

Regulatory T cells in allergic diseases



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

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Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations: T. Chatila has received research support from the National Institutes of Health and consultancy fees from Merck. M. Noval Rivas declares no relevant conflict of interest.

Activity Objectives:

1. To understand the distinction between natural regulatory T (nTreg) and induced regulatory T (iTreg) cells and their role in induction of tolerance.
2. To describe how regulatory T (Treg) cells are beneficial in promoting tolerance.
3. To describe how a proallergic environment can derange the Treg cell response to aggravate and perpetuate disease.
4. To understand the influence of inflammation on Treg cell subsets.
5. To describe the relationship between type 2 innate lymphoid cells (ILC2s), T_H2 cells, and Treg cells.

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

List of CME Exam Authors: Mahta Mortezaei, MD, Jessica Stern, MD, and R. John Looney, MD.

Disclosure of Significant Relationships with Relevant Commercial Companies/Organizations: The exam authors disclosed no relevant financial relationships.

The pathogenesis of allergic diseases entails an ineffective tolerogenic immune response to allergens. Regulatory T (Treg) cells play a key role in sustaining immune tolerance to allergens, yet mechanisms by which Treg cells fail to maintain tolerance in patients with allergic diseases are not well understood. We review current concepts and established mechanisms regarding how Treg cells regulate different components of allergen-triggered immune responses to promote and maintain tolerance.

We will also discuss more recent advances that emphasize the “dual” functionality of Treg cells in patients with allergic diseases: how Treg cells are essential in promoting tolerance to allergens but also how a proallergic inflammatory environment can skew Treg cells toward a pathogenic phenotype that aggravates and perpetuates disease. These advances highlight opportunities for novel therapeutic strategies that aim to re-establish tolerance in patients with chronic allergic diseases by promoting Treg cell stability and function. (*J Allergy Clin Immunol* 2016;138:639-52.)

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Disclosure of potential conflict of interest: T. A. Chatila has received research support from the National Institutes of Health and consultancy fees from Merck. M. Noval Rivas declares no relevant conflict of interest.

Received for publication April 26, 2016; revised June 7, 2016; accepted for publication June 10, 2016.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2016.06.003>

Key words: Asthma, food allergy, regulatory T cells, forkhead box P3, IL-4, T_H2 cells

The increased prevalence in allergic diseases has become a major health problem in affluent and rapidly developing societies. Over the last 150 years, accelerated social and environmental changes augured by the industrial revolution that profoundly altered patterns of human activity, living arrangements, diet, and infections all came to influence the increase in and severity of allergic disorders.¹ In the United States food allergy prevalence reaches up to 8% among children and 5% of the adult population, whereas 8.6% of children and 7.4% of adults are affected by asthma.^{2,3} The dramatically increased burden of allergic diseases has exacted considerable morbidity on those with these disorders and resulted in substantial financial costs incurred by affected

Abbreviations used

CNS:	Conserved noncoding region
CTLA-4:	Cytotoxic T-lymphocyte-associated protein 4
DC:	Dendritic cell
FOXP3:	Forkhead box P3
GF:	Germ-free
ICOS:	Inducible T cell costimulator
ILC2:	Type 2 innate lymphoid cell
IL-4R α :	IL-4 receptor α chain
iTreg:	Induced regulatory T
LAG-3:	Lymphocyte activation gene 3
MyD88:	Myeloid differentiation primary response gene 88
nTreg:	Natural regulatory T
ROR:	Retinoic acid–related orphan receptor
SCFA:	Short-chain fatty acid
STAT:	Signal transducer and activator of transcription
Tconv:	Conventional T
TCR:	T-cell receptor
TLR:	Toll-like receptor
T _R 1:	Type 1 regulatory T
Treg:	Regulatory T

subjects and their health care systems. Although therapies for allergic diseases have improved over the years with the introduction of agents aimed at combating inflammatory processes, as well as providing symptomatic relief, those therapies have remained, for the most part, noncurative.

Allergic diseases arise in response to normally innocuous environmental agents, including aeroallergens and foods. They involve the participation of components of the innate and adaptive immune responses, such as type 2 innate lymphoid cells (ILC2s), mast cells, basophils, and eosinophils, as well as activated T_H2 cells and B cells switched to the production of IgE.^{4,5} Immune regulatory mechanisms normally operating to maintain allergen tolerance break down for reasons that still remain unknown.

The dramatic increase in the prevalence of allergic disease during past decades indicates a strong influence of environmental factors acting on genetically susceptible hosts to promote disease.^{6,7} Emerging studies emphasize the interaction of environmental factors, including diet, antibiotic use, and others, with components of the immune system affecting their function and modifying the outcome of the immune response.⁸ They also support the idea that commensal bacteria play a central role in the regulation of allergic diseases and that they dynamically interact with host genetic background and environmental factors to promote or disrupt oral tolerance.^{9–11} Genetic and immunologic evidence also reinforce the idea of a pivotal role for regulatory T (Treg) cells in promoting tolerance to allergens and preventing allergic disorders.^{12–16} In this review we will discuss recent advances demonstrating the “dual potential” of Treg cells in patients with allergic diseases: how Treg cells are beneficial in promoting tolerance but also how the proallergic environment can derange the Treg cell response to aggravate and perpetuate disease.

NATURAL AND INDUCED FORKHEAD BOX P3-POSITIVE Treg CELLS

Treg cells were initially described as a population of CD4⁺ T cells expressing the IL-2 receptor α chain (CD25) and

CD45RB, which are able to protect mice from autoimmune diseases.^{17,18} Afterward, the establishment of Treg cells as a distinct CD4⁺ T-cell subpopulation was empowered by identification of the forkhead winged helix transcription factor forkhead box P3 (FOXP3) as a specific Treg cell maker essential to their function.^{19,20} FOXP3 is required for the differentiation of Treg cells, as evidenced by the generation of aberrant Treg cells lacking in regulatory function in mice with loss-of-function mutations in *Foxp3*.^{21,22} FOXP3 deficiency results in the development of a multiorgan lymphoproliferative autoimmune disease, referred to as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, in human subjects and *scurfy* in mice.^{12,23–26} Expression of FOXP3 into human and murine conventional CD4⁺Foxp3[−] non-Treg cells by means of retroviral gene transfer converts naive T cells into Treg cells.¹⁹ It is now well established that Treg cells enforce tolerance to both self-antigens and the “extended self,” the latter encompassing commensal flora and innocuous environmental antigens, such as allergens.^{27–30}

A major population of Treg cells arises in the thymus and is known as CD4⁺FOXP3⁺ natural regulatory T (nTreg) cells (also known as thymus-derived regulatory T cells), which chiefly mediate tolerance to self-antigens (Fig 1).³¹ A second population of CD4⁺FOXP3⁺ Treg cells arises extrathymically in peripheral lymphoid tissues from a pool of naive conventional CD4⁺FOXP3[−] T cells (conventional T [Tconv] cells) after exposure to antigens and in the presence of TGF- β .³² These induced regulatory T (iTreg) cells (also known as peripheral regulatory T cells) are particularly enriched in the gastrointestinal tract and in the lungs during chronic inflammation, with specificities directed against microbial antigens or environmental allergens (Fig 1).^{33–35} The generation of iTreg cells at the intestinal mucosa is facilitated by the large abundance of TGF- β and retinoic acid, a vitamin A metabolite, both secreted by the CD103⁺CD11c⁺ dendritic cells (DCs).^{36–38} In lung tissues resident macrophages (CD45⁺CD11c⁺MHC class II^{low}F4/80⁺) constitutively expressing TGF- β and retinoic acid are the main subset of cells driving iTreg cell induction from naive CD4⁺ Tconv cells (Fig 1).³⁹ Both FOXP3⁺ nTreg and iTreg cell subsets play a key function in the maintenance of peripheral tolerance by suppressing reactivity to self-antigens and by containing the amplitude of immune responses to foreign antigens.

