

The role of Treg cell subsets in allergic disease

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Abstract

Allergic diseases are caused by a hypersensitivity reaction to an external substance that is normally not harmful to the body. An imbalance between type 2 immune response and regulatory T cells (Tregs) has been found to be effective in immunopathology of allergic diseases. Tregs can inhibit type 2 immune cells such as T helper 2 (Th2), type 2 innate lymphoid cells and IgE-producing B cells; meanwhile, they induce tolerogenic dendritic cells, regulatory B cells and IgG4-producing B cells. Tregs play a critical role in maintaining immune tolerance to allergens that regulate the type 2 immune response in patients with allergic diseases. Allergen-specific immunotherapy (AIT) is the only causal treatment modality to reduce allergic symptoms by altering the immune response to allergens. A key feature of AIT is to induce and maintain immune tolerance to allergens that enhances functionality, while inducing and maintaining Tregs in allergic patients. In this review, we discuss the six subsets of Tregs, natural (nTregs), inducible Treg (iTregs), inducible costimulatory (ICOS⁺ Tregs), Tr1, CD8⁺ Tregs and IL-17-producing Tregs, and their role in allergic disease and allergen immune tolerance. We also discuss specific markers of dysregulated Tregs in allergy such as, immunoglobulin-like transcript (ILT) 3, chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) and ST2. These novel molecules on Tregs provide an opportunity for novel treatment strategies aimed at changing the function of Tregs in allergic diseases.

Key words: Treg, allergic disease, allergen-specific immunotherapy, AIT, allergy, immune tolerance, IL-10

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Introduction

Allergic diseases are a group of complex disorders caused by inappropriate immune responses towards allergens. Allergy is caused by an imbalance between regulatory cells and type 2 immune responses.¹ Type 2-immunity is an immune response induced by an allergen, where naïve T CD4⁺ cells differentiate into T helper (Th) 2 effector cells and is additionally characterized by immunoglobulin (Ig) E-producing B cells, eosinophils, type 2 innate lymphoid cells (ILC2) and activated mast cells and basophils. Type 2 cytokines are interleukin (IL)-4, IL-5, IL-9 and IL-13, which are generally produced by ILC2 and Th2. IL-4 induces Th2 cell differentiation and induces isotype switching to IgE production in B cells. IL-5 is responsible for the maturation and recruitment of eosinophils.² IL-9 is produced by Th9 which induces eosinophilic inflammation, mast cell growth, mucus hypersecretion, and airway hyperresponsiveness (AHR). IL-13 regulates the proliferation of

IgE-producing B cells, mucus hypersecretion and AHR and opens the epithelial tight junction barrier.³⁻⁵ Epithelial cells of airways, skin and gut are the first line of defense against allergens. Damaged epithelial cells release alarmin cytokines, such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). These cytokines can directly activate ILC2 to produce type 2 cytokines.^{3,4} Moreover, IL-33 and TSLP directly activate mast cells. TSLP stimulates Th0 cells and dendritic cells (DCs) to induce a Th2-like process. TSLP also promotes B cell proliferation.¹ Other stimuli such as toll-like receptor (TLR) 2 and TLR-4 can also stimulate the release of TSLP from epithelial cells.^{6,7}

Regulatory cells are essential in maintaining immunological self-tolerance, in preventing autoimmune diseases and in regulating immune responses to foreign antigens. Regulatory cells induce immune tolerance by producing IL-10, TGF- β and

IL-35.⁸ In addition, they function via surface CTLA-4 and PD1-PDL interaction. IL-10 is an immune-regulatory cytokine with suppressor functions in many effector cells and different types of inflammatory reactions.^{9,10} Current evidence supports the potential role of IL-10-secreting regulatory T (Treg) and B (Breg) cells in inducing and maintaining allergen tolerance.¹¹ Allergen-specific immunotherapy (AIT) is an immune tolerance inducing treatment that is effective in reducing symptoms of allergic rhinitis, asthma, venom and food allergy.¹²⁻¹⁹ Therefore, it is the only treatment that enhances allergen-specific Treg and Breg cells.²⁰⁻²² An increasing number of allergen-specific Treg and Breg cells during AIT have been associated with the improvement of allergic symptoms in a successful AIT.²³⁻²⁵ AIT also induces IgA, IgG2 and IgG4-producing B cells, whereas decrease Th2, ILC2, IgE-producing B cells and mast cells activation.²⁶⁻²⁹ Furthermore, a sustained Treg response after discontinuation of AIT is associated with improved clinical response, decreased eosinophil counts and serum specific-IgE levels, measured during high exposure allergen season.³⁰ In this review, we aimed to clarify the underlying mechanisms of Tregs in allergic diseases and allergen immune tolerance, in the light of recent data on the Treg subsets in allergic diseases and AIT.

