achieve lasting relief. Similarly, oral mucosal Langerhans cells bind allergens after resorption, which significantly increased their migratory capacity but attenuated their maturation.[54] Allergen challenge promoted the release of TGF-β1 and IL-10 by oral mucosal Langerhans cells themselves, as well as by cocultured T cells.

The tolerogenic function of different types of ***dendritic cells*** (DCs) depends on certain maturation stages and subsets of different ontogenies and can be influenced by immunomodulatory agents. A role for DCs in the induction of different subsets of Treg cells in defined microenvironments has been supported by several studies. In intestinal lamina propria, several subsets of DCs reside and are in close contact with commensal bacteria and food antigens/allergens.[55,56] DCs from the lamina propria of the small intestine and from the mesenteric lymph nodes are noticeably better than splenic DCs at inducing the expression of forkhead box protein 3 (FOXP3) in naive T cells in the presence of exogenous TGF-β.[55,56] Treg cells can be induced in the microenvironment of tumors and chronic infections caused by DCs that promote them. In some cases DCs conditioned by $FOXP3^+$ Treg cells; pathogen-derived molecules, such as filamentous hemagglutinin[57]; and exogenous signals, such as histamine through HR2,[50] adenosine,[58] vitamin D3 metabolites,[59] or retinoic acid[60] can induce new populations of Treg cells. Antigen presentation by partially mature airway DCs that express IL-10 induce the formation of T_R1-like cells, which inhibit subsequent inflammatory responses.[61] In addition, depletion and adoptive transfer of pulmonary plasmacytoid DCs has demonstrated an important role for these cells in protection from allergen sensitization and asthma development in mice.[62]

Virus-like particles as a novel, modular, and acellular antigen-presenting system and as strong adjuvants are able to modulate the responses of allergen-specific T cells. Displaying Fel d 1 on virus-like particles prevents type I hypersensitivity despite greatly enhanced immunogenicity and represents a novel therapy for cat allergy. A single vaccination was sufficient to induce protection in mice.[63,64]

Treg cells and peripheral T-cell tolerance to allergens

The induction of a tolerant state in peripheral T cells represents an essential step in allergen SIT (Fig 1). Peripheral T-cell tolerance is characterized mainly by generation of allergen-specific Treg cells[65-67] and initiated by IL-10 and TGF-β, which are increasingly produced by the antigen-specific Treg cells.[65-68] Subsets of Treg cells with distinct phenotypes and mechanisms of action include the naturally occurring, thymic selected $CD4^+$***CD25***$^+$ Treg cells and the inducible T_R1 cells.[69] Different studies show roles for both subsets, suggesting an overlap particularly in the inducible subsets of Treg cells in human subjects. Their first effect is realized by suppression of allergen-specific T_H2 and T_H1 cells. The suppression by these cells could partially be blocked by the use of neutralizing antibodies against secreted or membrane-bound IL-10 and TGF-β. In coherence with this, it has been shown that $CD4^+CD25^+$ Treg cells from atopic donors have a reduced capability to suppress the proliferation of $CD4^+CD25^-$ T cells.[70] The presence of local $FOXP3^+CD25^+CD3^+$ cells in the nasal mucosa, their increased numbers after immunotherapy, and their association with clinical efficacy and suppression of seasonal allergic inflammation strengthen the concept of allergen tolerance based on Treg cells in human subjects.[71] These findings were coined by tracking specific T cells with allergen class II tetramers: clinical tolerance induction in human subjects is associated with a marked loss of IL-4–producing T cells and the acquisition of IL-10–producing and $FOXP3^+$ antigen-specific $CD4^+$ T cells.[72] In addition to conventional immunotherapy, ***peptide immunotherapy*** in patients with allergic asthma generates IL-10–dependent immunologic tolerance associated with linked ***epitope*** suppression.

Treatment with selected epitopes from a single allergen resulted in suppression of responses to other ("linked") epitopes within the same molecule.[73]

IL-10–producing antigen-presenting cells, such as B cells[74] and DCs,[61] as well as clonally expanded IL-10–producing allergen-specific T_R1 cells,[66,75] all contribute to the suppressive effects of IL-10 in different models. IL-10 suppresses T cells by blocking CD2, CD28, and ***inducible costimulator*** (ICOS) costimulatory signals in a rapid signal transduction cascade.[76] In the presence of IL-10, a direct inhibition on CD2, CD28, and ICOS signaling in T cells occurs through use of Src homology 2 domain-containing tyrosine phosphatase (SHP-1) by IL-10.[76,77] SHP-1 rapidly binds to CD28 and ICOS and dephosphorylates them.[76] Supporting these findings, spleen cells from SHP-1–deficient mice show increased proliferation with CD2, CD28, and ICOS stimulation in comparison with that seen in wild-type mice, which was not suppressed by IL-10. Generation of dominant negative SHP-1–overexpressing T cells or silencing of the SHP-1 gene by small inhibitory RNA both altered SHP-1 functions and abolished the suppressive effect of IL-10.[76-78] Interestingly, the suppressive effect of IL-10 was not observed in other IL-10 family cytokines, such as IL-19, IL-20, IL-22, and IL-24.[79] In addition to T cells, IL-10 also exerts an inhibitory effect on activated monocytes and macrophages.[80] It has been shown in monocytes and DCs that IL-10 suppresses costimulatory molecules and downregulates MHC class II molecules and antigen-presenting cell capacity.[81] Furthermore, IL-10 induces the expression of the suppressor of cytokine-signaling 3 gene *(SOCS3)*, which might play a role in the inhibition of the IFN-γ–induced tyrosine phosphorylation of signal transducer and activator of transcription 1.[82]