Because of their different origins, the T-cell receptor (TCR) repertoires of thymic nTreg and peripheral iTreg cells are largely nonoverlapping and biased toward self and nonself antigens, respectively.⁴⁰ However, iTreg cells are known to be less stable than nTreg cells and can lose FOXP3 expression and produce cytokines, such as IFN- γ and IL-17, under inflammatory conditions.^{41,42} This lack of stability can be explained by the methylation status of the conserved noncoding region (CNS) 2 of the *Foxp3* gene. The *FOXP3* CNS2 locus, which acts to maintain Treg cell lineage identity under inflammatory conditions, is known to be stably hypomethylated in nTreg cells, whereas it is incompletely demethylated in iTreg cells.^{43–46}

One difficulty for the functional and genetic study of iTreg and nTreg cells is the lack of unique and specific markers allowing the distinction between those 2 populations and their identification *in vivo*. nTreg and iTreg cells express similar levels of shared Treg cell markers, such as FOXP3, cytotoxic T-lymphocyte antigen 4 (CTLA-4), glucocorticoid-induced TNFR-related protein

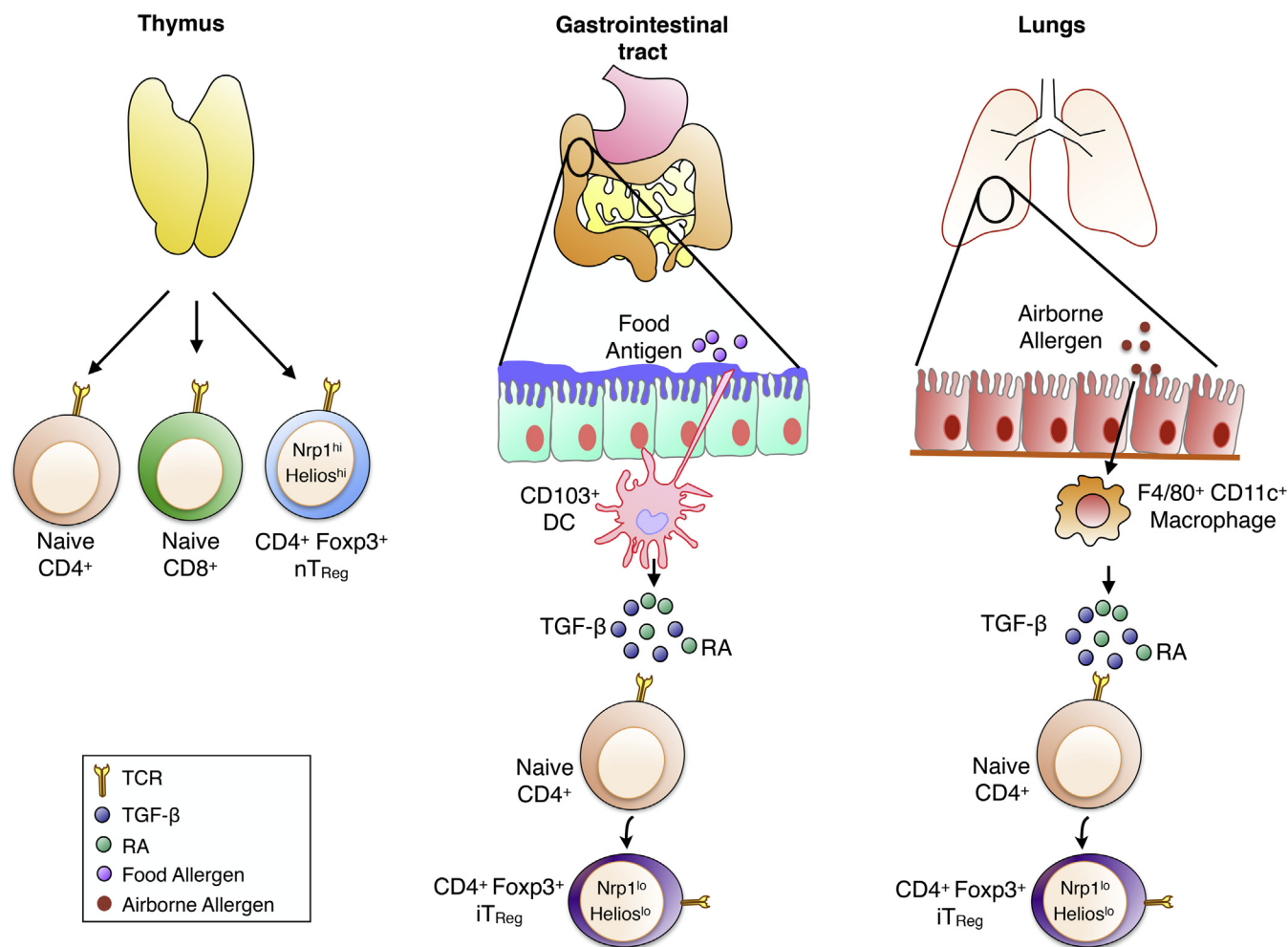


FIG 1. Natural and induced Foxp3⁺ Treg cell subsets. The Treg cell pool is composed of 2 different subpopulations, nTreg and iTreg cells, both expressing the transcription factor Foxp3, which is crucial for their development and regulatory function. Foxp3⁺ neuropilin-1 (*Nrp-1*)^{high} Helios^{high} nTreg cells arise in the thymus and mediate tolerance to self-antigens. Foxp3⁺ Nrp-1^{low} Helios^{low} iTreg cells, which mediate tolerance to foreign antigens, are induced extrathymically from naive CD4⁺ Foxp3[−] Tconv cells in the presence of TCR stimulation, TGF-β, and retinoic acid (RA) by either CD103⁺ DCs at the intestinal mucosa or F4/80⁺ CD11c⁺ macrophages at airway epithelial surfaces.

(GITR), inducible costimulator (ICOS), CD103, and CD25. However, many of those markers are also upregulated by activated CD4⁺ T cells under inflammatory conditions, and their expression level does not allow distinction between nTreg and iTreg cells.⁴⁷ The use of Helios and neuropilin-1 (Nrp-1) has been proposed to specifically discriminate nTreg from iTreg cells because expression of those markers is higher in nTreg compared with iTreg cells.^{48–50} Although Nrp-1 can be upregulated in the context of an inflammatory environment, Helios^{low} expression has been extensively used as an *in vivo* marker that distinguishes iTreg from nTreg cells.^{50–52}

In addition to FOXP3⁺ Treg cells, CD4⁺ type 1 regulatory T (T_R1) cells represent another subset of Treg cells defined by the expression of IL-10 and the surface markers lymphocyte activation gene 3 (LAG-3) and CD49b in the face of absent FOXP3 and CD25 expression.⁵³ The relationship between FOXP3⁺ Treg cells and T_R1 cells remains obscure, with both subsets using common effector pathways, including IL-10, TGF-β, and CTLA-4.⁵⁴ Unlike FOXP3⁺ Treg cells, T_R1 cells

are not uniquely defined by one transcription factor, such as FOXP3, but express a number of transcription factors common to other T-cell populations, including c-Maf, aryl hydrocarbon receptor, and others.⁵⁴ Many studies that have referred to IL-10-producing Treg cells as T_R1 cells did not discriminate between the 2 populations by appropriate staining for differentiating markers, including FOXP3. In this review we will focus on FOXP3⁺ Treg cells because their role in the regulation of allergic disease is far more well defined.

