

Mechanisms and treatment of allergic disease in the big picture of regulatory T cells

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Activity Objectives

1. To review the role of different regulatory T (Treg) cells in controlling immune responses.

2. To understand how effector T cells regulate the healthy immune response.

3. To understand how Treg cells affect the outcome of allergen-specific immunotherapy.

4. To review the strategies for drug development to prevent and cure allergic diseases.

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Various populations of regulatory T (Treg) cells have been shown to play a central role in the maintenance of peripheral homeostasis and the establishment of controlled immune responses. Their identification as key regulators of immunologic processes in peripheral tolerance to allergens has opened an important era in the prevention and treatment of allergic diseases. Both naturally occurring CD4⁺CD25⁺ Treg cells and inducible populations of allergen-specific, IL-10-secreting Treg type 1 (T_R1) cells inhibit allergen-specific effector cells in experimental models. Skewing of allergen-specific effector T cells to a regulatory phenotype appears to be a key event in the development of healthy immune response to allergens and successful outcome in allergen-specific immunotherapy.

Forkhead box protein 3-positive CD4⁺CD25⁺ Treg cells and T_R1 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_H1, T_H2, and T_H17 cells; suppression of allergen-specific IgE and induction of IgG4; suppression of mast cells, basophils, and eosinophils; interaction with resident tissue cells and remodeling; and suppression of effector T-cell migration to tissues. Current strategies for drug development and allergen-specific immunotherapy exploit these observations, with the potential for preventive therapies and cure for allergic diseases. (*J Allergy Clin Immunol* 2009;123:735-46.)

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

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The immune system is a highly interactive network, which makes its decisions on the basis of all body tissues, infections, normal flora bacteria, and almost any environmental agents. Extensive progress has been made in the understanding of mechanisms of allergic disease with the complex interaction of

Abbreviations used

CCR:	CC chemokine receptor
CTLA:	Cytotoxic T lymphocyte-associated antigen
DC:	Dendritic cell
DP:	D prostanoid
FoxP3:	Forkhead box protein 3 (FOXP3: human, FoxP3: mice)
HR:	Histamine receptor
ICOS:	Inducible costimulator
IPEX:	X-linked immune dysregulation, polyendocrinopathy, and enteropathy syndrome
LAP:	Latency-associated protein
NK:	Natural killer
SHP:	Src homology 2 domain-containing protein tyrosine phosphatase
SIT:	Specific immunotherapy
SLIT:	Sublingual specific immunotherapy
Treg:	Regulatory T

effector T cells, natural killer (NK) T cells, other effector cells, resident tissue cells, and regulatory T (Treg) cells. In recent years, Treg cells have become a prime target for strategies aimed at inducing tolerance. Immune tolerance in the context of allergy can be defined as persistence of efficacy after discontinuation of treatment, implying an altered allergen-specific memory T- and B-cell response.¹⁻⁴ In addition, prevention of new antigen sensitizations⁵ and prevention of progression to more severe disease, such as the development of asthma after allergic rhinitis,⁶ are essential clinical implications of immune tolerance. The pivotal role of Treg cells in inducing and maintaining immune tolerance has been demonstrated in that their adoptive transfer was shown to prevent or cure several T cell-mediated diseases, including allergy, asthmatic lung inflammation, autoimmune diseases, and allograft rejection, by restoring immune tolerance to allergens, self-antigens, or alloantigens in animal models. In the clinical setting allergen-specific immunotherapy (SIT) has been the only treatment that induces specific Treg cells in human subjects. The role of Treg cells in allergen-specific tolerance, their interaction with other cells in the inflamed tissues, and their role in antibody regulation have been demonstrated in several studies. This review article discusses the role of Treg cells in the treatment of allergy, as well as the healthy immune response to allergens, in the big picture of immune regulation.

EFFECTOR T-CELL SUBSETS

Depending on the adjuvanticity of the substances coexposed with the antigen and the status of the cells and cytokines in the microenvironment, CD4⁺ naive T cells can differentiate into T_H1, T_H2, T_H9, or T_H17 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 (Fig 1). During the development of allergic disease, effector T_H2 cells produce IL-4, IL-5, IL-9, and IL-13,^{1-3,7,8} and probably other recently identified cytokines, such as IL-25, IL-31, and IL-33, contribute to T_H2 response and inflammation.⁹⁻¹³ These cytokines play roles in the production of allergen-specific IgE, eosinophilia, the permissiveness of endothelium for the recruitment of inflammatory cells to inflamed tissues, the production of mucus, and the decreased threshold of contraction of smooth muscles.¹⁴ T_H1 cells also

efficiently contribute to the effector phase in allergic diseases^{15,16} or dampen allergic inflammation, depending on the specific disease model and stage of inflammation.¹⁷ They play a role in apoptosis of the epithelium in asthma and atopic dermatitis,^{15,16} and a predominant T_H2 profile in atopic diseases might be a result of the increased tendency for activation-induced cell death of high IFN- γ -producing T_H1 cells.¹⁸ The discovery of the T_H17 cell is filling 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.¹⁹⁻²³ Neutralization of IL-17 and T_H17-related functions resolves tissue pathology in autoimmune models, improves joint destruction in experimental arthritis, and reduces neutrophil infiltration in an experimental asthma model while increasing eosinophil infiltration.²⁴⁻²⁷ In addition to currently established subsets of effector T cells, several novel subsets of T cells are being identified. For example, distinct T_H2 cell subgroups have been demonstrated to express 1 or 2 of the lineage-specific cytokines but not others.²⁸⁻³⁰ These T cells play a predominant role in either increased eosinophilia or induction of IgE. This depends on whether IL-5 or IL-4, IL-13, or both is the overriding cytokine, respectively. With the identification of new T_H2-like cytokines, such as IL-25, IL-31, and IL-33, it is conceivable that novel functional subgroups of the T_H2 cell subset can be identified.⁹⁻¹³ Supporting the concept of expectation of new T_H cell subsets, it was shown in 2 very recent studies that TGF- β can govern effector T-cell differentiation along a new pathway.^{31,32} 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-10.³² In other words, IL-4 blocks the generation of TGF- β -induced Foxp3⁺ Treg cells and instead induces T_H9 cells.³¹ Adoptive transfer of T_H9 cells into recombination-activating gene 1-deficient mice induced colitis and peripheral neuritis. T_H9 cells lack suppressive function and constitute a distinct population of effector T cells that promote tissue inflammation.^{31,32}

Treg CELLS

During the last 13 years, the concept of Treg cells has received general attention by the scientific community, and excitement about the possibility of these cells in therapeutic applications for the treatment of diseases that are associated with a dysfunction in T-cell regulation has been increased. Many investigations have firmly established the involvement of Treg cells in controlling various aspects of inflammation.³³⁻³⁵ Models of allergic inflammation in mice and various studies in human subjects have demonstrated their essential role in the control of allergic disease (Fig 2). Treg cells are able to inhibit the development of allergic T_H2 responses and play a major role in allergen SIT.^{36,37} Subsets of Treg cells with distinct phenotypes and mechanisms of action include the naturally occurring, thymus-selected CD4⁺CD25⁺ forkhead box protein 3 (FoxP3)⁺ Treg cells and the inducible type 1 Treg cells (T_R1).^{38,39} In addition, subsets of CD8⁺ T cells,⁴⁰⁻⁴² $\gamma\delta$ T cells,⁴³ CD4⁺CD8⁺ T cells,⁴⁴ IL-10-producing B cells,⁴⁵ IL-10-producing NK cells,⁴⁶ IL-10-producing dendritic cells (DCs),⁴⁷ and macrophage subsets with suppressive properties⁴⁸ might contribute to suppressive and regulatory events.³⁹ The triangle interaction of Treg-T effector cells and DCs is important, but T effector and Treg cells were also shown to directly interact with neutrophils,²² B cells,⁴⁹ NK cells,⁵⁰ and NKT cells.⁵¹ In this context investigations of the mechanisms