TGF-β is essential for the maintenance of immunologic self-tolerance.[83] TGF-β induces the conversion of naive $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ T cells by means of the induction of FOXP3,[84] and TGF-β signaling is required for *in vivo* expansion and immunosuppressive capacity of $CD4^+CD25^+$ T cells.[85] In addition, runt-related transcription factors 1 and 3 play an essential role in FOXP3 development both in human subjects and mice.[86] However, the exact suppressive mechanisms behind TGF-β activation of Smad pathways remain to be elucidated.

Treg cells in healthy immune response to allergens in subjects exposed to high doses

Two high-dose allergen exposure models have been studied in human subjects. These are immune responses to bee venom allergens in beekeepers and immune responses to cat allergens in cat owners.[69,87] If a detectable immune response is mounted, T_R1 cells specific for common environmental allergens consistently represent the dominant subset in healthy subjects (Box 1). They use multiple suppressive mechanisms, IL-10 and TGF-β as secreted cytokines, and ***cytotoxic T lymphocyte antigen 4*** and programmed death 1 as surface molecules. Healthy and allergic subjects exhibit all three (ie, T_H1-, T_H2-, and T_R1-type allergen-specific subsets in different proportions).[75] Accordingly, a change in the dominant subset and the balance between T_H2 and Treg cells might lead to either allergy development or recovery.

It was found in allergic children that Treg cells increase during the pollen season.[88] Whether these $CD4^+CD25^{high}$ T cells directly contribute to inflammation or their increased levels keep the inflammation at low levels remains an important research question. Circulating allergen-specific $CD4^+CD25^{high}FOXP3^+$ Treg cells do not show a major difference between nonatopic and atopic subjects.[89] However, it was demonstrated that FOXP3 expression shows a negative correlation with IgE, eosinophilia, and IFN-γ levels, and the $FOXP3^+/CD4^+$ ratio is significantly low in patients with asthma and atopic dermatitis.[90] $CD4^+CD25^+$ Treg cells have been associated with the spontaneous remission of cow's milk allergy. Children who outgrew their allergy (tolerant children) had higher frequencies of circulating $CD4^+CD25^+$ T cells and decreased *in vitro* proliferative responses to ***bovine β-lactoglobulin*** in PBMCs compared with children who maintained clinically active allergy.[91] Peripheral tolerance uses multiple mechanisms to suppress allergic inflammation. Treg cells contribute to the control of allergen-specific immune responses by means of (1) suppression of antigen-presenting cells that support the generation of effector T cells; (2) suppression of T_H2 and T_H1 cells; (3) suppression of allergen-specific IgE and induction of IgG4; (4) suppression of mast cells, basophils, and eosinophils; and (5) interaction with resident tissue cells and remodeling (Fig 2).[69]

Modulation of allergen-specific IgE and IgG responses during allergen SIT

Peripheral T-cell tolerance is rapidly induced during SIT; however, there is no evidence for B-cell tolerance in the early course.[65] Allergen SIT induces a transient increase in serum specific IgE levels, followed by a gradual decrease over months or years of treatment (Fig 1).[92,93] In pollen-sensitive patients desensitization prevents the increase of serum specific IgE levels during the pollen season.[94] The changes in IgE levels cannot explain the diminished responsiveness to specific allergen because of SIT because the decrease in serum IgE levels is relatively late and does not correlate with clinical improvement after SIT.

Subclasses of IgG antibodies, especially IgG4, are thought to capture the allergen before reaching the effector cell–bound IgE and thus to prevent the activation of mast cells and basophils. IgG4 antibodies can be viewed as a marker of introduced allergen dose, and they have the ability to modulate the immune response to allergen. However, the relationship between the efficacy of SIT and the induction of allergen-specific IgG subgroups remains a controversial issue, with serum concentrations of allergen-specific IgG correlating with clinical improvement in some studies but not in others.[95,96] Allergen-specific IgG might be directed against the same epitopes as IgE, resulting in direct competition for allergen binding and a "blocking" effect. The concept of blocking antibodies has been re-evaluated. Analysis of the IgG subtypes induced by means of SIT has shown specific increases in IgG1 and particularly IgG4, with levels increasing 10- to 100-fold.[97,98] There is accumulating evidence that SIT also influences the blocking activity on IgE-mediated responses by IgG4. Results suggest that successful SIT is associated with an increase in IgG-blocking activity that is not solely dependent on the quantity of IgG antibodies.[99,100] In a recent study inhibition by IgG required Fcγ receptor IIB. One IgG against a single epitope on the major allergen was able to block the degranulation of basophils from subjects with cat allergy. The inhibitory potential of IgG antibodies increased when larger allergen-IgG complexes were formed.[101] It seems to be relevant rather to measure the blocking activity, affinity, or both of allergen-specific IgG or IgG subsets, particularly IgG4 and also IgG1, instead of their levels in sera.