Treg cells

Treg cells represent 5–10% of circulating CD4⁺ T cell population in healthy humans. Treg cells can be classified into natural (nTregs), inducible Treg (iTregs), inducible costimulatory (ICOS⁺ Tregs), Tr1, CD8⁺ Tregs and IL-17-producing Tregs (Table 1). It has to be noted here that some of these Treg cell subsets are functionally overlapping or synergize each other. These Tregs share some characteristics, including expression of IL-10 and transforming growth factor-beta (TGF-β).³¹ TGF-β regulates T cell proliferation, differentiation and apoptosis.

The cell surface marker CD25 or IL-2 receptor alpha chain is also expressed on Tregs.³² Indeed, IL-2 is the main factor for survival of Tregs, which maintains peripheral immunological tolerance. Forkhead box P3 (FOXP3) is a transcription factor that controls the differentiation and functions of CD4⁺CD25⁺ Tregs.³³ FOXP3 also induces the expression of anti-inflammatory cytokine IL-10 through a mechanism in cooperation with STAT3.³⁴

Natural Treg cells

The thymic or natural Treg (nTreg) cells (CD4⁺CD25⁺CD127⁻FOXP3⁺) have originated from those that have high-affinity interactions with self-peptide/MHC class II complexes during T cell development in thymus. nTregs produce IL-10 and TGF-β and represent one of the most substantial subsets of Tregs. The role of Tregs in the immunopathology of allergic diseases is shown in Figure 1. Tregs can suppress proliferation and activation of effector Th cells, such as Th2 or Th17. Moreover, nTregs play a role in allergen-specific immune reactions, including suppression of inflammatory DCs that activate the formation of effector T cells, while supporting tolerogenic DCs. Tregs can inhibit functions and migration of Th1, Th2, Th9 and Th17 cells. In addition, Tregs can suppress allergen-specific IgE production, while inducing IgG4-secreting B cells and IL-10-producing Bregs. Furthermore, Tregs can suppress the activation of ILC2s, NKT cells, mast cells, basophils, and eosinophils.³⁵

Tregs have suppressive mechanisms by producing inhibitory cytokines such as IL-10, IL-35 and TGF-β. FOXP3⁺ Tregs show a variety of different mechanisms for implementing their suppressive actions. They release perforin and granzyme A, B and K to induce cytolysis of T, B, and NK cells; with cell-cell contact they compete with effector T cells and antigen-presenting cells for stimulatory signals such as CTLA-4, PD-1,

Table 1. Characterization of Treg subsets defined by specific marker and cytokines

Subset	Specific marker	Cytokine production
natural Treg (nTreg)	CD4 ⁺ CD25 ⁺ CD127 ⁻ FOXP3 ⁺ Helios ⁺ CD39 ⁺ CD73 ⁺ CTLA4 ⁺ Nrp1 ⁺	IL-10, TGF-β, IL-35
induced Treg (iTreg)	CD4 ⁺ CD25 ⁺ CD127 ⁻ FOXP3 ⁺ CD39 ⁺ CD73 ⁺ CTLA4 ⁺	IL-10, TGF-β, IL-35
Inducible costimulatory Treg (ICOS ⁺ Treg)	CD4 ⁺ CTLA4 ⁺ FOXP3 ⁺ ICOS ⁺	IL-10, TGF-β
IL-10-producing Tr1 cells (Tr1)	CD4 ⁺ CD25 ⁺ LAG3 ⁺ CD49b ⁺	IL-10
CD8 ⁺ Treg	CD8 ⁺ CD25 ⁺ FOXP3 ⁺ CD28 ⁺ CTLA-4 ⁺ CD122 ⁺ CD137 ⁺ CD103 ⁺	IL-10, TGF-β, IL-16, IFN-γ
IL-17A-producing Treg cells	CD4 ⁺ FOXP3 ⁺ RORγt ⁺ CCR6 ⁺ CD49d ⁺ IL-1R-β ⁺ CD161 ⁺ HLA-DR ⁻	IL-10, IL-17A