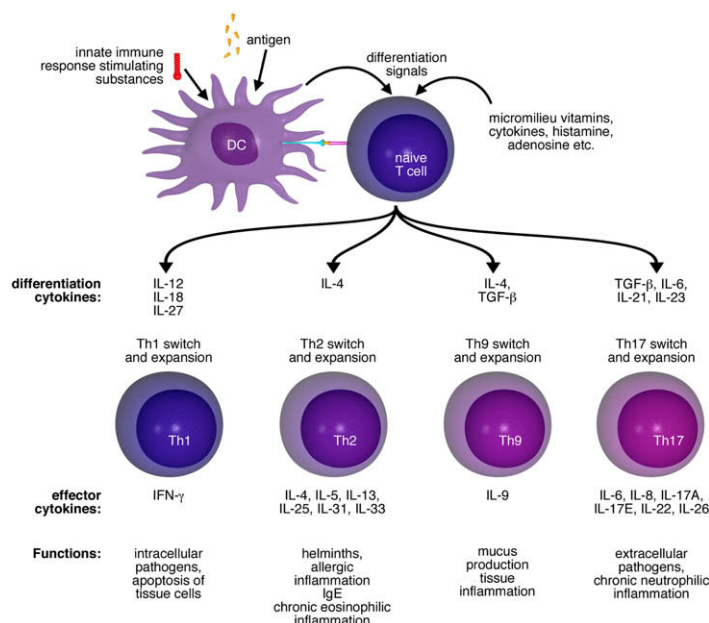


FIG 1. Effector T-cell subsets. After antigen presentation by DCs, naive T cells differentiate into Th1, Th2, Th9, and Th17 effector subsets. Their differentiation requires cytokines and other cofactors that are released from DCs and also expressed in the micromilieu. T-cell activation in the presence of IL-4 enhances differentiation and clonal expansion of Th2 cells, perpetuating the allergic response. IL-12, IL-18, and IL-27 induce Th1 cell differentiation; IL-4 and TGF- β induce Th9 differentiation; and IL-6, IL-21, IL-23, and TGF- β induce the differentiation of Th17 cells.

of Treg cell function have identified an ever-growing list of molecules and interactions that contribute to their suppressive functions. Depending on the nature of the immune response, the eliciting agent, the genetic and immunologic background of the host, and the site of inflammation, different cellular and molecular mechanisms might play dominant roles in immune regulation. As discussed in this review, understanding the immune mechanisms that prevent disease occurrence in nonallergic individuals and evidence for healing of altered regulatory mechanisms in allergic diseases offers promise for new immune interventions.

MECHANISMS OF Treg CELL GENERATION

There has been long-term belief, as well as experimental evidence, that the immune system has inducible peripheral mechanisms of immune tolerance to allergens. DCs not only control immunity but also maintain peripheral tolerance, 2 complementary functions that would ensure the integrity of the organism in an environment full of pathogens and allergens. The tolerogenic function of DCs depends on certain maturation stages and subsets of different types of ontogeny 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 in and are in close contact with commensal bacteria and food antigens/allergens.^{52,53} 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 Foxp3 in naive T cells in the presence of exogenous TGF- β .^{52,53} In addition, DCs expressing CD103 in these 2 compartments can induce Foxp3⁺ T cells in the absence of any exogenous factors, and these *de novo* induced Foxp3⁺ Treg cells express the gut-homing receptors CC chemokine receptor (CCR) 9 and $\alpha 4\beta 7$

integrin.^{52,53} Furthermore, Treg cells can be induced in a microenvironment of tumors and chronic infections because of DCs that promote them. The consequences of these are the maintenance of immune tolerance to tumor antigens, microbial persistence, and limitation of collateral tissue damage.⁵⁴ Immature DCs control peripheral tolerance by inducing the differentiation of Treg cells.⁵⁵ In some cases DCs conditioned by Foxp3⁺ Treg cells; pathogen-derived molecules, such as filamentous hemagglutinin⁵⁶; and exogenous signals, such as histamine through the histamine receptor (HR) 2,⁵⁷ adenosine,⁵⁸ vitamin D3 metabolites,⁵⁹ or retinoic acid,⁶⁰ can induce new populations of Treg cells.

Related to the prevention and development of asthma, airway DCs control the pulmonary immune response and determine tolerance and immunity to newly encountered antigens. Immature DCs are distributed throughout the lungs and capture allergens and migrate to the T-cell area of mediastinal lymph nodes within 12 hours.⁶¹ They express a partially mature phenotype with an intermediate array of costimulatory molecules and induce T-cell tolerance.⁶² Antigen presentation by partially mature airway DCs that express IL-10 induces the formation of T_R1-like cells, which inhibit subsequent inflammatory responses.⁴⁷ In addition, depletion and adoptive transfer of pulmonary plasmacytoid DCs has demonstrated an important role for these cells in protection against allergen sensitization and asthma development in mice.⁶³ Prostaglandin D₂ binds to the D prostanoid (DP) 1 and DP2 receptors and is seen as a critical mediator of asthma, causing vasodilation, bronchoconstriction, and inflammatory cell influx.⁶⁴ The inhalation of a selective DP1 agonist suppresses the cardinal features of asthma-like lung inflammation in mice by targeting the function of lung DCs. An increase in Foxp3⁺CD4⁺ Treg cells that suppressed inflammation in an IL-10-dependent way was observed in mice treated with DP1 agonist or receiving DP1 agonist-treated DCs.⁶⁴ Studies of IL-10 production in the human

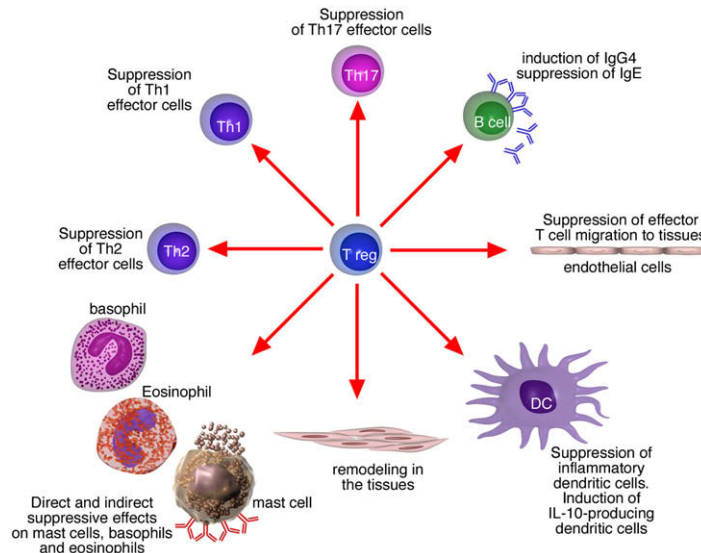


FIG 2. FoxP3⁺CD4⁺CD25⁺ and T_R1 cells contribute to the control of allergen-specific immune responses in several major ways. Suppression of DCs that support the generation of effector T cells; suppression of T_H1, T_H2, and T_H17 cells; suppression of allergen-specific IgE and induction of IgG4, IgA, or both; suppression of mast cells, basophils, and eosinophils; interaction with resident tissue cells and remodeling; and suppression of effector T-cell migration to tissues.