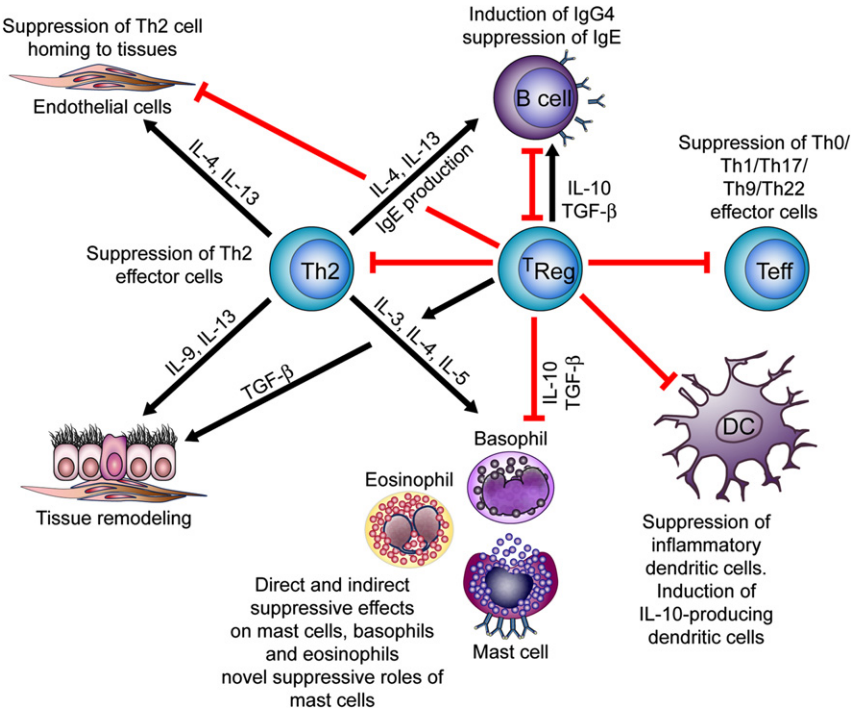

FIG 2. $FOXP3^+CD4^+CD25^+$ and T_R1 cells contribute to the control of allergen-specific immune responses in several major ways: suppression of DCs that support the generation of effector T cells; suppression of T_H2 and other effector cells; suppression of allergen-specific IgE and induction of IgG4, IgA, or both; suppression of mast cells, basophils, and eosinophils; interaction with resident tissue cells and remodeling; and suppression of effector T-cell and other inflammatory cell migration to tissues.

There are several features of IgG4 that might play a role in its noninflammatory role. The IgG4 hinge region has unique structural features that result in a lower affinity for certain Fcγ receptors, and the ability to separate and repair by means of dynamic Fab arm exchange leads to bispecific antibodies that are functionally monomeric.[102,103] Furthermore, IgG4 does not fix complement and is capable of inhibiting immune complex formation by other ***isotypes***, giving this isotype anti-inflammatory characteristics. In a clinical trial with 5 recombinant ***Phleum species allergen mixtures***, all treated subjects had very strong allergen-specific IgG4 and also increased IgG1 antibody responses. Some patients who were not initially sensitized to Phl p 5 had strong specific IgG4, but not IgE antibody responses specifically against that allergen.[97] This demonstrates that extract-based antibody measurements might provide the wrong information and studies on mechanisms of allergen SIT should be performed with single allergens.

It is highly possible that the decrease in IgE/IgG4 ratio during allergen SIT is a feature of skewing from allergen-specific T_H2 to Treg cell predominance. IL-10 is a potent suppressor of both total and allergen-specific IgE, whereas it simultaneously increases IgG4 production.[66,104] Thus IL-10 not only generates tolerance in T cells but also regulates specific isotype formation toward a noninflammatory phenotype. The healthy immune response to Der p 1 is associated with increased specific IgA and IgG4 levels, small amounts of IgG1, and almost undetectable IgE antibodies in serum (Box 1).[67] In the same study house dust mite SIT did not significantly change specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1, and IgG4 levels was observed.[67] The reason for the long time gap between the change in T-cell subsets but not IgE levels is not easily explainable by the half-life of this antibody. In this context the role of bone marrow–resident IgE-producing plasma cells with a very long lifespan remains to be investigated.[105]

Suppression of effector cells and inflammatory responses during allergen SIT

Allergen SIT efficiently modulates the thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release.[106] Treg cells play a role in this finding to some extent. IL-10 was shown to reduce proinflammatory cytokine release from mast cells.[107] In addition, IL-10 downregulates eosinophil function and activity and suppresses IL-5 production by human resting T_H0 and T_H2 cells.[108] During birch pollen SIT, there are reduced plasma levels of eosinophil cationic protein, a marker of eosinophil activation, as well as decreased chemotactic factors for eosinophils and neutrophils correlated with decreased bronchial hyperreactivity and clinical improvement.[109] Inhibition by allergen SIT of the seasonal increase in eosinophil priming has also been demonstrated.[110]