Table 2. Molecular and functional features of Tregs

Treg markers	Mechanism of suppression
CD25, CTLA-4, PD-1	Suppressive cytokines: IL-10, TGF-β, IL-35
TGF-β, IL-10, CD49b, LAG-3, LAP	Metabolic disruption: CD25, cAMP, ADR2, HR2, CD39, CD73
Granzyme A, B and K, CD122, CD103	Target molecule DCs: CTLA-4, PD-1, TGF-βR, IL-10R
ICOS, GARP, Neuropilin-1, Gpr83, ECM1, Helios, GITR	Cytolysis, Granzymes A, B and K perforin

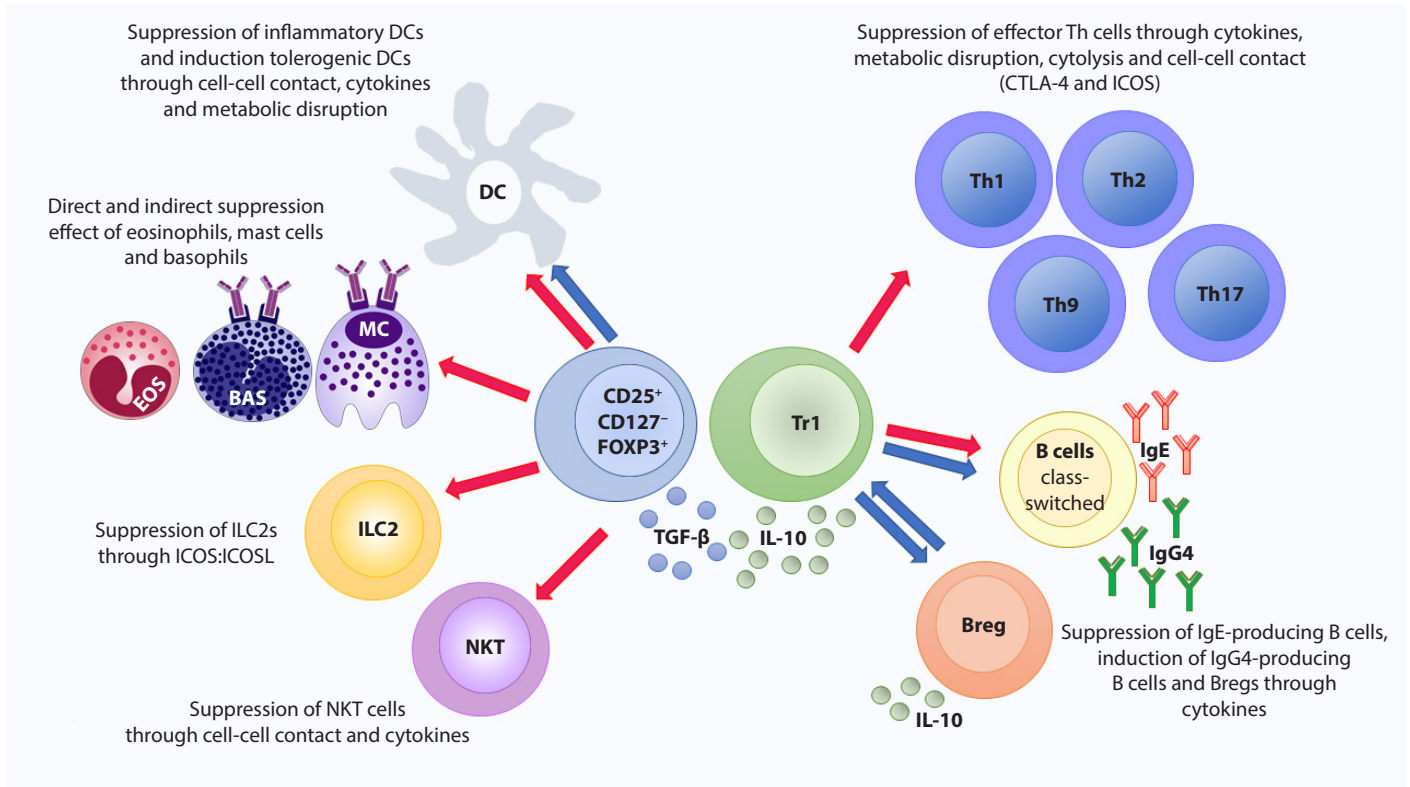


Figure 1. Tregs in allergic diseases. Tregs inhibit type 2 immune responses and contribute to the control of allergic diseases through several pathways. Red arrows display the regulatory and suppressive effects that Tregs exert on inflammatory dendritic cells (DCs), mast cells, basophils, eosinophils, type 2 innate lymphoid cells (ILC2), NKT