lung demonstrate that there is significantly less IL-10 present in the lungs of patients with asthma than in those of control subjects.⁶⁵ Polymorphisms in the IL-10 gene promoter are similarly associated with low IL-10 levels and more severe asthma.⁶⁶ Inverse correlations between IL-10 levels and disease severity are also observed in allergic patients. For example, wheal size from skin prick tests with allergen was positively associated with *in vitro* IL-5 and IFN- γ responses and negatively associated with IL-10 levels.⁶⁷ Although molecular mechanisms of Treg cell generation *in vivo* remain to be elucidated, some existing therapies for allergic diseases, such as allergen SIT, treatment with glucocorticoids, and β_2 -agonists, seem to function to promote the numbers and activity of IL-10-secreting T_R1-like cells.^{1,68,69}

IL-10 IN IMMUNOREGULATION AND ALLERGIC DISEASE

Suppression of antigen-specific immune responses by IL-10, a known suppressive cytokine of T-cell proliferation and cytokine production, is essential in peripheral tolerance to allergens, autoantigens, transplantation antigens, and tumor antigens.⁷⁰ Multiple mechanisms have been proposed (Table I). In mice IL-10 administration before allergen treatment induces antigen-specific T-cell unresponsiveness and demonstrates the pivotal role of IL-10 in the establishment of peripheral T-cell tolerance.⁷¹ Moreover, inhibition of graft-versus-host disease by IL-10 and allograft rejection in patients with severe combined immunodeficiency undergoing HLA-mismatched bone marrow transplantation provide further evidence for a key role of this cytokine in the induction and maintenance of peripheral tolerance.⁷² Similarly, inappropriate stimulation of tumor-reactive human T cells was shown to result from increased endogenous IL-10 production by these cells, indicating a role for IL-10 in tumor-specific tolerance.⁷³ IL-10-producing antigen-presenting cells, such as B cells⁴⁵ and DCs,⁴⁷ as well as clonally expanded IL-10-producing allergen-specific T_R1 cells,^{36,74} all contribute to the suppressive effects of IL-10.

IL-10 inhibits the proliferative T-cell response in PBMCs to various allergens; however, it does not suppress the proliferative responses of T cells that were stimulated by anti-CD3.⁷⁵⁻⁷⁷ IL-10 inhibits the T-cell proliferation within a certain range of low numbers of triggered T-cell receptors that T cells require for costimulation. 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, direct inhibition on CD2, CD28, and ICOS signaling in T cells occurs through use of Src homology 2 domain-containing protein tyrosine phosphatase (SHP-1) by IL-10.^{76,77} On binding to its receptor, IL-10 activates 2 tyrosine kinases: Janus kinase 1 and tyrosine kinase 2. Tyrosine kinase 2 acts as a constitutive reservoir for SHP-1 tyrosine phosphatase in resting T cells. After activation, SHP-1 rapidly binds to CD28 and ICOS and dephosphorylates them within 1.5 to 3 minutes. In consequence, the binding of phosphatidylinositol 3-kinase to either costimulatory molecule no longer occurs, and downstream signaling is inhibited.⁷⁶ Supporting these findings, spleen cells from SHP-1-deficient mice showed 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-10.⁷⁵⁻⁷⁷ 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-24.⁷⁸ In addition to T cells, IL-10 also exerts inhibitory effect on activated monocytes and macrophages.⁷⁹ 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.⁸⁰ Furthermore, IL-10 induces the expression of the suppressor of cytokine signaling 3 gene, which might play a role in the inhibition of the IFN- γ -induced tyrosine phosphorylation of signal transducer and activator of transcription 1.⁸¹

TABLE I. Mechanisms of action of IL-10 and TGF- β

IL-10	TGF- β
Blocks CD2, CD28, and ICOS costimulatory pathways	Suppresses specific T _H 1, T _H 2, and T _H 17 cells
Inhibits DC maturation	Induces FoxP3 and suppressive function of Treg cells
Decreases MHC class II and costimulatory ligand expression on DCs and monocytes	Downregulates Fc ϵ RI expression on Langerhans cells
Reduces release of proinflammatory cytokines by mast cells	Associates with CTLA-4 expression on T cells
Suppresses IgE	Suppresses IgE
Induces IgG4 and IgA	Induces IgA
Induces IL-10–producing DCs	Induces T _H 9, T _H 17, and Treg cells under different conditions
Synergistic effect in <i>in vivo</i> suppression with TGF- β , CTLA-4, and PD-1	Synergistic effect in <i>in vivo</i> suppression with IL-10, CTLA-4, and PD-1

TABLE II. Components of immune response to allergens and possible involvement of Treg cells in healthy and allergic individuals

Allergen-specific T-cell response	
Healthy	No T-cell proliferation or cytokine response, no sensitization caused by no exposure or low-dose exposure T _H 0 response in PBMCs and specific T-cell clones with low frequency T _R 1, particularly IL-10–dominating response with relatively high frequency
Allergic	T _H 2 response with varying quantities of IL-4, IL-5, and IL-13; detectable IL-10; and IFN- γ
Specific antibodies in serum	
Healthy	Not detectable, no sensitization caused by no exposure or low-dose exposure Detectable IgG1, IgG4, and IgA
Allergic	High amounts of IgG4, detectable IgG1, and detectable IgA and IgE Relatively high amounts of IgE together with low or high amounts of IgG1, IgG4, and IgA
Clinical response	
Healthy	Healthy, no response on skin tests and allergen challenges Skin prick test and specific IgE positivity, clinical disease cannot be induced by provocation tests Skin prick test and specific IgE positivity, clinical disease can be induced by provocation tests and is dose dependent
Allergic	Allergic disease in various clinical forms, remissions and exacerbations related to tissue and specific immune response activation

TGF- β IN IMMUNOREGULATION AND ALLERGIC DISEASE

The TGF- β superfamily consists of more than 35 members, with TGF- β 1 being the proteotypic member. Three TGF- β members (TGF- β 1, TGF- β 2, and TGF- β 3) are present in mammals.⁸² TGF- β is an important pleiotropic cytokine with potent immunoregulatory properties and is essential for the maintenance of immunologic self-tolerance in the CD4⁺ T-cell compartment (Table I).⁸³ TGF- β 1 is synthesized and produced as a latent form that requires activation to be functional. A homodimer of the TGF- β 1 propeptide, the latency-associated protein (LAP), is constitutively associated with the mature TGF- β 1, which prevents TGF- β 1 from binding to its receptors. TGF- β 1 activation proceeds through the degradation of LAP or the alteration of LAP conformation; this can be mediated by proteases, including plasmin, matrix metalloproteinases, thrombospondin 1, and the integrins α v β 6 or α v β 8.⁸⁴ TGF- β 1–deficient mice have the phenotype, caused by activated CD4⁺ T cells, of a rapidly wasting syndrome leading to death by the age of 3 or 4 weeks.^{85,86} The failure to rescue the inflammatory phenotype under germ-free conditions suggests that the T cell–driven inflammation is of autoimmune origin.⁸⁷ TGF- β induces the conversion of naive CD4⁺CD25[–] T cells into CD4⁺CD25⁺ T cells by the induction of FoxP3,⁸⁸ and TGF- β signaling is required for *in vivo* expansion and immunosuppressive capacity of CD4⁺CD25⁺ T cells.⁸⁹ The TGF- β superfamily can act on virtually all mammalian cell types by engaging an intracellular cascade of Smad family proteins through ligand-induced activation of TGF- β receptor kinases.⁹⁰ Activated Smad complexes accumulate in the nucleus to participate in transcriptional activation of target genes, some of which stimulate tumorigenesis, whereas others suppress it. TGF- β receptors are also

able to activate Smad-independent signaling mechanisms, including mitogen-activated protein kinases and phosphoinositide 3-kinase.⁹¹ However, the exact suppressive mechanisms behind TGF- β activation of Smad pathways remain to be elucidated.