MECHANISMS OF Treg CELL SUPPRESSION

The suppressive functions of Treg cells are essential to control autoimmunity, allergic, and inflammatory reactions and responses to infectious agents and tumors. Foxp3⁺ nTreg and iTreg cells are characterized by a nonoverlapping TCR repertoire, resulting in a division of labor in which nTreg and iTreg cells regulate immune responses targeting “self” antigens and “nonself” infectious or innocuous agents, respectively.^{40,55}

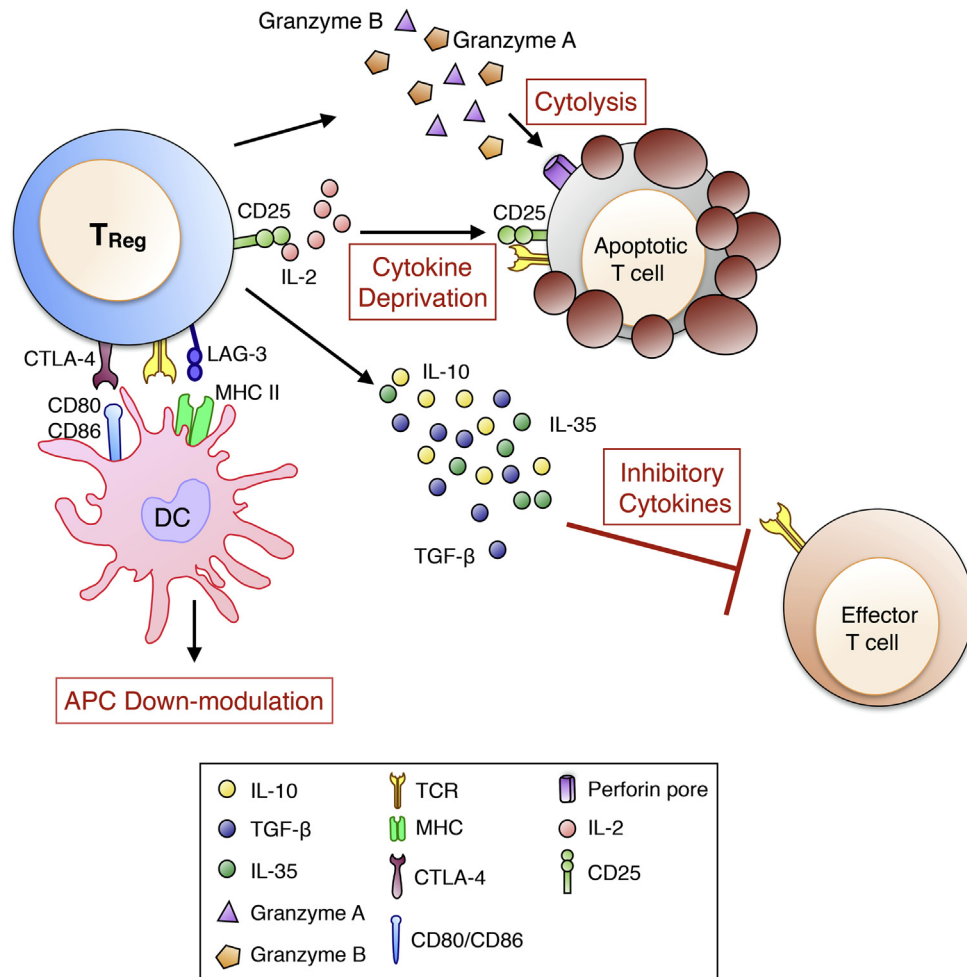


FIG 2. Mechanisms of Foxp3⁺ Treg cell-mediated suppression. Foxp3⁺ Treg cells mediate tolerance to allergens through diverse suppressive mechanisms. These include T-cell cytotoxicity through a granzyme-dependent mechanism; IL-2 deprivation; production of inhibitory cytokines, including IL-10, IL-35, and TGF- β capable of blocking the proliferation of effector T (*Teff*) cells; and downmodulation of antigen-presenting cells (APCs) through LAG-3-MHC class II and CTLA-4-CD80/CD86 interactions.

Treg cell suppressive functions are mediated by multiple mechanisms that involve either the release of inhibitory cytokines (IL-10, TGF- β , and IL-35)⁵⁶⁻⁵⁹ and cytolytic molecules (granzymes A and B)⁶⁰⁻⁶² or the downmodulation of antigen-presenting cells (CTLA-4) and lymphocyte-activation gene 3 (LAG-3),^{63,64} deprivation of trophic cytokines (IL-2 through CD25),⁶⁵ and modulation of metabolic pathways (CD73 and CD39, Fig 2).⁶⁶

Expression of select transcription factors and receptors enables Treg cell suppressive functions under inflammatory conditions. GATA-3 expression by Treg cells is triggered by TCR activation and is required to maintain FOXP3 expression and allow accumulation of Treg cells at inflamed sites.⁶⁷ More recently, it has been demonstrated that Helios expression by Treg cells is key to support their suppressive functions and phenotypic stability during inflammation.⁶⁸

Treg cell functions can also be regulated by endogenous danger signals or alarmins released by epithelial cells at the mucosal barrier. Colonic Treg cells express the IL-33 receptor (ST2), allowing them to respond to epithelial cell IL-33 production resulting from tissue damage by amplifying their regulatory functions and restraining intestinal inflammation.⁶⁹

ROLE OF Treg CELLS IN TISSUE REPAIR

In addition to their immunosuppressive functions and capacities to restrict the intensity of immune responses, Treg cells can also control nonimmunologic processes, such as tissue repair, resulting from extensive inflammation. Studies have identified the presence of Treg cells in a wide variety of nonlymphoid organs, such as the skin, intestinal mucosa, lungs, and visceral adipose tissues.⁷⁰ In mice Treg cells accumulate and remain in skeletal muscle after acute injury, and their depletion results in increased muscle damage.⁷¹ Treg cell production of amphiregulin, an epidermal growth factor family member known to promote healing and tissue regeneration in injured lung and muscle tissues, appears to prevent the tissue damage.^{71,72}

Treg CELLS AND ALLERGIC DISEASES

Allergic diseases reflect a failure to develop tolerance toward a specific allergen, provoking the emergence of an allergen-specific CD4⁺ T_H2 cell response, generation of allergen-specific IgE, and recruitment of effector cells to the gastrointestinal tract or lung tissue.^{4,73} In human subjects loss-of-function mutations affecting *FOXP3* result in the development of immune dysregulation,

polyendocrinopathy, enteropathy, X-linked syndrome, which is characterized not only by autoimmunity but also severe allergic inflammation, including atopic dermatitis, food allergy, asthma, increased serum IgE levels, and peripheral eosinophilia.^{12,13,74} *Foxp3* mutant mice spontaneously exhibit allergic airways inflammation, atopic dermatitis skin-like disease, and increased serum IgE levels independently of their genetic background.⁷⁵ By using another genetic murine model, DERE mice, which express the diphtheria toxin receptor under the control of the *Foxp3* gene, Hadis et al⁷⁶ have demonstrated that Treg cell depletion in ovalbumin (OVA)-tolerant DERE mice was sufficient to break oral tolerance.