cells, effector T helper cells, and IgE-producing B cells. Blue arrows show the contribution of Tregs to the induction of immune tolerance that Tregs exert on tolerogenic DCs, Bregs and IgG4-producing B cells.

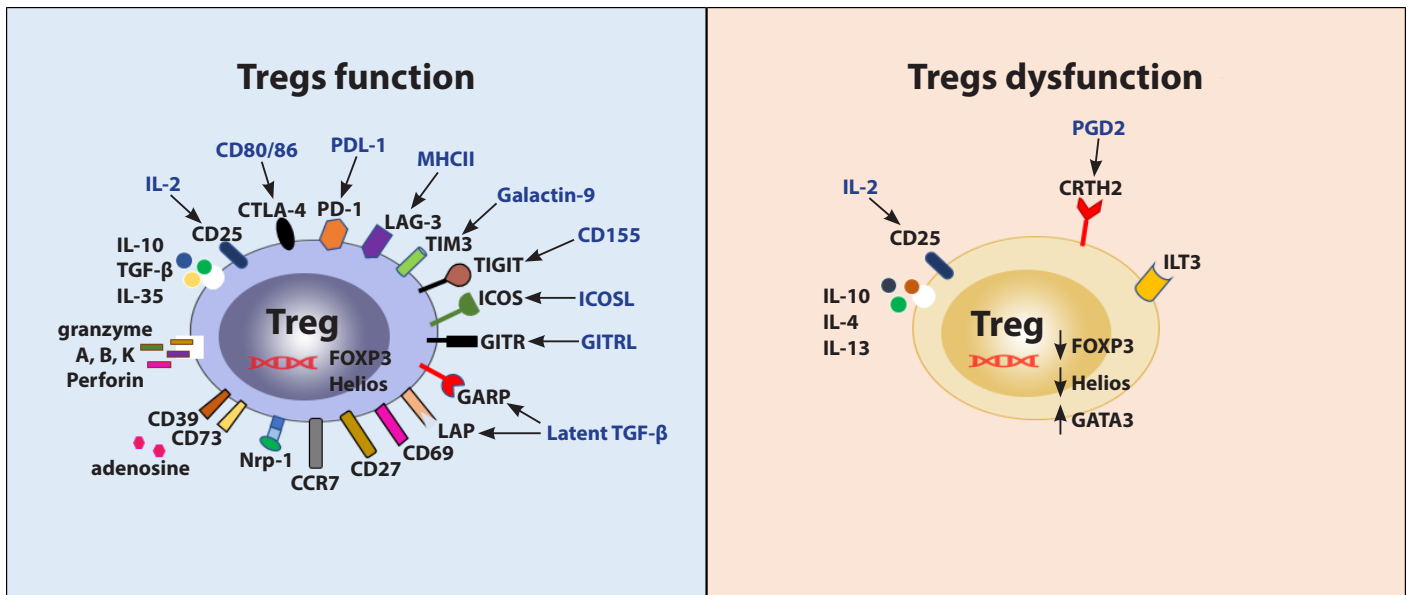


Figure 2. The marker of functional and dysfunctional Tregs. Tregs have high expression levels of surface markers associated with suppression such as CTLA-4, PD-1, LAG-3, TIM-3, LAP, GARP, TIGIT, ICOS, GITR, CD39, CD73, CD69, CD27, CCR7 and Nrp-1. Helios is a transcription factor that is upregulated in activated Tregs. CRTH2 and ILT3 are expressed on Tregs and related with the inadequate Treg suppression to effector T cells. The expression of transcription factor decreased FOXP3 and Helios, whereas increased GATA3 were found in impaired immunosuppression Tregs.