In contrast to its known T cell–suppressive activity, some reports imply a role for TGF- β in the pathogenesis of asthma, particularly in the remodeling of injured lung tissue in human subjects.⁹² The increased allergic inflammation observed after blocking of cytotoxic T lymphocyte–associated antigen (CTLA) 4 is clearly associated with decreased TGF- β levels in the bronchoalveolar lavage fluid of these animals.⁹³ Evidence to support the contribution of TGF- β to airway remodeling in asthma is derived from animal model studies with mice deficient in Smad-3, which mediates signaling in response to TGF- β ,⁹⁴ or studies with mice administered an anti-TGF- β antibody.⁹⁵ Neutralizing either TGF- β or TGF- β signaling through Smad-3 significantly reduces peribronchial fibrosis, airway smooth muscle proliferation, and mucus production without an associated significant change in levels of airway inflammation. TGF- β is expressed in the airway in patients with asthma and stimulates fibroblasts to produce extracellular matrix proteins (collagen and fibronectin).^{96,97} In addition, TGF- β decreases the production of enzymes that degrade the extracellular matrix (collagenase) and increases the production of proteins that inhibit enzymes that degrade the extracellular matrix (tissue inhibitor of metalloprotease).⁹⁷ The net effect of TGF- β acting on fibroblasts is to increase the production of extracellular matrix proteins. There is evidence that the subepithelial fibrosis component of airway remodeling in asthma is mediated through induction of TGF- β expression, with consequent activation of myofibroblasts to produce extracellular matrix

proteins, such as collagen.⁹² TGF- β expression correlates with the degree of subepithelial fibrosis, and levels of TGF- β are significantly increased in patients with severe asthma who have prominent airway eosinophilic inflammation.⁹⁸ An important role for eosinophil-expressed TGF- β in airway remodeling in human subjects with asthma is also suggested from studies in which TGF- β levels in the lungs of patients with asthma were reduced by depleting eosinophils that express TGF- β .⁹⁹ In these studies with anti-IL-5 (which depletes eosinophils), there was a parallel decrease in levels of airway TGF- β , eosinophil TGF- β expression, and associated airway remodeling.⁹⁹ These studies suggest that eosinophil expression of TGF- β is an important contributor to airway remodeling in human asthma.

Treg CELLS IN ALLERGEN-SPECIFIC IMMUNE RESPONSE

Allergen-specific T-cell response in healthy individuals and Treg cells

Studies on T-cell response to allergens in healthy individuals have demonstrated a wide range of immune responses, from no detectable T-cell response to involvement of active peripheral tolerance mechanisms mediated by different subsets of Treg cells (Table II). In a high number of healthy individuals, T cells do not show any proliferative response to allergens in PBMC cultures. This can be due to a low frequency of specific T cells because of a lifetime lack of exposure. If a detectable allergen-specific T-cell response is mounted in a nonallergic individual, active suppression against allergens takes place in cultures by T_R1 cells or CD4⁺FoxP3⁺ Treg cells.^{37,57,74,100}

The human *in vivo* relevance of Foxp3 is understood after the discovery of X-linked immune dysregulation, polyendocrinopathy, and enteropathy syndrome (IPEX).^{101,102} Various allergic and autoimmune phenotypes spontaneously develop in patients with IPEX when CD4⁺CD25⁺ Treg cells are nonfunctional, indicating the presence of a population of professional suppressor cells. IPEX is characterized by an allergic phenotype with dermatitis and hyper-IgE syndrome and an autoimmune phenotype with enteropathy, type I diabetes, thyroiditis, hemolytic anemia and thrombocytopenia, and results from mutations of FOXP3. A patient was described with clinical manifestations of IPEX who had a normal FOXP3 gene but CD25 deficiency caused by autosomal recessive mutations in this gene.¹⁰³ This patient exhibited defective IL-10 expression from CD4 lymphocytes, whereas a FOXP3-deficient patient expressed normal levels of IL-10. These data show that CD25 deficiency results in an IPEX-like syndrome and suggests that FOXP3 is not required for normal IL-10 expression by human CD4 lymphocytes, whereas CD25 expression is important. Similarly, deletion in the forkhead domain of FoxP3 in scurfy mice results in failure to develop thymus-derived naturally occurring Treg cells and presents with lymphoproliferative disease, hyper-IgE levels, and eosinophilia in the absence of T_H2 skewing, with the death of mice around 3 weeks.¹⁰⁴⁻¹⁰⁶ The relevance of immune suppressive function of FOXP3⁺CD4⁺CD25⁺ Treg cells in human allergies has been studied. Impaired skin infiltration of CD4⁺CD25⁺FOXP3⁺ T cells was observed in acute atopic dermatitis lesions,¹⁰⁷ and FOXP3 mRNA expression was significantly increased in asthmatic patients receiving glucocorticoid treatment.⁶⁹ Similar findings were observed in patients with chronic rhinosinusitis with nasal polyps, with decreased FOXP3 expression and a downregulation of TGF- β 1 levels compared

with values seen in control subjects and patients with chronic rhinosinusitis without nasal polyps.¹⁰⁸ Supporting these findings, the accumulation of FoxP3⁺ Treg cells in local draining lymph nodes of the lung correlates with spontaneous resolution of chronic asthma in a murine model.¹⁰⁹ CD25^{hi}CD4⁺ T cells expressing FoxP3 and cutaneous lymphocyte-associated antigen were increased in patients with atopic dermatitis and were linked to disease severity.¹¹⁰ Two subtypes of CD25^{hi} T cells were identified on the basis of differential expression of CCR6. Despite a regulatory phenotype, activated CD25^{hi} T cells that lack expression of CCR6 were found to promote T_H2 responses.¹¹⁰

It was found in allergic children that Treg cells increase during the pollen season.¹¹¹ Whether these CD4⁺CD25^{hi} 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^{hi}Foxp3⁺ Treg cells do not show a major difference between nonatopic and atopic individuals.¹¹² 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.¹¹³ Another study on healthy immune response to allergens demonstrated that 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 values seen in children who maintained clinically active allergy.¹¹⁴ Studies have been reported in other diseases in the same line. The *in vitro* proliferative response to nickel of human CD4⁺ T cells from healthy nonallergic individuals was strongly augmented when CD4⁺CD25⁺ Treg cells were depleted.¹¹⁵

In a high-dose allergen exposure model in human subjects, repeated exposure of nonallergic healthy beekeepers to bee venom antigens during the beekeeping season represents a valuable model to ascertain mechanisms of T-cell tolerance induction. After multiple bee stings, venom antigen-specific T_H1 and T_H2 cells show clonality and switch toward IL-10-secreting T_R1 cells, which results in diminished T cell-related cutaneous late-phase responses in parallel with suppressed allergen-specific T-cell proliferation and T_H1 and T_H2 cytokine secretion.⁵⁷ This regulation is marked as long as venom exposure persists and returns to initial levels within 2 to 3 months after bee stings. In the same model the upregulation of HR2 on specific T_H2 cells suppresses allergen-stimulated T cells and increases IL-10 production.⁵⁷

By using IFN- γ -, IL-4-, and IL-10-secreting allergen-specific CD4⁺ T cells that resemble T_H1-, T_H2-, and T_R1-like cells, respectively, it was shown that both healthy and allergic individuals exhibit all 3 subsets, although in different proportions. In healthy individuals who show detectable IgG antibodies against allergen, T_R1 cells represent the dominant subset for common environmental allergens, whereas a high frequency of allergen-specific IL-4-secreting T cells is found in allergic individuals. Therefore the frequency of effector T_H2 cells or T_R1 cells is decisive in the development of allergy or a healthy immune response.⁷⁴ Allergen-specific T_R1 cells use IL-10, TGF- β , CTLA-4, and PD1 as suppressor molecules.^{57,74}

Human CD4⁺ T_H1 cells predominantly express HR1 and CD4⁺ T_H2 cells predominantly express HR2, which results in their differential regulation by histamine.¹¹⁶ Histamine induces the production of IL-10 by DCs.¹¹⁷ In addition, histamine induces IL-10

production by T_H2 cells^{57,118} and enhances the suppressive activity of TGF- β on T cells.¹¹⁹ 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.^{57,116} Apparently, these findings suggest that HR2 might represent an essential receptor that participates in peripheral tolerance to allergens.