In a model of myocarditis, IL-10 gene transfer significantly reduces mast cell density, local histamine concentration, and mast cell growth and prevents mast cell degranulation.[111] Treg cells directly inhibited the FcεRI-dependent mast cell degranulation through cell-cell contact involving OX40–OX40 ligand interactions between Treg cells and mast cells, respectively. When activated in the presence of Treg cells, mast cells showed increased cyclic AMP concentrations and reduced Ca^{++} influx.[112] In this way several aspects of immediate-type hypersensitivity reactions are inhibited by Treg cells.

Long-term SIT is associated with reduction of not only the immediate response to allergen provocation but also the

late-phase reaction in the nasal and bronchial mucosa or in the skin. The mechanism of late-phase reaction is different from that of the mast cell–mediated immediate reaction and involves the recruitment, activation, and persistence of eosinophils and activated T cells at the sites of allergen exposure.[5] Successful SIT results not only in the increase of allergen concentration necessary to induce an immediate response or late-phase reaction in the target tissue but also in decreased response to nonspecific stimulation. Bronchial, nasal, and conjunctival hyperreactivity to nonspecific stimuli, which seems to reflect underlying mucosal inflammation, decreases after SIT and correlates with clinical improvement.[113] In addition, protein expression signatures in serum during sublingual immunotherapy (SLIT) showed an increase in apolipoprotein A-IV, which decreases histamine release from basophils.[114] Recently, it was demonstrated that allergin-1, which contains an ***immunoreceptor tyrosine-based inhibitory motif*** (ITIM)–like domain preferentially expressed on mast cells, suppressed IgE-mediated degranulation of bone marrow–derived mast cells.[115] Mice deficient in allergin-1 experienced enhanced passive systemic and cutaneous anaphylaxis. Mast cells are not only enhancers of allergic inflammation. In certain models they play a role in downmodulating the allergic inflammation in which IL-10 plays an important role.[116,117]

Mechanisms of SLIT

Sustained and disease-modifying effects of SLIT have been confirmed in large-scale, randomized, double-blind, placebo-controlled trials and also in children.[118-122] In addition, it represents very high activity for novel treatment modalities, and several novel immunotherapies are under development, including oral immunotherapy for food allergy.[53,123-126] Oral mucosal tissue has a natural tolerogenic character without any acute inflammation in spite of high bacterial colonization and rapid wound healing without scar development. Lack of inflammatory cells around mucosal tissue and high permeability for allergens suggest a method of action for sublingual allergen immunotherapy.[127] The first step of SLIT is to uptake the allergen by Langerhans cells[128] within the oral mucosa through high-affinity surface IgE receptors.[129] This leads to secretion of IL-10 and induction of T cells with a regulatory phenotype *in vitro*.[130] The mechanism of action of SLIT has been found in the same direction as in injection immunotherapy associated with increases in sublingual FOXP3-expressing cells and increased allergen-specific IgG4, IgA, and serum inhibitory activity for IgE-facilitated allergen binding to B cells.[131]

CONCLUSION

Allergen SIT and high-dose allergen exposure models represent efficient tools for the understanding of the mechanisms of action of curative treatment of allergic diseases and now enlighten the complex interactions of effector cells with tissues and Treg cells. In addition to allergy, these mechanisms might have implications in autoimmunity, organ transplant tolerance, tumor cell growth, parasite survival/clearance, and chronic infections. Suppression of an immune response by T_R1 or $FOXP3^+$ Treg cells seems to show beneficial effects in the case of allergic reactions. However, it might be harmful in other cases, such as tumor antigen tolerance during cancer development or immune tolerance to chronic infectious agents, which prevents complete neutralization. Changes in the fine balance between allergen-specific Treg cells and T_H2, T_H17, T_H22, and/or T_H1 cells is very crucial in the development and also treatment of allergic diseases. Taking these findings into account, along with the recent advances in the knowledge of Treg cells and related peripheral tolerance mechanisms, developments of safer approaches and better treatment of allergy, asthma, and other immune-mediated diseases will soon be made. In addition to the treatment of established allergy, it is essential to consider prophylactic approaches using similar mechanisms before the initial sensitization takes place. Preventive vaccines that induce Treg cell responses could be developed, and allergen-specific Treg cells, which will become predominant, might in turn dampen allergic inflammation, ensuring a well-balanced immune response.

What do we know?