Furthermore, *in vivo* depletion of CD4⁺CD25⁺ T cells in peanut-sensitized mice by means of anti-CD25 mAb results in impaired oral tolerance development and leads to heightened allergy.⁷⁷ The central role of Treg cells in oral tolerance development to food allergen has been confirmed in human studies in which children who had outgrown milk allergy exhibited higher frequencies of milk protein-specific CD4⁺CD25⁺ Treg cells and in which the emergence of allergen-specific Treg cells is highly correlated with a favorable disease outcome.^{78,79}

Among the Treg cell populations, Foxp3⁺ iTreg cells play an essential role in maintaining tolerance at environmental interfaces, including the small and large intestines and lung respiratory mucosa.³² Allergen-specific iTreg cells are involved in controlling inflammation severity and the IL-4 T_H2 cell immune response.³⁵ Accordingly, the development of allergic reactions can result from decreased induction, impaired function, or both of allergen-specific iTreg cells in genetically allergy-prone subjects. This proposition is supported by a study that took advantage of mice lacking *CNS1*, an intronic *Foxp3* enhancer that contains binding sites for multiple transcription factors, such as nuclear factor of activated T cells and Smad family member 3 (Smad3), and is required for the differentiation of iTreg cells *in vivo*.^{80,81} Despite decreased iTreg cell expansion, *CNS1*-deficient mice do not have a fatal autoimmune lymphoproliferative disease. Nonetheless, with time, *CNS1*-deficient mice have a proallergic phenotype associated with T_H2 cell-induced pathologies at mucosal surfaces.⁸¹ Using mice with a gain-of-function mutation in the IL-4 receptor α (IL-4R α) chain (*Il4raF709* mice), which heightens susceptibility to oral sensitization, we have recently demonstrated that food allergy development is associated with impaired generation and function of allergen-specific Treg cells.⁸² Patients with peanut allergy undergoing successful oral immunotherapy leading to peanut tolerance induction demonstrated increased numbers of circulating allergen-specific iTreg cells with heightened suppressive capacities and augmented stability, as evidenced by increased demethylation of CpG islands within the *FOXP3* gene.⁸³ Evidence also points to a reduced frequency of Treg cells associated with allergic asthma.^{84,85} Compared with healthy control subjects, frequencies of pulmonary CD4⁺CD25^{high} Treg cells in the bronchoalveolar lavage fluid were significantly decreased in untreated asthmatic children.⁸⁶ Four weeks of inhaled corticosteroid treatment were sufficient to restore the Treg cell compartment in the blood and bronchoalveolar lavage fluid.⁸⁶

A specific requirement for the cytokines IL-10 and TGF- β 1 expressed by Treg cells in the control of allergic responses has emerged. Kearley et al⁸⁷ have demonstrated that adoptively transferred allergen-specific Treg cell suppressive functions

during allergic airway inflammation rely on their capacity to induce IL-10 production by CD4⁺ T cells. However, subsequent studies by Rubstov et al⁸⁸ used a genetic approach to show that Treg cell-specific deletion of *Il10* promoted allergic airway inflammation, thereby suggesting that Treg cell-derived IL-10 plays a “privileged” nonredundant role in the induction of immune tolerance in patients with allergic airway inflammation.⁸⁸ IL-10 has immunosuppressive functions and can modulate the activity of a key cell subset involved in allergic reactions, such as mast cells,^{82,89} T_H2 T cells,⁹⁰ eosinophils, and DCs.⁹¹ Similar to IL-10, TGF- β 1 specifically expressed by Treg cells also appears to play a privileged role in regulation of allergic responses. In mice Treg cell-specific deletion of *Tgfb1* heightens susceptibility to food allergy (M.N.R. and T.C., unpublished data). The respective role of Treg cell IL-10 and TGF- β 1 in the regulation of different allergic responses remains to be fully mapped.

In addition to the defect in allergen-specific iTreg cell induction, aberrations of the Treg cell compartment during allergic disease can also be attributed to a decrease in or failure of their suppressive functions. *In vitro* studies with peripheral Treg (CD4⁺CD25⁺) cells isolated from the blood of atopic and nonatopic patients demonstrated that atopic Treg cells can be distinguished from nonatopic Treg cells by decreased capacities to suppress allergen-driven proliferation of effector CD4⁺ T cells, as well as their T_H2 cell cytokine secretion.⁹²

Treg CELL REGULATION OF THE INNATE IMMUNE RESPONSE IN PATIENTS WITH ALLERGIC DISEASES

Treg cells can exert their immunosuppressive functions on a broad variety of different cell types, including innate immune cells. Accumulating evidence demonstrates that Treg cells control the immediate hypersensitivity response by acting directly on mast cells and blocking their degranulation (Fig 3).^{82,89,93,94} After allergen sensitization, triggering of mucosal mast cells through the high-affinity receptor for IgE (Fc ϵ RI) will induce the release of preformed mediators and elicit an IgE-mediated hypersensitivity response.⁹⁵ One mechanism by which Treg cells modulate IgE-mediated mucosal mast cell degranulation and decrease mast cell effector mediators release is through the OX40–OX40 ligand pathway.⁹³ Direct cell-to-cell contact between OX40 expressed on Treg cells and OX40 ligand on mast cells leads to increased intracellular levels of cyclic AMP and results in blockage of extracellular Ca²⁺ influx and mast cell mediator release inhibition.⁹³

IL-4 production by mast cells is critical in patients with food-induced allergic reactions. IgE interaction with the Fc ϵ RI expressed on mast cells acts as an amplifier of the T_H2 cell and IgE responses during allergic sensitization.⁸⁹ Importantly, dysregulated IgE mast cell activation and their subsequent IL-4 production profoundly inhibits allergen iTreg cells generation during allergic processes.⁸² This IL-4 inhibition of iTreg cell generation is mediated through increased intracellular levels of GATA-3, which acts as a FOXP3 inhibitor in early T-cell differentiation.⁹⁶ In contrast, food allergy-prone mice that lack the α chain of the high-affinity IgE receptor Fc ϵ RI (*Il4raF709* Fc ϵ 1a^{-/-}) were protected from anaphylaxis.⁸² This protection was reflected in decreased mast cell expansion and degranulation and inhibition of the conventional CD4⁺ cell response, which is

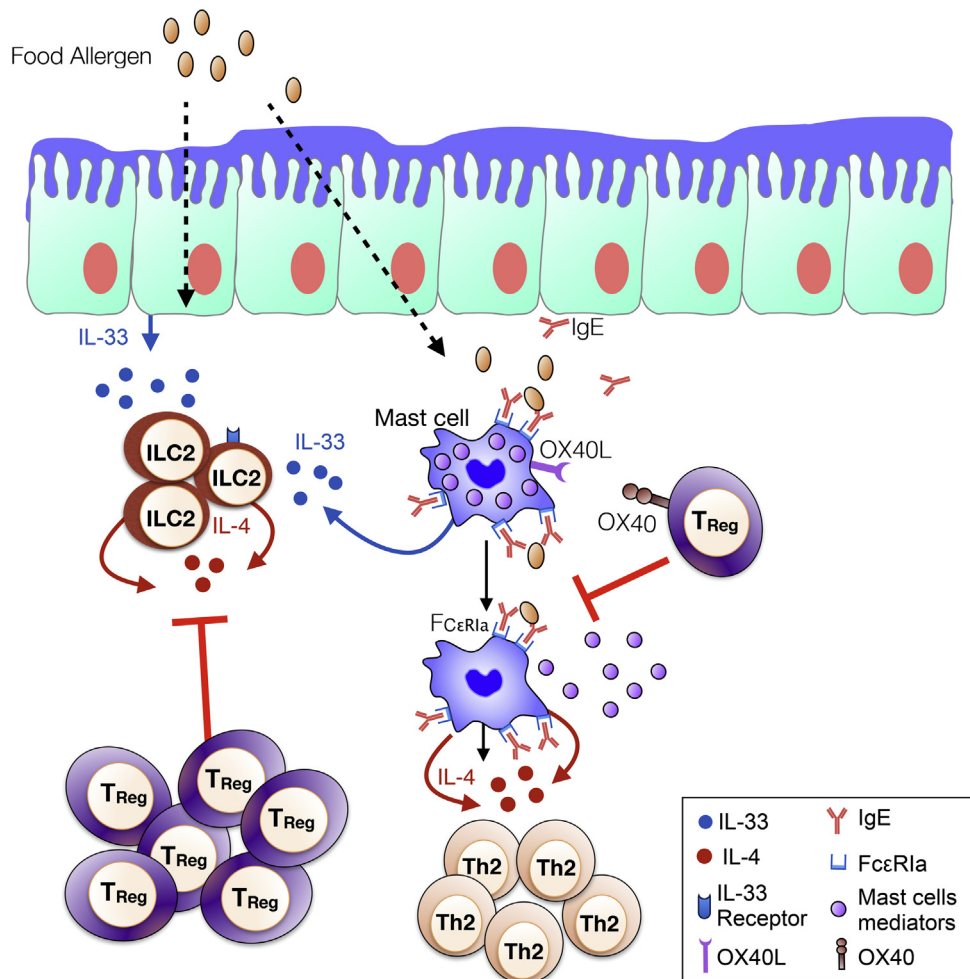


FIG 3. Regulation and suppression of allergic innate immune responses by Treg cells. Treg cells control innate immune cell subsets involved in promoting allergy. Treg cells block mast cell activation and release of preformed anaphylactic mediators through OX40–OX40L ligand–OX40L-mediated interactions. Treg cells also impede IL-33–driven ILC2 expansion in the intestinal mucosa and subsequent IL-4 production. Adapted from the graphical abstract of Noval Rivas et al.⁹⁴