Allergen SIT and Treg cells

The induction of a tolerant state in peripheral T cells represents an essential step in allergen SIT. Peripheral T-cell tolerance is characterized mainly by generation of allergen-specific Treg cells and suppressed proliferative and cytokine responses against the major allergen.¹²⁰ It is initiated by the autocrine action of IL-10 and TGF- β , which are increasingly produced by the antigen-specific T_R1 cells.^{36,37,121} 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 . However, these cells do express CD4 and CD25, raising the question of whether these are inducible T_R1 cells that have upregulated CD25 or naturally occurring $CD4^+CD25^+$ Treg cells that produce suppressive cytokines.¹²² There is some evidence in adults that circulating $CD4^+CD25^+$ Treg cells and IL-10- and TGF- β -secreting T_R1 cells represent overlapping populations. 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 after allergen SIT.^{37,100} It has been suggested that upregulation of $CD4^+CD25^+$ Treg cells plays a role in allergen SIT. Recently, it was shown that grass pollen immunotherapy increased the expression of mucosal and peripheral T-cell IL-10¹²³ and TGF- β .¹²⁴ The demonstration of local FOXP3⁺CD25⁺ T cells in the nasal mucosa and their increased numbers after immunotherapy supported the role of Treg cells in the induction of allergen-specific tolerance in human subjects.¹²⁵ Their increased numbers correlated with clinical efficacy and suppression of seasonal allergic inflammation.¹²⁵

The findings observed in sublingual specific immunotherapy (SLIT) seem to be similar to those seen with injection SIT. Increases in serum allergen-specific IgG4 levels,^{126,127} decreases in allergen-stimulated T-cell proliferation,¹²⁸ induction of IL-10 in T cells,¹²⁸⁻¹³⁰ suppression of T_H2 cells,¹²⁹ and decreased eosinophilia and eosinophil migration to the nose in response to allergen challenge^{131,132} have been reported. However, a significant number of studies have not detected any immunologic changes.^{126,133} In a recent study after 4 weeks of SLIT, higher frequencies of circulating $CD4^+CD25^+$ T cells were detected together with increased FoxP3 and IL-10 and reduced IL-4 and IFN- γ mRNA expression compared with expression seen before SLIT.¹³⁰ Proliferation to all 3 antigens was markedly reduced but increased significantly after depletion of $CD25^+$ cells or addition of anti-IL-10 antibodies. Neither TGF- β levels nor cell-cell contact-mediated suppression of $CD4^+CD25^+$ cells changed during the course of SLIT. Current understanding of mechanisms of allergen SIT, particularly the role of Treg cells in peripheral tolerance, opens a new window for novel treatment strategies.

To date, clinical trials of peptide immunotherapy have been performed for 2 different allergies, and evidence for the induction of peripheral T-cell tolerance to whole allergen has been demonstrated. Synthetic peptide-based vaccines from the major cat allergen Fel d 1 and bee venom-derived phospholipase A₂ have been developed and clinically evaluated.¹³⁴⁻¹³⁶ Mixtures of short

peptides demonstrated downregulation of systemic T_H1 and T_H2 cell responses to allergen,¹³⁴ together with concomitant induction of IL-10 production.^{136,137}

Allergen-specific antibody response in nonallergic individuals and allergen SIT: Relationship to Treg cells

Allergen-specific antibody response in healthy individuals ranges from no detectable antibodies to low or sometimes relatively high amounts of specific IgG4, IgG1, and IgA response in the presence or absence of low amounts of specific IgE (Table II).^{37,138} No exposure or no efficient antigen presentation (exposure in small doses) might be responsible for no antibody response. Low amounts of exposure leading to immunologic ignorance caused by no accessibility, short persistence of the allergens in secondary lymphoid organs, or both has been demonstrated in mouse models.¹³⁹ Short persistence and low dose might not be as demonstrative in human subjects as in mice because a single bee sting can induce IgE and IgG and relatively very small doses of T-cell epitope peptides injected into skin can initiate allergic reactions.^{135,140,141} One honeybee sting releases approximately 50 to 140 μ g of venom per sting and contains 5 to 15 μ g of the major allergen phospholipase A₂. A single honeybee sting is sufficient to induce IgE or IgG production against phospholipase A₂.¹⁴¹ Wasp venom has approximately 10 to 20 times less protein content (1.7-3.1 μ g) compared with honeybee venom; however, again one wasp sting is sufficient to induce IgE- or IgG-type antibodies against single allergic components, some of which are less than 10% of the protein content.¹⁴¹ This means that 0.1 to 0.3 μ g of allergen directly injected into the skin is sufficient to initiate an antibody response. However, it has to be noted here that the immune response does not only depend on the allergen dose as a single protein antigen. Many other molecules in the content of the venom, such as hyaluronidase, can show adjuvant effects and influence the diffusion of venom.¹⁴²

Interestingly, nonallergic beekeepers have approximately 1000 times higher specific IgG4 ratios versus specific IgE ratios compared with those seen in allergic individuals.¹⁴³ The level of specific IgG4 primarily reflects exposure. In beekeepers bee venom-specific IgG4 levels correlate to the number of annual stings and to the number of years spent in beekeeping. High-dose exposure appears to induce clinical tolerance. Forty-five percent of beekeepers who were stung less than 25 times a year had a history of systemic allergic reactions to bee venom when compared with no allergy in those with more than 200 stings per year.¹⁴¹ High-dose exposure, increased specific IgG4 levels, and protection against allergy have also been demonstrated in high contact with cats and the low occurrence of asthma and allergies in children.¹⁴⁴ IgG4 has unique structural and functional features among IgG subsets. Its heavy chains can exchange in a way that leads to functional monomeric antibodies, it has low affinity for certain Fc γ receptors, and it does not activate complement.¹⁴⁵ IgG4 antibodies exchange Fab arms by swapping a heavy chain and attached light chain (half-molecule) with a heavy/light chain pair from another molecule, which results in bispecific antibodies.¹⁴⁶ As such, IgG4 is uniquely capable of inhibiting immune complex formation by other isotypes and thus might have an anti-inflammatory role in immunity.