In the course of allergen SIT:

- **Increased IL-10–secreting T_R1-like cells are observed.**
- **Increased suppressive capacity of T_R1 and $CD4^+CD25^+$ Treg cells is observed.**
- **Decreased allergen-specific T-cell proliferation and decreased T_H1 and T_H2 cytokine levels are detected.**
- **Increased IL-10 and TGF-β are released from T cells.**
- **Increased specific IgG4 levels are determined in serum.**
- **Multiple suppressor factors, such as IL-10, TGF-β, IL-10 receptor, TGF-β receptor, cytotoxic T lymphocyte antigen 4, programmed death-1, and HR2 play a role.**
- **Decreased clinical and experimental late-phase responses are observed.**
- **Decreased numbers of tissue mast cells, eosinophils, and their mediators are detected.**
- **Increased IL-10, TGF-β, and $CD4^+CD25^+FOXP3^+$ Treg cell numbers are found in nasal mucosa.**
- **IL-10 and T_R1 cells, as well as IgG4, play a role in tolerance to high-dose exposure to bee venom and cat allergen.**
- **Peptide immunotherapy, SLIT, and high-dose allergen exposure induce similar Treg cell–related mechanisms.**
- **IL-10, T_R1 cells, and $CD4^+CD25^+$ T cells induce IgG4 and suppress IgE *in vitro*.**
- **Linked suppression to epitopes presented with the same MHC class II molecule occurs.**

What is still unknown?

- **Molecular mechanisms of how Treg cells are generated *in vivo***
- **Allergen SIT vaccine adjuvants that specifically induce Treg cells**
- ***In vivo* lifespan of Treg cells induced by allergen SIT**
- **How to balance the possible deleterious role of Treg cells, such as immune tolerance to tumor antigens and chronic infectious agents**
- **Clinical effects of vitamin D3, retinoic acid, HR2, and adenosine receptor–targeting strategies in the induction of Treg cells and benefit to patients**
- **Contribution of resident tissue cells to immune tolerance**

- **Mechanisms of spontaneous healing, remission, and exacerbation of allergic disease and the contributions of Treg cells in these processes**
- **Most of the local events during SLIT and epicutaneous SIT**
- **Early molecular markers and predictors of starting, stopping, and success**
- **Whether there are differences in the mechanisms of high-dose and low-dose allergen SIT**
- **Mechanisms of long-term maintenance of allergen tolerance**