consistent with the key function of the IgE/FcεRI axis in not only mediating anaphylaxis but also driving the food allergen–associated cell response.^{82,89} Moreover, FcεRI deficiency completely corrected the impaired allergen-specific iTreg cell generation.⁸² Similar results were obtained by targeting IgE production (IgE^{−/−} mice or anti-IgE treatment) or using mast cells ablation genetic murine models.⁸⁹ The key role of Treg cells in inhibiting degranulation of mast cells and their Th2 cell cytokine secretion is thus critical to the prevention of food allergy (Fig 3).

ILC2s, a population of mucosal innate cells, are simultaneously characterized by a lack of antigen specificity (absence of TCR and B-cell receptor) and lymphoid traits, as demonstrated by a shared developmental origin and phenotypic traits with T cells.⁹⁷ ILC2s produce large amounts of Th2 cell cytokines and are linked to allergic disorders, such as asthma, chronic rhinosinusitis, and atopic dermatitis.^{98–101} In mice ILC2s can be identified based on the expression of CD25 (IL-2Rα), IL-33R (ST2), and CD127 (IL-7Rα).¹⁰² ILC2s are located in the blood and various organs, such as the spleen, gastrointestinal tract, liver, lungs, and lymph nodes.^{103,104} The transcription factor GATA-3 is

required for differentiation of ILC2s, their stability, and Th2 cell cytokine production.^{105,106} Halim et al¹⁰³ demonstrated that ILC2s are required for the development of protease allergen papain–induced airway inflammation because ILC2-deficient mice (retinoic acid–related orphan receptor [ROR] α^{−/−}) were incapable of mounting an effective Th2 cell immune response and had reduced type 2 lung inflammation. The critical role of ILC2s in triggering Th2 cell adaptive immune responses involves their production of IL-13, which promotes migration of DCs to the draining lymph nodes and enhance the conversion of naive CD4⁺ T cells into Th2 cells.¹⁰³

The role of ILC2s in patients with food allergy has been less well documented. It appears that IL-13 production by ILC2s enhances allergic mucosal inflammation and promotes IgE-mediated experimental food allergy.¹⁰⁷ Food allergy development is associated with defective allergen-specific Treg cell induction, consequently resulting in disease promotion.⁸² We have recently demonstrated that increased IL-33 production at the intestinal mucosa during food allergy promotes ILC2 expansion, which further enhances the IgE-mediated food-induced allergic response through their IL-4 production.⁹⁴

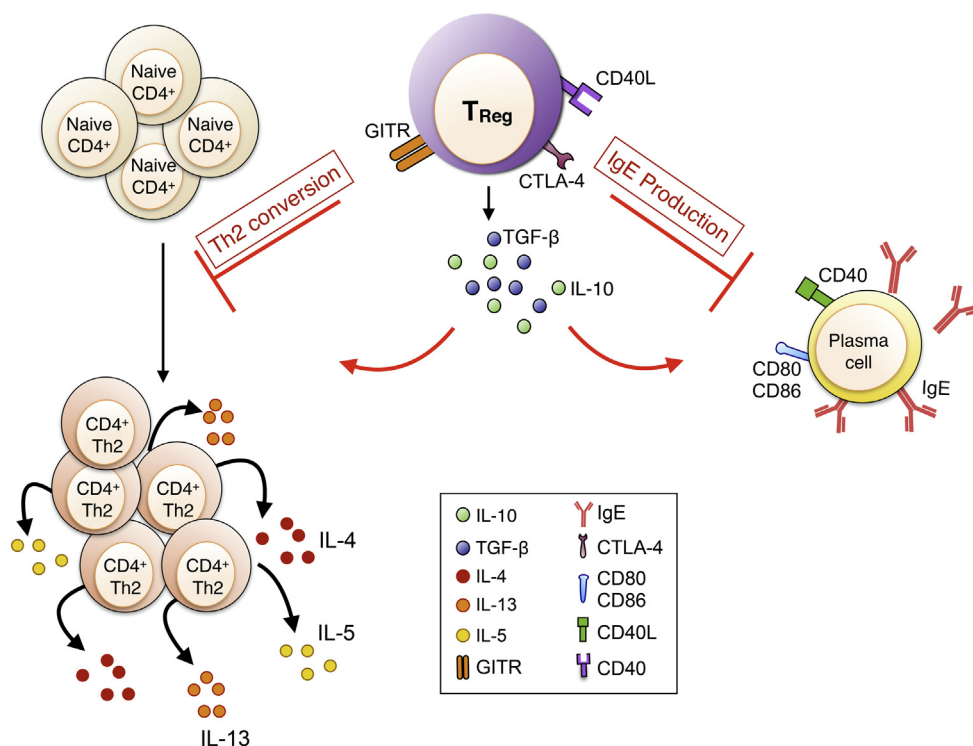


FIG 4. Treg cell-mediated suppression of the adaptive allergic immune response. Treg cells regulate allergen-specific T_H2 immune responses and B-cell IgE production. GITR stimulation of Treg cells increased their suppressive functions, leading to blockade of naive $CD4^+$ Tconv cell conversion into allergen-specific T_H2 T cells. Treg cells are also able to control B cells and block their IgE production through a direct CTLA-4- and cell contact-dependent mechanism and through production of immunosuppressive cytokines, such as IL-10.

ILC2-derived IL-4 inhibits Treg cell response and promotes mast cells activation. Reciprocally, Treg cells block ILC2 expansion and suppress their IL-4 production (Fig 3).⁹⁴

Together, these findings point to the disruption of Treg cell control of mast cells and ILC2s as a key mechanism in the pathogenesis of food allergy. At steady state, Treg cells control both mast cells and ILC2s by restricting their capacity to promote food allergy. Perturbation of this regulatory interaction will subsequently result in a dysregulated proallergic innate immune response, skewing the immunologic balance toward food allergy.