Although peripheral tolerance has been demonstrated in specific T cells, the ability of B cells to produce specific IgE

antibodies is not eliminated during allergen SIT.¹²⁰ SIT frequently induces a transient increase in serum specific IgE levels, however, followed by a gradual decrease over months or years of treatment.^{147,148} In pollen-sensitive patients allergen SIT prevents the increase of serum specific IgE levels during the pollen season.¹⁴⁹ Serum levels of both specific IgE and IgG4 antibodies increase during the early phase of treatment. However, the increase in antigen-specific IgG4 levels is more striking, and therefore the ratio of specific IgE to IgG4 significantly decreases between 6 months to 3 years. A similar change in specific isotype ratio has been observed in SIT of various allergies.^{120,123} Moreover, IL-10 produced and progressively secreted during allergen SIT appears to counterregulate synthesis of antigen-specific IgE and IgG4 antibodies. IL-10 potentially suppresses both total and allergen-specific IgE production while simultaneously increasing IgG4 production.^{36,150} Therefore IL-10 not only generates T-cell tolerance, it also regulates specific isotype formation and skews the specific IgE response toward an IgG4-dominated phenotype.

Direct influence of T_R1 and Foxp3⁺ Treg cells on B cells and the induction of IgG4 and suppression of IgE has been demonstrated in cell cultures of peripheral blood cells of healthy individuals.⁴⁹ In a mouse model of food allergy, it was observed that antigen-specific secretory IgA antibody levels in the gut were decreased, suggesting a role for secretory IgA in peripheral tolerance to foods. Peyer's patch CD31 cells were primarily involved by favoring IgA production through the release of IL-10 and TGF- β , and low IL-10 production in Peyer's patches favored the symptoms of food allergy.¹⁵¹ In conjunction with the role of TGF- β and IL-10 in IgA regulation in childhood asthma with house dust mite allergy, 6 and 12 months of sublingual immunotherapy downregulated the specific IgE response while slightly increasing specific IgA levels.¹⁵²

There is accumulating evidence that SIT also influences the blocking activity on IgE-mediated responses caused by IgG4, and cellular assays are commonly used to investigate these changes. An assay that detects allergen/IgE binding by using flow cytometry has been used to detect "functional" SIT-induced changes in IgG antibody activity. 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.¹⁵³ It seems to be relevant to measure the blocking activity of allergen-specific IgG or IgG subsets, particularly IgG4 and also IgG1, rather than the crude levels in sera. In this context the role of anti-IgE treatment in the induction phase of allergen SIT on safety and efficacy has been questioned. Anti-IgE mAb pretreatment enhances the safety of SIT for allergic rhinitis and might be an effective strategy to permit more rapid and higher doses of allergen immunotherapy.^{154,155} Its function on long-term efficacy is still under investigation.

Suppression of effector cells and inflammatory responses by Treg cells

Allergen SIT efficiently modulates the thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release.¹⁵⁶ IL-10 was shown to reduce proinflammatory cytokine release from mast cells.¹⁵⁷ In addition, IL-10 downregulates eosinophil function and activity and suppresses IL-5 production by human resting T_H0 and T_H2 cells.¹⁵⁸ Although demonstrated 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.¹⁵⁹ It was

recently explored whether Treg cells influence the immediate hypersensitivity response of mast cells.¹⁶⁰ Treg cells directly inhibited the Fc ϵ RI-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 adenosine monophosphate concentrations and reduced Ca^{++} influx. The *in vivo* depletion or inactivation of Treg cells caused enhancement of the anaphylactic response.¹⁶⁰ The demonstrated crosstalk between Treg cells and mast cells defines a key mechanism controlling mast cell degranulation. Loss of this interaction might contribute to the severity of allergic responses.

Long-term SIT is associated with a 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 mast cell-mediated immediate reaction and involves the recruitment, activation, and persistence of eosinophils and activated T cells at the sites of allergen exposure. Successful allergen SIT results not only in the increase of allergen concentration necessary to induce an immediate or late-phase reaction in the target tissue but also in the decreased responses to nonspecific stimulation. Bronchial, nasal, and conjunctival hyperreactivity to nonspecific stimuli, which seem to reflect underlying mucosal inflammation, decrease after SIT and correlate with clinical improvement.¹⁶¹ During birch pollen SIT, reduced plasma levels of eosinophil cationic protein, a marker of eosinophil activation, as well as chemotactic factors for eosinophils and neutrophils, correlated with decreased bronchial hyperreactivity and clinical improvement.¹⁶² Inhibition by SIT of the seasonal increase in eosinophil priming has also been demonstrated.¹⁶³ In biopsy specimens taken during grass pollen SIT, decreased eosinophil and mast cell infiltration in nasal and bronchial mucosa after SIT correlated with the anti-inflammatory effect.¹⁶⁴

CONCLUSION

Understanding the mechanisms of action of allergic diseases might enlighten the complex interactions of effector cells with tissues and Treg cells. Peripheral T-cell tolerance to allergens, which is also achieved by means of successful allergen SIT, represents the key mechanism in healthy immune responses to allergens. Both built-in and inducible components of the immune system characterized by CD4⁺CD25⁺FoxP3⁺ Treg and T_R1 cells, suppressive cytokines (eg, IL-10 and TGF- β), and noninflammatory antibody isotypes (eg, IgG4 and IgA) organize these responses. In addition to allergy, these mechanisms might have implications in autoimmunity, organ transplantation 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 and immune tolerance to chronic infectious agents, which prevents complete neutralization. Changes in the fine balance between allergen-specific Treg and T_H2 , T_H17 , and/or T_H1 cells are very crucial in both the development and treatment of allergic diseases. In addition to the treatment of established allergy, it is essential to consider prophylactic approaches 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. Taking these findings into account along with recent advances in knowledge of Treg cells and related peripheral tolerance mechanisms, developments of safer approaches and better treatment of allergy, asthma, and other immune-mediated diseases are coming.

What do we know?

Allergen SIT induces:

- Increased IL-10-secreting T_R1-like cells
- Increased suppressive capacity of T_R1 and CD4⁺CD25⁺ Treg cells
- Decreased allergen-specific T-cell proliferation
- Decreased T_H1 and T_H2 cytokines
- Increased IL-10 levels in venom and IL-10 and TGF-β levels in aeroallergen SIT
- Increased specific IgG4 levels (specific IgG4 is induced by IL-10 and T_R1 and CD4⁺CD25⁺ Treg cells)
- Multiple suppressor factors, such as IL-10, TGF-β, IL-10 receptor, TGF-β receptor, CTLA-4, PD-1, and HR2
- Decreased clinical and experimental late-phase response
- Decreased tissue mast cells and eosinophils and their mediators
- Increased IL-10, TGF-β, and CD4⁺CD25⁺FoxP3⁺ Treg cells in nasal mucosa

Also:

- IL-10 and T_R1 cells, as well as IgG4, play a role in tolerance to high-dose allergen exposure in bee venom and cat.
- Peptide immunotherapy and SLIT induce similar Treg-related mechanisms.
- Treatment with glucocorticoids and β₂-agonists promote the numbers and activity of IL-10-secreting T_R1-like cells.

What is still unknown?