lymphocyte activation gene 3 (LAG3) and TIM3; and they use metabolic disruption mechanisms such as cAMP, adenosine receptor 2, histamine receptor 2 (HR2), CD39 and CD73.³⁵ Functional suppressor Tregs also express CD27, CCR7, T cells expressing the coinhibitory molecule (TIGIT), glycoprotein A repetitions predominant (GARP), inducible costimulatory (ICOS), latency associated peptide (LAP), glucocorticoid-induced tumor necrosis factor-related receptor (GITR) and CD69 (Table 2 and Figure 2).¹¹

Helios⁺ and Helios⁻ Tregs are phenotypically and functionally distinct and express unique TCR repertoires.³⁶ Helios is a marker of T cell activation and expression.³⁷ Helios also enhances induced Treg cell function in collaboration with FOXP3.³⁸ The percentage of FOXP3⁺Helios⁺ Tregs in healthy controls and patients with stable allergic asthma was higher than in patients with exacerbated asthma.³⁹ AIT was shown to increase allergen-specific FOXP3⁺Helios⁺ Tregs and that correlates with the reduction of allergic symptoms.²³ Furthermore, the presence of local FOXP3⁺CD25⁺ Tregs in the nasal mucosa increases after AIT. They are associated with clinical efficacy and suppression of seasonal allergic inflammation. In a mouse model, AIT also induces systemic and local Tregs in skin of atopic dermatitis mice that correlate with the efficacy of AIT.⁴⁰ Thus, it can be said that the concept of local allergen tolerance in the peripheral tissues may depend on Treg responses.^{41,42}

In developing Tregs in the thymus, a framework of Treg-specific epigenetic marks is anchored. This epigenetic framework is accompanied by the expression of FOXP3, which together shape the function and phenotype of mature thymus-derived Tregs.⁴³ The recent study in nTregs showed genes with Treg-specific DNA hypomethylation tend to be upregulated in Tregs in the steady-state. In contrast, the genes with FOXP3 binding regions tend to be downregulated in activated Tregs.⁴⁴ Thus, the two events seem to display different but collateral roles in Treg-specific gene expression.

Induced Treg cells (iTregs)

iTregs are peripherally induced Tregs by abundant TGF- β , and retinoic acid that is produced by DCs and macrophages.^{45,46} iTregs and nTreg express similar levels of shared Treg cell markers, such as FOXP3, CTLA-4, GITR, ICOS, CD103 and CD25.⁴⁷ Helios and neuropilin-1 (Nrp-1) are highly expressed in nTregs compared to iTregs and they are used to distinguish nTregs from iTregs.^{48,49} Moreover, iTregs are known to be less stable than nTregs, because they may lose FOXP3 expression and produce cytokines, such as IFN- γ and IL-17 because of local inflammatory conditions and innate immune response stimulating substances in the microenvironment.⁵⁰ iTregs and nTregs can be converted from their suppressor property into pathogenic effector cells. These effects can be mediated in different pathways such as IL-6-induced IL-17 production in iTregs but not in nTregs.⁵¹ In a mouse allergic asthma-like lung inflammation model, iTregs are significantly more tolerogenic than nTregs. iTregs reduce active Th2 response by 72-86%, whereas nTregs reduce these responses by 41-56%.⁵² Furthermore, increased demethylation of the Treg-specific demethylation region in the *Foxp3* locus in nTregs but not iTregs was reported in mice study.⁵³

A recent study in atopic children showed that the number of circulating memory Tregs (CD45RA⁻) were higher compared to healthy children.²⁷ However, the study did not investigate the FOXP3 expression in Tregs and Treg suppressor function. Myeloid-derived suppressor cells (CD45⁺CD33⁺CD14⁺HLA-DR^{-low}CD15⁻) can suppress effector T cells and promote Treg expansion. In adult asthmatic patients, the percentage of myeloid-derived suppressor cells is small, compared to healthy controls and has been associated with decreased FOXP3⁺ Tregs in peripheral blood.⁵⁴