By processing and presenting antigens to naive T cells, DCs are key initiators and master regulators of the allergen-specific immune response. Treg cells also directly act on DCs by downmodulating their surface expression of CD80/CD86 expression and subsequently blocking generation of an allergen-specific T_H2 cell immune response. Treg cell suppression of DCs appears to be mediated through CTLA-4, LAG-3, and leukocyte function-associated antigen 1.^{63,64,108} Through ICOS-ICOSL interactions, DCs, mostly plasmacytoid DCs, have the capacities to prime naive T cells and induce their differentiation into IL-10-secreting Treg cells.^{109,110}

Treg CELL REGULATION OF THE ADAPTIVE IMMUNE RESPONSE IN PATIENTS WITH ALLERGIC DISEASES

Allergic disorders are characterized by increased dysregulated and aberrant immune responses mediated by the T_H2 cell

cytokines IL-5, IL-4, and IL-13. Treg cells also have the capacity to regulate allergen-induced adaptive T- and B-cell responses through diverse mechanisms, either soluble or membrane-bound suppressive molecules (Fig 4). Treg cells constitutively express CTLA-4, a negative costimulatory molecule essential to their suppressive functions. Mice deficient for CTLA-4 exhibit a lethal multiorgan lymphoproliferative disease.¹¹¹ Treg cell-specific deletion of CTLA-4 by means of crossing *Foxp3-Cre* with *CTLA-4^{fl/fl}* mice leads to an autoimmune disease characterized by an increased T_H2 cell immune response, as evidenced by increased IL-4 production by $CD4^+$ *Foxp3*[−] Tconv cells and increased serum IgE levels.⁹¹ OVA-specific nTreg cells are efficient in controlling *in vitro* T_H2 cell immune responses and IL-4 production by inhibiting the polarization of naive $CD4^+$ T cells into T_H2 cells through a GITR-dependent suppressive mechanisms.¹¹² Circulating $CD4^+$ *CD25*⁺ Treg cells isolated from the blood of atopic human subjects were also less efficient *in vitro* than $CD4^+$ *CD25*⁺ Treg cells of healthy control subjects in controlling the T_H2 cell cytokine production by effector $CD4^+$ T cells.⁹² Furthermore, frequencies of allergen-specific Treg cells secreting IL-10 with suppressive functions were predominant in PBMCs from healthy subjects, whereas frequencies of T_H2 $CD4^+$ IL-4-secreting T-cell frequencies were overrepresented in allergic subjects.⁹⁰ Effector $CD4^+$ IL-4⁺ T_H2 cells and suppressive Treg IL-10⁺ cells are present in both healthy subjects and allergic patients, and their ratio frequencies determine either tolerance induction or allergic response development.⁹⁰

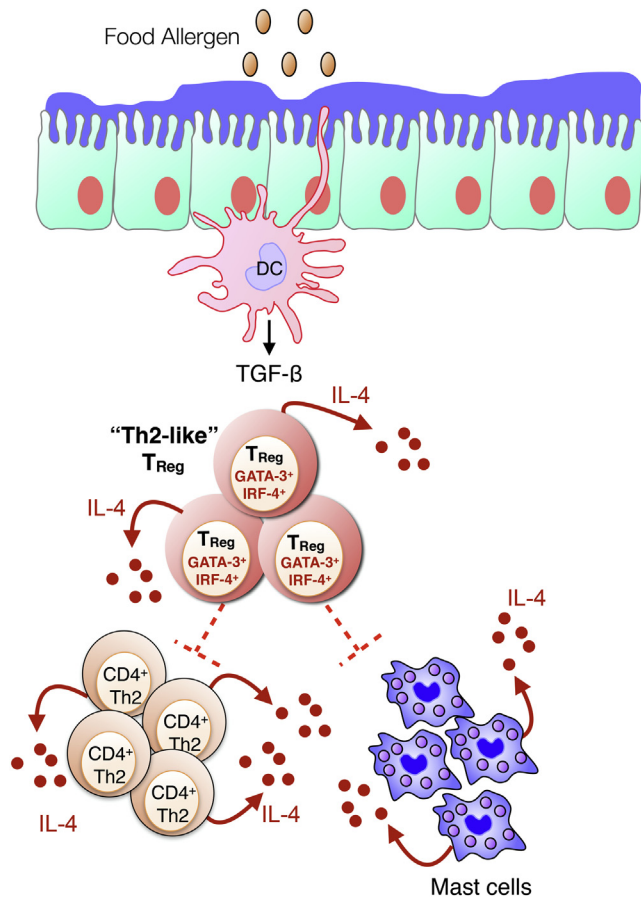


FIG 5. Pathogenic “Th₂ cell-like” Treg cell reprogramming in patients with food allergy. Food allergy is characterized by a decreased induction of allergen-specific iTreg cells at the intestinal mucosa. Induced allergen-specific Treg cells in patients with food allergy are prone to acquire a pathogenic skewed “Th₂-like” phenotype, resulting in increased GATA-3 expression and IL-4 secretion. “Th₂ cell-like” iTreg cells are dysfunctional and lacking in suppressor function. They are not able to control the effector T (*T_H2*) cell immune response and mast cell expansion, perpetuating in the allergic phenotype.

Through their production of IgE, B cells are essential in the development of allergic immune responses. IgE responses are highly dependent on immune response T_H polarization; the T_H2 cell cytokines IL-4 and IL-13 and CD40–CD40 ligand cognate interactions are 2 signals required for class-switching and IgE production by B cells.¹¹³ *In vitro* peripheral allergen-specific Treg cells from healthy subjects repress B-cell IgE production by inducing IgG₄ class-switching.¹¹⁴ Treg cells can also exercise their suppressive functions through the release of immunosuppressive cytokines, such as IL-10. B-cell suppression by Treg cells appears to be cell-to-cell contact mediated and probably occurs through CTLA-4 and TGF-β1.¹¹⁴

PATHOGENIC Treg CELL T_H REPROGRAMMING IN ALLERGIC DISEASES

An important problem in patients with chronic allergic diseases relates to the mechanisms that enable persistence of inflammation in the face of Treg cell responses.¹¹⁵ In the course of regulating T_H cell immune responses, Treg cells appropriate partial or “aborted” forms of the transcriptional programs of the target

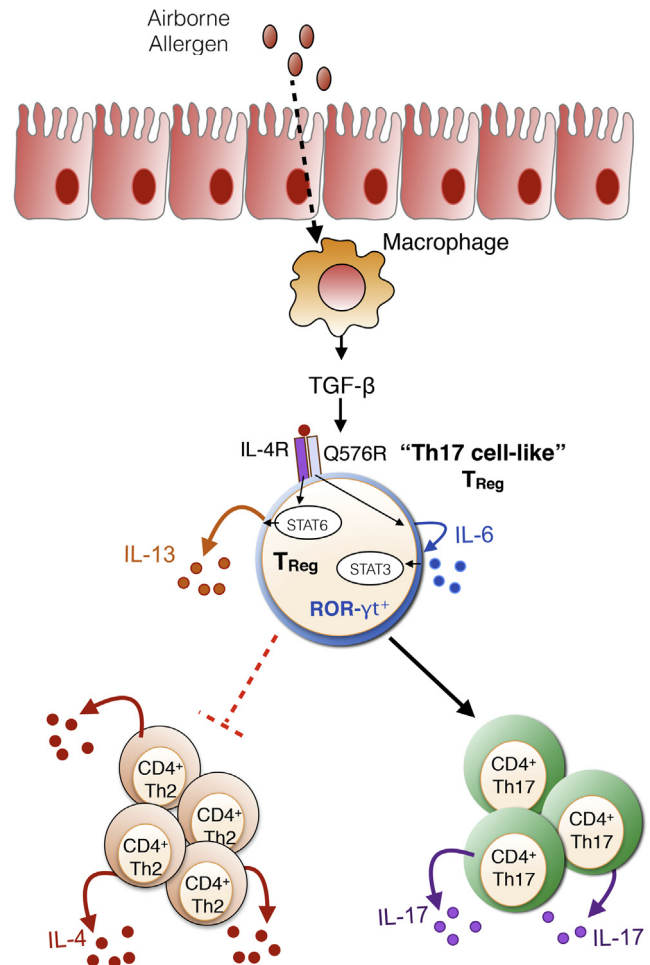


FIG 6. Pathogenic “Th₁₇ cell-like” Treg cell reprogramming by the IL-4Rα Q576R allele. Human IL-4Rα Q576R is associated with increased asthma severity. Signaling through the IL-4Rα Q576R allele on iTreg cells induces dual activation of STAT6 and STAT3, the latter through an autocrine IL-6 production loop. The IL-6–STAT3 axis promotes pathogenic “Th₁₇ cell-like” Treg cell reprogramming, resulting in RORγt expression and IL-17 secretion by the reprogrammed Treg cells.