REFERENCES

1. Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. Nat Rev Drug Discov 2009;8:645-60.
2. Akdis CA. Allergy and hypersensitivity: mechanisms of allergic disease. Curr Opin Immunol 2006;18:718-26.
3. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 2007;119:780-91.
4. Akdis M. Healthy immune response to allergens: T regulatory cells and more. Curr Opin Immunol 2006;18:738-44.
5. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol 2006;6:761-71.
6. Durham SR, Walker SM, Varga E-V, Jacobson MR, O'Brien F, Noble W, et al. Long-term clinical efficacy of grass-pollen immunotherapy. N Engl J Med 1999;341:468-75.
7. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 2001;31:1392-7.
8. Moller C, Dreborg S, Ferdousi HA, Halken S, Host A, Jacobsen L, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 2002;109:251-6.
9. Wang M, Takeda K, Shiraishi Y, Okamoto M, Dakhama A, Joetham A, et al. Peanut-induced intestinal allergy is mediated through a mast cell-IgE-FcepsilonRI-IL-13 pathway. J Allergy Clin Immunol 2010;126:306-16, e1-12.
10. Berin MC, Shreffler WG. T(H)2 adjuvants: implications for food allergy. J Allergy Clin Immunol 2008;121:1311-22.
11. Chatila TA, Li N, Garcia-Lloret M, Kim HJ, Nel AE. T-cell effector pathways in allergic diseases: transcriptional mechanisms and therapeutic targets. J Allergy Clin Immunol 2008;121:812-25.
12. Kang CM, Jang AS, Ahn MH, Shin JA, Kim JH, Choi YS, et al. Interleukin-25 and interleukin-13 production by alveolar macrophages in response to particles. Am J Respir Cell Mol Biol 2005;33:290-6.
13. Wang YH, Angkasekwinai P, Lu N, Voo KS, Arima K, Hanabuchi S, et al. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. J Exp Med 2007;204:1837-47.
14. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol 2004;5:752-60.
15. Bilsborough J, Leung DY, Maurer M, Howell M, Boguniewicz M, Yao L, et al. IL-31 is associated with cutaneous lymphocyte antigen-positive skin homing T cells in patients with atopic dermatitis. J Allergy Clin Immunol 2006;117:418-25.
16. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov 2008;7:827-40.
17. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. J Allergy Clin Immunol 2010;125:752-4.
18. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. J Allergy Clin Immunol 2009;123:1047-54.
19. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 2004;113:395-400.
20. Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. Science 2002;295:336-8.
21. Akkoc T, de Koning PJ, Ruckert B, Barlan I, Akdis M, Akdis CA. Increased activation-induced cell death of high IFN-gamma-producing T(H)1 cells as a mechanism of T(H)2 predominance in atopic diseases. J Allergy Clin Immunol 2008;121:652-8, e1.
22. Trautmann A, Akdis M, Kleemann D, Altznauer F, Simon HU, Graeve T, et al. T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. J Clin Invest 2000;106:25-35.
23. Trautmann A, Schmid-Grendelmeier P, Krüger K, Crameri R, Akdis M, Akkaya A, et al. T cells and eosinophils cooperate in the induction of bronchial epithelial apoptosis in asthma. J Allergy Clin Immunol 2002;109:329-37.
24. Meyer N, Zimmermann M, Bürgler S, Bassin C, Woehrl S, Moritz K, et al. IL-32 is expressed by human primary keratinocytes and modulates keratinocyte apoptosis in atopic dermatitis. J. Allergy Clin Immunol 2010;125:858-65, e10.
25. Solarewicz-Madejek K, Basinski TM, Crameri R, Akdis M, Akkaya A, Blaser K, et al. T cells and eosinophils in bronchial smooth muscle cell death in asthma. Clin Exp Allergy 2009;39:845-55.
26. Burgler S, Ouaked N, Bassin C, Basinski TM, Mantel PY, Siegmund K, et al. Differentiation and functional analysis of human T(H)17 cells. J Allergy Clin Immunol 2009;123:588-95, e1-7.
27. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007;445:648-51.
28. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6:1133-41.
29. 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.
30. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6:1123-32.
31. Makihara S, Okano M, Fujiwara T, Kariya S, Noda Y, Higaki T, et al. Regulation and characterization of IL-17A expression in patients with chronic rhinosinusitis and its relationship with eosinophilic inflammation. J Allergy Clin Immunol 2010;126:397-400, e1-11.
32. Martin P, Gomez M, Lamana A, Marin AM, Cortes JR, Ramirez-Huesca M, et al. The leukocyte activation antigen CD69 limits allergic asthma and skin contact hypersensitivity. J Allergy Clin Immunol 2010;126:355-65, e1-3.
33. Sergejeva S, Ivanov S, Lotvall J, Linden A. Interleukin-17 as a recruitment and survival factor for airway macrophages in allergic airway inflammation. Am J Respir Cell Mol Biol 2005;33:248-53.
34. Hellings PW, Kasran A, Liu Z, Vandekerckhove P, Wuyts A, Overbergh L, et al. Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. Am J Respir Cell Mol Biol 2003;28:42-50.
35. Bush KA, Farmer KM, Walker JS, Kirkham BW. Reduction of joint inflammation and bone erosion in rat adjuvant arthritis by treatment with interleukin-17 receptor IgG1 Fc fusion protein. Arthritis Rheum 2002;46:802-5.
36. Rangachari M, Mauermann N, Marty RR, Dirnhofer S, Kurrer MO, Komnenovic V, et al. T-bet negatively regulates autoimmune myocarditis by suppressing local production of interleukin 17. J Exp Med 2006;203:2009-19.
37. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, et al. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. Nat Immunol 2008;9:1347-55.
38. Veldhoen M, Uyttenhove C, van Snick J, Helmby H, Westendorf A, Buer J, et al. Transforming growth factor-beta "reprograms" the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. Nat Immunol 2008;9:1341-6.
39. Nograles KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, et al. IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. J Allergy Clin Immunol 2009;123:1244-52, e2.
40. Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. J Allergy Clin Immunol 2009;123:735-48.
41. Tarzi M, Klunker S, Texier C, Verhoef A, Stapel SO, Akdis CA, et al. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. Clin Exp Allergy 2006;36:465-74.
42. Alexander C, Ying S, Kay BA, Larche M. Fel d 1-derived T cell peptide therapy induces recruitment of CD4+ CD25+; CD4+ interferon-gamma+ T helper type 1 cells to sites of allergen-induced late-phase skin reactions in cat-allergic subjects. Clin Exp Allergy 2005;35:52-8.
43. Woo HY, Kim YS, Kang NI, Chung WC, Song CH, Choi IW, et al. Mechanism for acute oral desensitization to antibiotics. Allergy 2006;61:954-8.
44. 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.