- Molecular mechanisms of Treg cell generation *in vivo*
- Adjuvants that directly induce Treg cells
- Longevity of Treg cells after allergen SIT
- Whether adoptive transfer therapies will be possible in human subjects
- How to balance the harmful role of Treg cells, such as immune tolerance to tumor antigens and chronic infectious agents
- The use of Treg cells and their markers as a predictive determinant for patient selection and success of allergen SIT
- Clinical effects of vitamin D3, retinoic acid, HR2, and adenosine receptors targeting strategies in the induction of Treg cells and benefit to patients
- Contribution of tissue cells to immune tolerance
- Mechanisms of spontaneous healing and remissions and exacerbations of allergic disease and the contributions of Treg cells in these processes
- Mechanisms and means for short-term success in allergen SIT

REFERENCES

1. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2007;119:780-91.
2. Akdis M. Healthy immune response to allergens: T regulatory cells and more. *Curr Opin Immunol* 2006;18:738-44.
3. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006;6:761-71.
4. 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.
5. 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.
6. 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.
7. Berin MC, Shreffler WG. T(H)2 adjuvants: implications for food allergy. *J Allergy Clin Immunol* 2008;121:1311-22.
8. 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.
9. 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.
10. 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.
11. Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Pressnell SR, et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol* 2004;5:752-60.
12. 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.
13. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Drug Discov* 2008;7:827-40.
14. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol* 2004;113:395-400.
15. Trautmann A, Akdis M, Kleemann D, Altnauer 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.
16. Trautmann A, Schmid-Grendelmeier P, Krüger K, Cramer 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.
17. 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.
18. Akkoc T, de Koning PJ, Ruckert B, Barlan I, Akdis M, Akdis CA. Increased activation-induced cell death of high IFN-γ-producing T(H)1 cells as a mechanism of T(H)2 predominance in atopic diseases. *J Allergy Clin Immunol* 2008;121:652-8.
19. 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 [Epub ahead of print].
20. 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.
21. 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.
22. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-β induces development of the T(H)17 lineage. *Nature* 2006;441:231-4.
23. 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.
24. 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.
25. 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.

26. 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.
27. 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.
28. Schmidt-Weber CB, Akdis M, Akdis CA. TH17 cells in the big picture of immunology. *J Allergy Clin Immunol* 2007;120:247-54.
29. Scott JT, Turner C, Mutapi F, Woolhouse ME, Chandiwana SK, Mdluluzi T, et al. Dissociation of interleukin-4 and interleukin-5 production following treatment for *Schistosoma haematobium* infection in humans. *Parasite Immunol* 2000;22:341-8.
30. Mary C, Auriault V, Faugere B, Dessein AJ. Control of *Leishmania infantum* infection is associated with CD8(+) and gamma interferon- and interleukin-5-producing CD4(+) antigen-specific T cells. *Infect Immun* 1999;67:5559-66.
31. 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.
32. Veldhoen M, Uttenhove C, van Snick J, Helmbj 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.
33. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994;265:1237-40.
34. Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. Regulatory interactions between CD45RBhigh and CD45RBlow CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J Exp Med* 1994;179:589-600.
35. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, De Vries JE, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;389:737-42.
36. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998;102:98-106.
37. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszczyk 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.
38. Robinson DS, Larche M, Durham SR. Tregs and allergic disease. *J Clin Invest* 2004;114:1389-97.
39. Akdis CA, Blaser K, Akdis M. Genes of tolerance. *Allergy* 2004;59:897-913.
40. Smith TR, Kumar V. Revival of CD8+ Treg-mediated suppression. *Trends Immunol* 2008;29:337-42.
41. Sigmund K, Ruckert B, Ouaked N, Burgler S, Speiser A, Akdis CA, et al. Unique phenotype of human tonsillar and in vitro-induced FOXP3+CD8+ T cells. *J Immunol* 2009;182:2124-30.
42. Hu D, Ikizawa K, Lu L, Sanchirico ME, Shinohara ML, Cantor H. Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat Immunol* 2004;5:516-23.
43. Seo N, Tokura Y, Takigawa M, Egawa K. Depletion of IL-10- and TGF-beta-producing regulatory gamma delta T cells by administering a daunomycin-conjugated specific monoclonal antibody in early tumor lesions augments the activity of CTLs and NK cells. *J Immunol* 1999;163:242-9.
44. Strober S, Cheng L, Zeng D, Palathumpram 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.
45. Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med* 2003;197:489-501.
46. Deniz G, Erten G, Kucuksezer UC, Kocacik D, Karagiannis C, Aktas E, et al. Regulatory NK cells suppress antigen-specific T cell responses. *J Immunol* 2008;180:850-7.
47. 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.
48. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. *J Leukoc Biol* 2006;80:1298-307.
49. 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.
50. Zimmer J, Andres E, Hentges F. NK cells and Treg cells: a fascinating dance cheek to cheek. *Eur J Immunol* 2008;38:2942-5.
51. Santodomingo-Garzon T, Han J, Le T, Yang Y, Swain MG. Natural killer T cells regulate the homing of chemokine CXCR3-positive regulatory T cells to the liver in mice. *Hepatology* 2008 [Epub ahead of print].
52. 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.
53. 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.
54. Belkaid Y. Role of Foxp3-positive regulatory T cells during infection. *Eur J Immunol* 2008;38:918-21.
55. 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.
56. 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.
57. Meiler F, Zunker S, 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.
58. Csoka B, Himer L, Selmecezy 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. Vermaelen KY, Carro-Muino I, Lambrecht BN, Pauwels RA. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J Exp Med* 2001;193:51-60.
62. Lambrecht BN, Pauwels RA, Fazekas De St Groth B. Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *J Immunol* 2000;164:2937-46.
63. 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.
64. Hammad H, Kool M, Soullie T, Narumiya S, Trottein F, Hoogsteden HC, et al. Activation of the D prostanoid 1 receptor suppresses asthma by modulation of lung dendritic cell function and induction of regulatory T cells. *J Exp Med* 2007;204:357-67.
65. Borish L, Aarons A, Rumblyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol* 1996;97:1288-96.
66. Lim S, Crawley E, Woo P, Barnes PJ. Haplotype associated with low interleukin-10 production in patients with severe asthma. *Lancet* 1998;352:113.
67. Heaton T, Rowe J, Turner S, Aalberse RC, de Klerk N, Suriyaarachchi D, et al. An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet* 2005;365:142-9.
68. Peek EJ, Richards DF, Faith A, Lavender P, Lee TH, Corrigan CJ, et al. Interleukin-10-secreting "regulatory" T cells induced by glucocorticoids and beta2-agonists. *Am J Respir Cell Mol Biol* 2005;33:105-11.
69. Karagiannis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, et al. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol* 2004;114:1425-33.
70. O'Garra A, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C. Strategies for use of IL-10 or its antagonists in human disease. *Immunol Rev* 2008;223:114-31.
71. Enk AH, Saloga J, Becker D, Mohamadizadeh M, Knop J. Induction of hapten-specific tolerance by interleukin 10 in vivo. *J Exp Med* 1994;179:1397-402.
72. Bacchetta R, Bigler M, Touraine JL, Parkman R, Tovo PA, Abrams J, et al. High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J Exp Med* 1994;179:493-502.
73. Becker JC, Czerny C, Brocker EB. Maintenance of clonal anergy by endogenously produced IL-10. *Int Immunol* 1994;6:1605-12.
74. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannis C, Cramer 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.
75. 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.