T_H cells by expressing their master transcription factors, such as T-box transcription factor for T_H1 cells and interferon regulatory factor 4 for T_H2 cells, and co-opting their function.^{116,117} Although under physiologic conditions such partial T_H cell programming remains restrained, such restraint is lost under the influence of chronic inflammation, leading to pathogenic reprogramming of Treg cells into T_H cells.^{118,119} In the context of allergic diseases, emerging evidence indicates that a sharply skewed inflammatory environment can overcome the allergen-specific Treg cell regulatory response and redirect those cells toward a pathogenic and proinflammatory phenotype (Figs 5 and 6). Recent studies from our laboratory have provided 2 examples of how allergen-specific Treg cells can acquire effector T-cell programs and in the process contribute to disease pathogenesis.

In the first set of studies, a tyrosine (Y) to phenylalanine (F) mutation at position 709 of murine IL-4Rα inactivated the receptor’s immunotyrosine inhibitory motif and resulted in augmented activation by IL-4 and IL-13 of the downstream transcription factor signal transducer and activator of

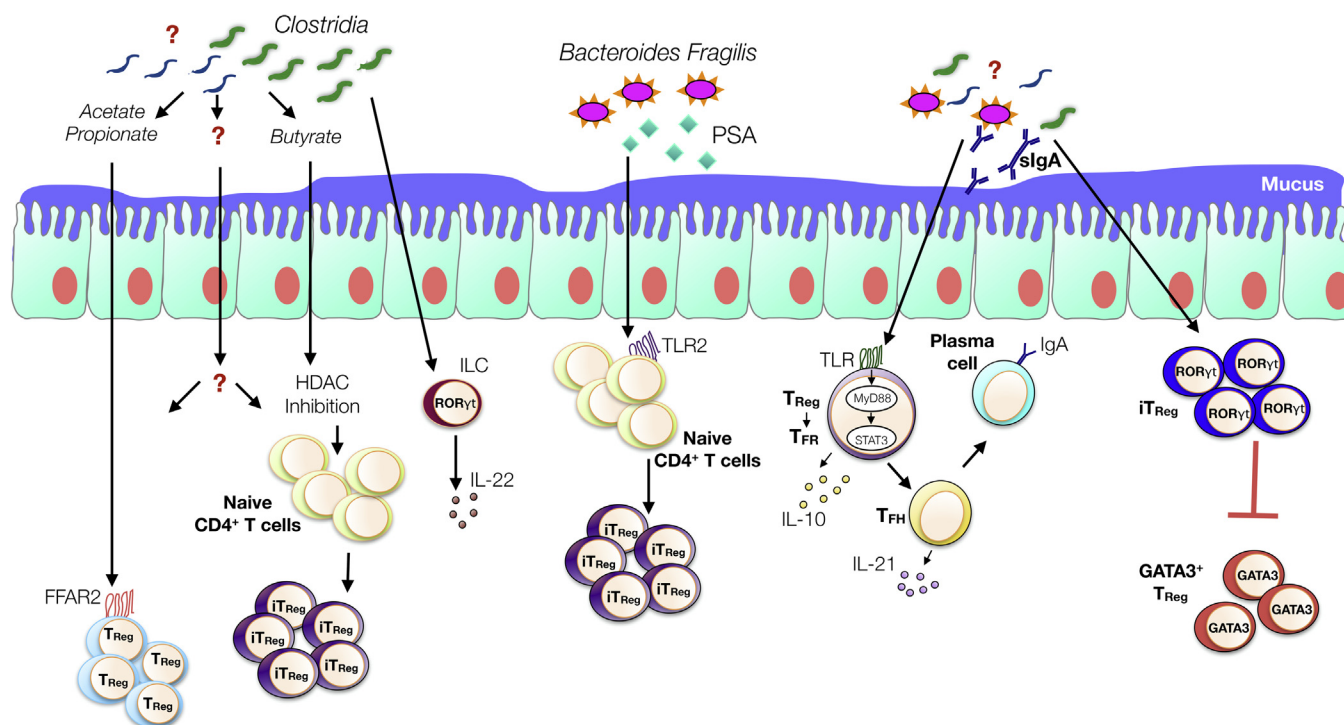


FIG 7. Microbiota-immune cell interactions shape oral tolerance. Metabolites, such as SFCAs, produced by bacterial fermentation of dietary fibers promote the proliferation and *de novo* induction of iTreg cells through FFAR2 (GPR43) receptor and histone deacetylase (HDAC) inhibition. Clostridial bacterial species promote the production of IL-22 by RORγt innate lymphoid cells, reinforcing oral tolerance by decreasing gut permeability and oral allergen uptake. *Bacteroides fragilis* production of polysaccharide A (PSA) promotes *de novo* iTreg cell generation through TLR2 signaling. MyD88/STAT3 sensing by Treg cells enforces oral tolerance by inducing and directing the follicular helper T-follicular Treg cell and IgA axis. The microbiota also promotes the emergence of RORγt-expressing iTreg cells. RORγt deficiency in Treg cells promotes a T_H2 environment and oral allergen sensitization, possibly by inducing iTreg cell reprogramming into "T_H2 cell-like" cells expressing GATA-3.

transcription (STAT) 6.^{120,121} This mutation, which models human polymorphisms that promote STAT6 activation through IL-4Rα, imparted on mice heightened susceptibility to allergic diseases, including food allergy and allergic airway inflammation, and reproduced a T_H2 cell–high disease “endotype” common in some patients with those disorders. Importantly, allergen-specific Treg cells became reprogrammed to express a T_H2 cell–like phenotype, including IL-4 production, all the while retaining their Foxp3 expression.⁸² Whereas the T_H2 cell master transcription factor GATA-3 normally plays a positive role in the accumulation of Treg cells at sites of inflammation and prevents their polarization into T_H17 cells, its abnormal expression in Treg cells might contribute to their T_H2 cell–like reprogramming under conditions of intense cell polarization.^{67,122,123} The significance of this reprogramming was underlined by the observation that Treg cell–specific deletion of the *Il4/Il13* genes restrained the induction of food allergy and allergic airway inflammation in these mice.⁸² Consistent with these results, human patients with food allergy manifest increased expression of T_H2 cell cytokines in their circulating allergen-specific Treg cells, which is indicative of their acquisition of a “T_H2 cell–like” effector T phenotype.⁸² Oral immunotherapy was associated with reversal of T_H2 cell–like reprogramming of allergen-specific Treg cells, which is coincident with their improved suppression function (data not shown).

A second example of allergen-specific Treg cell reprogramming came from studies on a human IL-4Rα allele that bears a glutamine (Q) to an arginine (R) substitution at position 576 (IL-4Rα-Q576R).¹²⁴ This allele is associated with asthma severity, whereas introduction of the Q576R substitution into murine IL-4Rα results in exaggerated allergic airway inflammation when the mice are sensitized and then challenged in their airways with allergens (Fig 6).^{125–127} Signaling through the IL-4Rα Q576R allele does not affect activation by IL-4R of its dedicated transcription factor STAT6, and T_H2 cell responses promoted through IL-4R are preserved. Nevertheless, in both human subjects and mice the Q576R substitution acts to create a novel branch of signalling through IL-4Rα that activates microtubule-associated protein kinases, leading to induction by IL-4 of IL-6 production (Fig 6).¹²⁸ The newly produced IL-6 destabilizes newly formed allergen-specific Treg cells toward the T_H17 cell lineage, thus giving rise to mixed T_H2–T_H17 cell responses in the context of allergic inflammation.¹²⁸ Inhibition of the capacity of allergen-specific Treg cells to differentiate into T_H17 cells, whether through neutralization of IL-6 or Treg cell–specific deletion of genes encoding the IL-6 receptor α chain (*Il6ra*) or the T_H17 cell master transcription factor RORγt, reversed the exaggerated allergic inflammation induced by IL-4Rα-Q576R mice (Fig 6).¹²⁸