45. Jutel M, Muller UR, Fricker M, Rihs S, Pichler WJ, Dahinden C. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. Clin Exp Allergy 1996;26:1112-8.
46. 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.
47. Jutel M, Akdis M, Akdis CA. Histamine, histamine receptors and their role in immune pathology. Clin Exp Allergy 2009;39:1786-800.
48. Muller UR, Jutel M, Reimers A, Zumkehr J, Huber C, Kriegel C, et al. Clinical and immunologic effects of H1 antihistamine preventive medication during honeybee venom immunotherapy. J Allergy Clin Immunol 2008;122:1001-7, e4.
49. Akdis CA, Simons FE. Histamine receptors are hot in immunopharmacology. Eur J Pharmacol 2006;533:69-76.
50. 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.
51. Bussmann C, Xia J, Allam JP, Maintz L, Bieber T, Novak N. Early markers for protective mechanisms during rush venom immunotherapy. Allergy 2010;65: 1558-65.
52. Crameri R, Rhyner C. Novel vaccines and adjuvants for allergen-specific immunotherapy. Curr Opin Immunol 2006;18:761-8.
53. Senti G, Graf N, Haug S, Ruedi N, von Moos S, Sonderegger T, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. J Allergy Clin Immunol 2009;124:997-1002.
54. Allam JP, Wurtzen PA, Reinartz M, Winter J, Vrtala S, Chen KW, et al. Phl p 5 resorption in human oral mucosa leads to dose-dependent and time-dependent allergen binding by oral mucosal Langerhans cells, attenuates their maturation, and enhances their migratory and TGF-beta1 and IL-10-producing properties. J Allergy Clin Immunol 2010;126:638-45, e1.
55. 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.
56. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med 2007;204:1775-85.
57. McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. J Exp Med 2002;195:221-31.
58. Csoka B, Himer L, Selmeczy Z, Vizi ES, Pacher P, Ledent C, et al. Adenosine A2A receptor activation inhibits T helper 1 and T helper 2 cell development and effector function. FASEB J 2008;22:3491-9.
59. Urry Z, Xystrakis E, Richards DF, McDonald J, Sattar Z, Cousins DJ, et al. Ligation of TLR9 induced on human IL-10-secreting Tregs by 1alpha,25-dihydroxyvitamin D3 abrogates regulatory function. J Clin Invest 2009;119:387-98.
60. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 2007;317:256-60.
61. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol 2001; 2:725-31.
62. de Heer HJ, Hammad H, Soullie T, Hijdra D, Vos N, Willart MA, et al. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. J Exp Med 2004;200:89-98.
63. Leb VM, Jahn-Schmid B, Kueng HJ, Schmetterer KG, Haiderer D, Neunkirchner A, et al. Modulation of allergen-specific T-lymphocyte function by virus-like particles decorated with HLA class II molecules. J Allergy Clin Immunol 2009;124: 121-8.
64. Schmitz N, Dietmeier K, Bauer M, Maudrich M, Utzinger S, Muntwiler S, et al. Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. J Exp Med 2009; 206:1941-55.
65. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Müller U, et al. Epitope specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. J Clin Invest 1996;98:1676-83.
66. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest 1998;102:98-106.
67. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. Eur J Immunol 2003;33: 1205-14.
68. 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.
69. Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol 2005;116:961-9.
70. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet 2004;363:608-15.
71. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. J Allergy Clin Immunol 2008;121:1467-72, e1.
72. Aslam A, Chan H, Warrell DA, Misbah S, Ogg GS. Tracking antigen-specific T-cells during clinical tolerance induction in humans. PLoS One 2010;5:e11028.
73. Campbell JD, Buckland KF, McMillan SJ, Kearley J, Oldfield WL, Stern LJ, et al. Peptide immunotherapy in allergic asthma generates IL-10-dependent immunological tolerance associated with linked epitope suppression. J Exp Med 2009; 206:1535-47.
74. Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. J Exp Med 2003;197:489-501.
75. 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.
76. 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.
77. 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.
78. Akdis CA, Joss A, Akdis M, Faith A, Blaser K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. FASEB J 2000;14:1666-8.
79. 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.
80. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991;174:1209-20.
81. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683-765.
82. Ito S, Ansari P, Sakatsume M, Dickensheets H, Vazquez N, Donnelly RP, et al. Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma- induced genes by suppressing tyrosine phosphorylation of STAT1. Blood 1999;93:1456-63.
83. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. Annu Rev Immunol 1998;16:137-61.
84. 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.
85. Huber S, Schramm C, Lehr HA, Mann A, Schmitt S, Becker C, et al. Cutting edge: TGF-beta signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory CD4+CD25+ T cells. J Immunol 2004;173:6526-31.
86. Klunker S, Chong MM, 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.
87. Custovic A, Simpson BM, Simpson A, Hallam CL, Marolia H, Walsh D, et al. Current mite, cat, and dog allergen exposure, pet ownership, and sensitization to inhalant allergens in adults. J Allergy Clin Immunol 2003;111:402-7.
88. Jartti T, Burmeister KA, Seroogy CM, Jennens-Clough ML, Tisler CJ, Salazar LP, et al. Association between CD4(+)CD25(high) T cells and atopy in children. J Allergy Clin Immunol 2007;120:177-83.
89. Maggi L, Santarlasci V, Liotta F, Frosali F, Angeli R, Cosmi L, et al. Demonstration of circulating allergen-specific CD4+CD25highFoxp3+ T-regulatory cells in both nonatopic and atopic individuals. J Allergy Clin Immunol 2007;120:429-36.
90. 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.
91. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. J Exp Med 2004;199:1679-88.
92. 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.
93. 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.