Inducible costimulatory (ICOS)⁺ Treg cells

ICOS has significant homology with the co-stimulatory molecule CD28 and the immune-checkpoint CTLA-4. ICOS⁺ and ICOS⁻ Tregs are different subsets due to their cytokine production capacity. ICOS⁺ Tregs can produce large amounts of IL-10, moderate amounts of TGF- β and another cytokines such as IL-17 and IFN- γ .^{55,56} ICOS signals together with FOXP3 and CTLA-4 and contributes to the survival and suppressive functions of Tregs.^{57,58} Tregs can suppress ILC2s through ICOS:ICOS-L to control airway inflammation together with the suppressor cytokines TGF- β and IL-10.^{59,60} The number of active ICOS⁺ Tregs was higher in the children living in traditional farms, with a low risk of atopy and asthma, compared with the children living in modern farms.⁶¹

Type 1 regulatory T cells (Tr1)

Tr1 cells secrete IL-10, which are not dependent on FOXP3 expression for their function. LAG3 and CD49b markers have been identified as additional cell-specific markers for human Tr1 cells.⁶²⁻⁶⁴ IL-10 can suppress Th2-type and Th17 cells. Besides, IL-10 can inhibit cytokine production of eosinophils, basophils, mast cells, antigen-presenting cells and dendritic cells. Moreover, IL-10 can support B cells that induce the development of Tr1, Br1 cells, and IgG4 upon differentiation of Br1 cells to plasma cells. IgG4 reduces allergic responses by blocking the allergen binding of IgE.⁶⁵ Moreover, it was suggested that IgG4 can drive M2a macrophage to regulatory M2b-like phenotype.⁶⁶

Patients with allergic diseases have lower percentage of antigen-specific Tr1 cells in peripheral blood compared to healthy subjects.⁶⁷ It was demonstrated that peanut-specific CD49b⁺LAG3⁺ Tr1 cells can be induced in vitro from healthy controls and patients with peanut allergy, but Tr1 cells from patients with peanut allergy are functionally defective.⁶⁸ Der p 2-specific Tr1 cells can be supported by Der p 2-pulsed DC-10, which can suppress Derp 2-specific Th2 cells.⁶⁹ Tr1 cells implicate to restore tolerance in allergy. Der p 1-specific Tr1 cells increase during AIT in patients allergic to house dust mites, correlated with the developing clinical tolerance.²³ Moreover, AIT may enhance Bet v 1-specific Tr1 cells associated with low skin prick test reactivity and reduced clinical symptoms to birch pollen.⁷⁰ IL-10 mRNA levels in whole blood cells remarkably correlate with house dust mite allergen immunotherapy efficacy.⁷¹ Tr1 Treg cells have been shown to be the main subset in high dose allergen tolerance models, such as bee venom allergen tolerance in multiple bee sting receiving bee keepers and cat allergen tolerance in cat owners.^{64,72}

CD8⁺ Treg cells

CD8⁺ Tregs can effectively block the overreacting immune response and maintain the body's immune homeostasis. This cell subset is generally identified as CD8⁺CD25⁺ cells and other markers including FOXP3, CD28, CTLA-4, CD122, CD137 and CD103 have been described.^{73,74} The cytotoxicity of CD8⁺ Tregs is due to the expression of MHC class Ib molecules, more specifically Qa-1 in mice and HLA-E in humans.⁷⁵ CD8⁺ Tregs secrete various cytokines and chemokines, including IL-10, TGF- β , IL-16, IFN- γ , and chemokine (C-C motif) ligand 4.⁷⁵ CD8⁺ Tregs stimulate tolerogenic antigen-presenting cells (APCs) by upregulating the expression of immunoglobulin-like transcript (ILT) 3 and ILT4. Moreover, CD8⁺ Tregs exhibit an inhibitory function by CTLA-4 dependent cell-contact. Tonsillar FOXP3⁺CD8⁺ Treg phenotype exhibits high CTLA-4 and CD45RO and low expression of CD127 and CD69.⁷⁴ FOXP3⁺CD8⁺ Tregs may inhibit the proliferation of CD4⁺ T cells in co-cultures.⁷⁴ The frequency of CD8⁺CD25⁺FOXP3⁺ cells in peripheral blood was lower in asthmatic patients compared to healthy controls.⁷⁶ In addition, the percentage of CD8⁺ Tregs correlated with peak expiratory flow rate and severity of asthma.⁷⁶ A significant increase was observed in Der p 2-specific CD8⁺FOXP3⁺ Treg cells expressing IL-10 and granzyme B, after house dust mite specific immunotherapy.⁷⁷ Furthermore, AIT expands CD8⁺CD25⁺CD137⁺ Tregs in the circulation and the nasal mucosa of the patients with allergic rhinitis.⁷⁸ Thus, CD8⁺ Tregs may cooperate with CD4⁺ Tregs to suppress the type 2 immune response by induced apoptosis of Th2 cells.