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. Oral HB, Kosenko 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.
79. 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.
80. 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.
81. 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.
82. Li MO, Flavell RA. Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10. *Immunity* 2008;28:468-76.
83. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Immunol* 1998;16:137-61.
84. Mazzieri R, Jurukovski V, Obata H, Sung J, Platt A, Annes E, et al. Expression of truncated latent TGF-beta-binding protein modulates TGF-beta signaling. *J Cell Sci* 2005;118:2177-87.
85. Diebold RJ, Eis MJ, Yin M, Ormsby I, Boivin GP, Darrow BJ, et al. Early-onset multifocal inflammation in the transforming growth factor beta 1-null mouse is lymphocyte mediated. *Proc Natl Acad Sci U S A* 1995;92:12215-9.
86. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992;359:693-9.
87. Boivin GP, Ormsby I, Jones-Carson J, O'Toole BA, Doetschman T. Germ-free and barrier-raised TGF beta 1-deficient mice have similar inflammatory lesions. *Transgenic Res* 1997;6:197-202.
88. 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.
89. 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.
90. Kunzmann S, Wohlfahrt JG, Itoh S, Asao H, Komada M, Akdis CA, et al. SARA and Hgs attenuate susceptibility to TGF-beta1-mediated T cell suppression. *FASEB J* 2003;17:194-202.
91. Massague J. How cells read TGF-beta signals. *Nat Rev Mol Cell Biol* 2000;1:169-78.
92. Broide DH. Immunologic and inflammatory mechanisms that drive asthma progression to remodeling. *J Allergy Clin Immunol* 2008;121:560-72.
93. Hellings PW, Vandenberghe P, Kasran A, Coorevits L, Overbergh L, Mathieu C, et al. Blockade of CTLA-4 enhances allergic sensitization and eosinophilic airway inflammation in genetically predisposed mice. *Eur J Immunol* 2002;32:585-94.
94. Le AV, Cho JY, Miller M, McElwain S, Golgotiu K, Broide DH. Inhibition of allergen-induced airway remodeling in Smad 3-deficient mice. *J Immunol* 2007;178:7310-6.
95. McMillan SJ, Xanthou G, Lloyd CM. Manipulation of allergen-induced airway remodeling by treatment with anti-TGF-beta antibody: effect on the Smad signaling pathway. *J Immunol* 2005;174:5774-80.
96. Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, et al. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003;111:1293-8.
97. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007;117:524-9.
98. Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, et al. Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1996;15:404-9.
99. Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029-36.
100. 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.
101. Chatila TA, Blaese 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 dysregulation syndrome. *J Clin Invest* 2000;106:R75-81.
102. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001;27:18-20.
103. Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. *J Allergy Clin Immunol* 2007;119:482-7.
104. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003;4:330-6.
105. Lin W, Truong N, Grossman WJ, Haribhai D, Williams CB, Wang J, et al. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J Allergy Clin Immunol* 2005;116:1106-15.
106. Ochs HD, Ziegler SF, Torgerson TR. FOXP3 acts as a rheostat of the immune response. *Immunol Rev* 2005;203:156-64.
107. Verhagen J, Akdis M, Traidl-Hoffmann C, Schmid-Grendelmeier P, Hijnen D, Knol EF, et al. Absence of T-regulatory cell expression and function in atopic dermatitis skin. *J Allergy Clin Immunol* 2006;117:176-83.
108. Van Bruene N, Perez-Novo CA, Basinska TM, Van Zele T, Holtappels G, De Ruyck N, et al. T-cell regulation in chronic paranasal sinus disease. *J Allergy Clin Immunol* 2008;121:1435-41.
109. Carson WF 4th, Guernsey LA, Singh A, Vella AT, Schramm CM, Thrall RS. Accumulation of regulatory T cells in local draining lymph nodes of the lung correlates with spontaneous resolution of chronic asthma in a murine model. *Int Arch Allergy Immunol* 2008;145:231-43.
110. Reefer AJ, Satinover SM, Solga MD, Lannigan JA, Nguyen JT, Wilson BB, et al. Analysis of CD25hiCD4+ "regulatory" T-cell subtypes in atopic dermatitis reveals a novel T(H)2-like population. *J Allergy Clin Immunol* 2008;121:415-22.
111. 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.
112. 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.
113. 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.
114. 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.
115. Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pita O, Girolomoni G. Human CD25+ regulatory T cells maintain immune tolerance to nickel in healthy, non-allergic individuals. *J Immunol* 2003;171:5760-8.
116. 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.
117. 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.
118. Osna N, Elliott K, Khan MM. Regulation of interleukin-10 secretion by histamine in TH2 cells and splenocytes. *Int Immunopharmacol* 2001;1:85-96.
119. 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.
120. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Muller 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.
121. 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.
122. 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.
123. 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.
124. Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, et al. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. *J Immunol* 2007;178:4658-66.
125. 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.

126. Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006;61:151-65.
127. Rossi RE, Monasterolo G, Coco G, Silvestro L, Operti D. Evaluation of serum IgG4 antibodies specific to grass pollen allergen components in the follow up of allergic patients undergoing subcutaneous and sublingual immunotherapy. *Vaccine* 2007;25:957-64.
128. Burastero SE, Mistrello G, Falagiani P, Paolucci C, Breda D, Roncarolo D, et al. Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. *Ann Allergy Asthma Immunol* 2008;100:343-50.
129. 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.
130. Bohle B, Kinaciyan T, Gerstmayr M, Radakovic A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007;120:707-13.
131. Passalacqua G, Albano M, Riccio A, Fregonese L, Puccinelli P, Parmiani S, et al. Clinical and immunologic effects of a rush sublingual immunotherapy to *Parietaria* species: a double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 1999;104:964-8.
132. Lue KH, Lin YH, Sun HL, Lu KH, Hsieh JC, Chou MC. Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. *Pediatr Allergy Immunol* 2006;17:408-15.
133. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005;60:4-12.
134. Muller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;101:747-54.
135. Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999;189:1885-94.
136. Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;360:47-53.
137. 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.
138. Pereira EA, Silva DA, Cunha-Junior JP, Almeida KC, Alves R, Sung SJ, et al. IgE, IgG1, and IgG4 antibody responses to *Blomia tropicalis* in atopic patients. *Allergy* 2005;60:401-6.
139. Zinkernagel RM, Ehl S, Aichele P, Oehen S, Kundig T, Hengartner H. Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. *Immunol Rev* 1997;156:199-209.
140. Müller UR, Mosbech H. Position paper: immunotherapy with hymenoptera venoms. *Allergy* 1993;48:36-46.
141. Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005;60:1339-49.
142. Girish KS, Kemparaju K. Inhibition of *Naja naja* venom hyaluronidase: role in the management of poisonous bite. *Life Sci* 2006;78:1433-40.
143. Carballido JM, Carballido-Perrig N, Kägi MK, Meloen RH, Wüthrich B, Heusser CH, et al. T cell epitope specificity in human allergic and non-allergic subjects to bee venom phospholipase A2. *J Immunol* 1993;150:3582-91.
144. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001;357:752-6.
145. Aalberse RC, Schuurman J. IgG4 breaking the rules. *Immunology* 2002;105:9-19.
146. 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.
147. Van Ree R, Van Leeuwen WA, Dieges PH, Van Wijk RG, De Jong N, Brewczynski 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.
148. 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.
149. Bousquet J, Maasch H, Martinot B, Hejjoui 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.
150. Punnonen J, De Waal Malefyt R, Van Vlasselaer P, Gauchat J-F, De Vries JE. IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. *J Immunol* 1993;151:1280-9.
151. Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. *J Allergy Clin Immunol* 2004;114:377-82.
152. Bahceciler NN, Arikian 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.
153. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. *Curr Opin Allergy Clin Immunol* 2004;4:313-8.
154. Casale TB, Busse WW, Kline JN, Ballas ZK, Moss MH, Townley RG, et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 2006;117:134-40.
155. Klunker S, Saggat LR, Seyfert-Margolis V, Asare AL, Casale TB, Durham SR, et al. Combination treatment with omalizumab and rush immunotherapy for ragweed-induced allergic rhinitis: inhibition of IgE-facilitated allergen binding. *J Allergy Clin Immunol* 2007;120:688-95.
156. 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.
157. 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.
158. 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.
159. 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.
160. 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.
161. 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.
162. Rak S, Hakansson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. *J Allergy Clin Immunol* 1990;86:706-13.
163. 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.
164. 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.