94. 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.
95. 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.
96. 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.
97. 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.
98. Reisinger J, Horak F, Pauli G, van Hage M, Cromwell O, Konig F, et al. Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. J Allergy Clin Immunol 2005;116:347-54.
99. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol 2004;4:313-8.
100. Till SJ, Francis JN, Nouri-Aria K, Durham SR. Mechanisms of immunotherapy. J Allergy Clin Immunol 2004;113:1025-35.
101. Uermosi C, Beerli RR, Bauer M, Manolova V, Dietmeier K, Buser RB, et al. Mechanisms of allergen-specific desensitization. J Allergy Clin Immunol 2010; 126:375-83.
102. Aalberse RC, Schuurman J. IgG4 breaking the rules. Immunology 2002;105:9-19.
103. van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science 2007;317:1554-7.
104. 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.
105. Radbruch A, Muehlinghaus G, Luger EO, Inamine A, Smith KG, Dorner T, et al. Competence and competition: the challenge of becoming a long-lived plasma cell. Nat Rev Immunol 2006;6:741-50.
106. Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-gamma production of T cells. J Allergy Clin Immunol 1999;103:326-32.
107. 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.
108. 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.
109. Rak S, Hakanson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. J Allergy Clin Immunol 1990;86:706-13.
110. 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.
111. Palaniyandi SS, Watanabe K, Ma M, Tachikawa H, Kodama M, Aizawa Y. Inhibition of mast cells by interleukin-10 gene transfer contributes to protection against acute myocarditis in rats. Eur J Immunol 2004;34:3508-15.
112. Gri G, Piconese S, Frossi B, Manfroi V, Merluzzi S, Tripodo C, et al. CD4+CD25+ regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. Immunity 2008;29:771-81.
113. Rak S, Lowhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. J Allergy Clin Immunol 1988;82:470-80.
114. Makino Y, Noguchi E, Takahashi N, Matsumoto Y, Kubo S, Yamada T, et al. Apolipoprotein A-IV is a candidate target molecule for the treatment of seasonal allergic rhinitis. J Allergy Clin Immunol 2010 [Epub ahead of print].
115. Hitomi K, Tahara-Hanaoka S, Someya S, Fujiki A, Tada H, Sugiyama T, et al. An immunoglobulin-like receptor, Allergin-1, inhibits immunoglobulin E-mediated immediate hypersensitivity reactions. Nat Immunol 2010;11:601-7.
116. Biggs L, Yu C, Fedoric B, Lopez AF, Galli SJ, Grimbaldeston MA. Evidence that vitamin D(3) promotes mast cell-dependent reduction of chronic UVB-induced skin pathology in mice. J Exp Med 2010;207:455-63.
117. Galli SJ, Grimbaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. Nat Rev Immunol 2008;8:478-86.
118. Amar SM, Harbeck RJ, Sills M, Silveira LJ, O'Brien H, Nelson HS. Response to sublingual immunotherapy with grass pollen extract: monotherapy versus combination in a multiallergen extract. J Allergy Clin Immunol 2009;124: 150-6, e1-5.
119. 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.
120. Horak F, Zieglmayer P, Zieglmayer R, Lemell P, Devillier P, Montagut A, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. J Allergy Clin Immunol 2009;124: 471-7, e1.
121. Skoner D, Gentile D, Bush R, Fasano MB, McLaughlin A, Esch RE. Sublingual immunotherapy in patients with allergic rhinoconjunctivitis caused by ragweed pollen. J Allergy Clin Immunol 2010;125:660-6, e1-6.
122. Bufe A, Eberle P, Franke-Beckmann E, Funck J, Kimmig M, Klimek L, et al. Safety and efficacy in children of an SQ-standardized grass allergen tablet for sublingual immunotherapy. J Allergy Clin Immunol 2009;123:167-73, e7.
123. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. J Allergy Clin Immunol 2009;124:292-300, e1-97.
124. Blumchen K, Ulbricht H, Staden U, Dobberstein K, Beschorner J, de Oliveira LC, et al. Oral peanut immunotherapy in children with peanut anaphylaxis. J Allergy Clin Immunol 2010;126:83-91, e1.
125. Thalhamer T, Dobias H, Stepanoska T, Proll M, Stutz H, Dissertori O, et al. Designing hypoallergenic derivatives for allergy treatment by means of in silico mutation and screening. J Allergy Clin Immunol 2010;125:926-34, e10.
126. Crameri R, Kundig TM, Akdis CA. Modular antigen-translocation as a novel vaccine strategy for allergen-specific immunotherapy. Curr Opin Allergy Clin Immunol 2009;9:568-73.
127. Marcucci F, Incorvaia C, Sensi L, Di Cara G, Cadario G, Cavaliere A, et al. Lack of inflammatory cells in the oral mucosa of subjects undergoing sublingual immunotherapy. Int J Immunopathol Pharmacol 2008;21:609-13.
128. Allam JP, Stojanovski G, Friedrichs N, Peng W, Bieber T, Wenzel J, et al. Distribution of Langerhans cells and mast cells within the human oral mucosa: new application sites of allergens in sublingual immunotherapy? Allergy 2008;63: 720-7.
129. Allam JP, Novak N, Fuchs C, Asen S, Berge S, Appel T, et al. Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcepsilonRI expression. J Allergy Clin Immunol 2003;112:141-8.
130. Allam JP, Peng WM, Appel T, Wenghoefer M, Niederhagen B, Bieber T, et al. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. J Allergy Clin Immunol 2008;121:368-74, e1.
131. Scadding GW, Shamji MH, Jacobson MR, Lee DI, Wilson D, Lima MT, et al. Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. Clin Exp Allergy 2010;40:598-606.