IL-17A-and IFN- γ -producing Treg cells

IL-17A-producing CD4⁺FOXP3⁺ Tregs express ROR γ t. ROR γ t is the Th17-specific transcription factor. Th17A producing Tregs can transform from conventional CD4⁺FOXP3⁺ Treg cells. IL-6, IL-21, IL-23, and IL-1 β are required to the differentiation of conventional Tregs into IL-17A-producing Tregs.⁷⁹ Expression of CCR6, CD49d, IL-1R- β , CD161, and the absence of HLA-DR, are used to identify cell surface markers of IL-17A-producing FOXP3⁺ Treg cells.⁸⁰ IL-17A-producing CD4⁺FOXP3⁺ Tregs can suppress the proliferation of CD4⁺ effector T cells through a cell-cell contact mechanism.⁸¹ Although, ROR γ t and FOXP3 expression have recently been associated with a reduced suppression function.⁸² The frequency of IL-17A-producing Tregs in allergic patients is increased compared to healthy controls.⁸³ In addition, IL-17A and ROR γ t expression was higher in patients with persistent allergic symptoms than in those with intermittent allergic symptoms, whereas FOXP3 expression was decreased.⁸⁴ Immunological changes in patients treated with AIT in the build-up phase are presented with a decrease of Th1, Th17 and CCR6⁺IL-17⁺FOXP3⁺ Tregs.⁸⁵

IFN- γ plays a vital role in both inductions of Tregs as well as immunosuppression mediated by IFN- γ -producing Tregs. IFN- γ -producing Tregs are only 0.04% of all CD4⁺ T cells in the circulating of healthy individuals and increases significantly during an immune response.⁸⁶ IFN- γ ⁺ Tregs are induced by IFN- γ and IL-12.⁸⁷ Type 1 interferon signaling attenuated Treg function in viral infection, tumor immunity and

organ transplant.^{88,89} However, the role of IFN- γ -producing Tregs in allergic diseases is required further study.

Dysregulated regulatory T cells

Dysregulated Tregs seems to play an essential role in the development or chronicity of allergic diseases. In patients with allergy and asthma, the *FOXP3* gene decreased compared to healthy controls.⁹⁰ In addition, several reports indicate that *FOXP3* polymorphisms and impaired Tregs function involve in the development of allergy and asthma.^{91,92} Infants with food allergy presented low number of nTregs at birth.^{23,93} Moreover, cesarean section was associated with a temporary reduction in nTregs at birth.⁹³ In allergic children, FOXP3⁺ Treg cell maturation was significantly delayed compared to mature and healthy children.⁹⁴ In vitro experiments have shown that, the suppressive function of Tregs in the peripheral blood of allergic patients is reduced compared to Treg cells of healthy controls.^{95,96} In patients with allergic asthma, FOXP3⁺ Tregs were found to be less in bronchoalveolar lavage and have impaired regulatory functions.⁹⁷ IL-27 is essential for controlling inflammatory responses of FOXP3⁺ Treg functions. In animal models, the intranasal administration of IL-27 could attenuate airway inflammation due to upregulated Th1 cells and Tregs because of repairing the STAT1 pathway.⁹⁸ Tregs of asthmatic patients exhibited blunted STAT1 phosphorylation following IL-27 stimulation.⁹⁹ The altered IL-27 response in Tregs may indicate inadequate Treg functions. Recently reported findings suggest that AIT restores the suppressive capacity of FOXP3⁺ Tregs in patients with aeroallergen allergy.¹⁰⁰

Tregs can lose their FOXP3 expression and acquire the ability to produce the corresponding Th cytokines depending on their microenvironment.¹⁰¹ GATA-3 expression in Tregs may contribute to Th2 responses by switching Tregs into Th2 cells under type 2 environment. In the animal model, GATA-3 was relevant regulators of Treg plasticity that inhibit Tregs from becoming novel APC-Tregs.¹⁰² The recent study in asthmatic patients revealed that the frequency of Tregs decreased in asthmatic patients with an impaired immunosuppression function and a Th2-like phenotype. That may be due to overexpression of GATA3 and FOXP3.¹⁰³ In the patients with immune dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, M3701FOXP3 mutation generated Th2-like Treg cells which expressing GATA3 and Th2 cytokines. Besides, this Tregs mutation had increased chromatin interaction at the Th2 locus.¹⁰⁴