MICROBIOME: Treg CELL INTERACTIONS IN PATIENTS WITH ALLERGIC DISEASES

Altered environmental exposures early in life might play a critical role in setting in motion the atopic diseases of childhood.¹²⁹ The hygiene hypothesis stipulates that increased allergic rates observed over the years result from reduced microbial exposures arising from lifestyle changes, such as family size reduction, use of antibiotics, and improved hygiene.¹³⁰ The influence of the intestinal microbiome in tolerance induction and allergy development is becoming more appreciated. The intestinal colonization of neonates starts at birth from the mother's vaginal flora because the microbiota composition of vaginally delivered infants is similar to that of the maternal vagina.¹³¹ Infants born by means of cesarean section have a different microbiota composition, mostly derived from maternal skin, and are at increased risks of asthma and allergy.¹³² The first months of life are a critical period for the intestinal flora to settle and stabilize because children exhibiting intestinal dysbiosis in this time window are at increased risk of asthma.¹³³ Exposure to a farm environment and the associated subsequent large diversity of environmental microbial signals reduces the risk of allergies.^{133,134} The importance of the microbial flora for allergic disease development is further emphasized by the observation that germ-free (GF) mice cannot be tolerized to oral antigens and develop a T_H2 cell-biased immune response.¹³⁵ In human subjects a polymorphism in the promoter of CD14, a high-affinity receptor for bacterial LPS and coreceptor of Toll-like receptor (TLR) 4, has been associated with the development of atopic disease.¹³⁶ Food-induced allergic responses are also aggravated in *Tlr4*^{-/-} or wild-type mice treated with antibiotics, and repopulation of commensal flora in antibiotic-treated mice results in reduced allergen-specific IgE and T_H2 cell cytokine responses.¹³⁷ These observations highlighted an important function of the intestinal microbial flora and microbial exposure in maintaining and shaping the immune response and inducing protection against the development of atopy.^{138,139}

Using *Il4raF709* food allergy-prone mice, we demonstrated that food allergy is associated with the emergence of an altered intestinal microbial flora.¹⁰ The microbiome of allergic *Il4raF709* mice exhibits decreased relative abundance of members from the Firmicutes phylum and increased abundance of bacteria belonging to the Proteobacteria phylum. Adoptive transfer of allergen-specific Treg cells prevented the development of food allergy in allergy-prone mice, as well as the emergence of food allergy-associated microbial dysbiosis.¹⁰ Importantly, disease susceptibility can be transferred from allergic *Il4raF709* mice to GF mice through transplantation of commensal flora from allergic donors. Allergy susceptibility transfer was associated with increased allergen-specific IgE production and expansion of IL-4-secreting T_H2 CD4⁺ T cells in GF mice reconstituted with the allergic microbial flora.¹⁰

The commensal flora can target different immune cell subsets belonging to the innate and/or adaptive allergic effector responses (Fig 7). In the steady state the microbial flora promotes intestinal IgA production through a Treg cell-intrinsic myeloid differentiation primary response gene 88 (MyD88)-dependent mechanism that enables the generation of iTreg cells in the gut and their differentiation into follicular helper T cells.¹⁴⁰ Depletion of the commensal flora by antibiotic treatment¹⁴¹ or the use of GF mice¹¹ is associated with development of T_H2 cell-

type allergic responses and higher serum IgE levels. T_H2 cell-type allergic immune responses were held in check by MyD88-dependent microbial sensing by B cells, which suppressed IgE responses.¹⁴¹ The commensal microbiota also influence the outcome of the allergic response modulating the innate lymphoid cells (Fig 7). Monocolonization of GF mice with anaerobic bacteria belonging to the Clostridia class blocks and protects from oral allergen sensitization by inducing IL-22⁺RORγt⁺ innate lymphoid cells at the intestinal mucosa.¹¹ ILC2s expand in the course of allergic disorders and have a pathogenic function in further promoting disease through their T_H2 cell-type cytokines secretion.^{102,107} However, how the intestinal or upper airways microbial flora could affect, regulate, or promote the ILC2 immune response requires further investigation.

The microbiota promotes either a tolerant or pro-T_H2 cell-type allergic immune response by interacting directly with immune cells and their TLRs or indirectly through the release of microbial products. Polysaccharide A, which is produced by the commensal bacterium *Bacteroides fragilis*, acts through a TLR2-dependent mechanisms to induce conversion of CD4⁺ T cells into functional iTreg cells with enhanced suppressive activities and increased IL-10 production.^{142,143} In mice clostridial species are among the most abundant gram-positive bacteria present at the intestinal mucosa. Colonization of GF mice with a mix of *Clostridium* species isolated from either murine or human feces resulted in a strong induction of iTreg cells in the colonic lamina propria of reconstituted mice.^{144,145} Through their production of IL-10, *Clostridium* species-induced Treg cells also controlled systemic IgE production by reducing *in vitro* the IL-4 production by splenic CD4⁺ T cells.¹⁴⁴

Short-chain fatty acids (SCFAs) produced by means of bacterial fermentation of dietary fibers act on T cells through a G protein-coupled receptor (GPR43) and protect mice from intestinal inflammation by expanding the pool of colonic Treg cells.¹⁴⁶ SCFAs also promote the generation of intestinal iTreg cells from naive CD4⁺ T cells through T cell-intrinsic epigenetic mechanisms.^{147,148} Butyrate, an SCFA known as a histone deacetylase inhibitor, increases Foxp3 protein acetylation, thereby conferring increased stability and enhanced suppressive function to *de novo*-generated intestinal iTreg cells.¹⁴⁸ Accordingly, a high-fiber diet results in modulation of the intestinal flora composition characterized by increased Bacteroidetes and decreased Firmicutes abundances, resulting in increased circulating SCFA levels and allergic airways inflammation protection.¹⁴⁹ More recently, it has been reported that the microbiome and oral antigen promote the induction of iTreg cells expressing RORγt at intestinal mucosal surfaces.¹⁵⁰ Specific ablation of RORγt in Treg cells resulted in increased frequencies of GATA-3⁺Foxp3⁺ Treg cells and increased production of IL-4 and IL-13 by CD4⁺Foxp3⁻ Tconv cells, leading to the conclusion that the microbiome controls the T_H2 cell immune response through expansion of Treg RORγt⁺ cells and regulation of DC activation.¹⁵⁰ Whether "proallergic" or "protolerant" bacterial species are linked to and directly affect the generation of those GATA-3⁺ or RORγt⁺ Treg cells needs to be further investigated. Because atopic diseases are associated with a defect in the generation of allergen-specific iTreg cells, it will be of interest to pursue investigation of these pathways to identify potential therapeutic targets to promote allergen-specific tolerance.

CONCLUSION

A dynamic view of Treg cells in allergic disease is emerging in which they play a central determinant role not only in tolerance induction but also, when destabilized and reprogrammed, in mediating disease pathogenesis, severity, and chronicity. Novel approaches to the re-establishment of tolerance are suggested by the results of preclinical models in which reinforcement of iTreg cell stability by interrupting their pathogenic programming might be of therapeutic benefit in patients with these disorders.

What do we know?

- Treg cells have a key role in promoting and maintaining tolerance to allergens by regulating both innate and adaptive allergen-triggered immune responses.
- Allergic diseases are associated with a failure to develop tolerance toward a specific allergen, leading to emergence of a pathogenic T_H2 immune response.
- A proallergic inflammatory environment might skew allergen-specific Treg cells toward a pathogenic phenotype that perpetuates and aggravates disease.
- Allergic responses are influenced by the commensal flora, acting in part through Treg cells.

What is still unknown?

- Mechanisms through which Treg cells do not maintain tolerance in patients with allergic diseases are not well understood and require further investigation.

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