ILT3 expression in FOXP3⁺ Treg cells represents a distinct subset of Tregs. Several lines of evidence demonstrate that they represent a dysregulated Treg cell subset. ILT3⁺FOXP3⁺ Tregs cannot control the maturation of DCs that increase Th2 response.¹⁰⁵ Protein kinase CK2 enables Tregs to suppress the type 2 immune response. Deletion of the beta subunit of CK2 results in proliferation of a subpopulation of Tregs characterized by the expression of the inhibitor receptor ILT3.¹⁰⁵ The incidence of ILT3⁺ Tregs in healthy controls is significantly lower than in allergic patients.¹⁰⁵ Recently, we reported that ILT3⁺ Tregs decreased significantly during AIT, although the patients showed allergen tolerance.²³ Moreover, we also found that ILT3⁺ Tregs show compromised suppressive function due to low FOXP3 and Helios expression.²³

Another dysfunctional Treg cell subset is chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)⁺ Tregs. These cells are Th2-like Tregs that combine inhibitory function and Th2-cytokine production. CRTH2 is a receptor that binds to the ligand prostaglandin D2 (PGD2) and binds to a surface marker of Th2 cells. In an *in vitro* experiment, CRTH2⁺ Tregs presented less suppressive function compared to CRTH2⁻ Tregs.¹⁰⁶ Moreover, CRTH2⁺ Tregs have been shown to produce greater amounts of IL-4 than CRTH2⁻ Tregs after PGD2 stimulation but there was no significant difference found in IL-10 levels. In addition, patients with allergic asthma had more CRTH2⁺ Tregs in peripheral blood than healthy controls.¹⁰⁶ Moreover, the number of CRTH2⁺ Tregs in peripheral blood is correlated with the ability to control asthma. After therapy of asthma patients, CRTH2⁺ Treg cell count and activated CRTH2⁺ Treg response to PGD2 stimulation are significantly decreased in patients with allergic asthma in parallel to improved lung functions. The use of IL-4 receptor alpha blockade in AIT has been a major question. It was demonstrated that AIT combined with IL-4 receptor alpha blockade can decrease Th2-biased Tregs (ST2⁺FOXP3⁺GATA3⁺) in allergic mice model.¹⁰⁷

IL-4 is a cytokine that induced naïve T cells to Th2 cells and activated Th2 to secrete IL-4 in a positive feedback loop. In the Tregs study, IL-4 inhibits TGF-β-induced FOXP3⁺ Tregs generates.¹⁰⁸ IL-4 inhibited Tregs differentiation due to suppress the expression indoleamine 2,3-dioxygenase on CD103⁺ DCs.¹⁰⁹ The other reported in the animal study, IL-4 had affected the function of Tregs by 1) protect T effector from Treg-mediated immune suppression, 2) IL-4 deprivation impaired the ability of Tregs to modulate the immune response, 3) IL-4 was associated with increased cell survival and granzyme expression of Tregs.¹¹⁰

In the animal model, loss of liver kinase B1 (LKB1) in Tregs has been shown to disrupt cell metabolism and function in mice leading to a fatal inflammatory disease characterized by excessive Th2 immune response.¹¹¹ Expression of the IL-33 receptor ST2 has been demonstrated in FoxP3⁺ Tregs in mice.¹¹² When exposed to IL-33, Tregs were found to upregulate GATA3 expression, as well as ST2 expression and produce type 2 cytokines.¹¹² Moreover, Tregs lose their capacity to suppress effector T cells in the presence of IL-33.¹¹² However, other reports have presented that ST2⁺ Tregs are superior to ST2⁻ Tregs in suppressing IL-33 independent *in vitro* CD4⁺ T cell proliferation.¹¹³ Data on ST2 expression in Tregs have not been reported in allergic patients. Bcl6 is required for the development of T follicular helper cells and T follicular regulatory (Tfr) cells. In mice, allergic airway inflammation, Bcl6 and Blimp1 played dual roles in regulating Tfr-cell activity in the germinal center and the development of ST2⁺ Tregs.¹¹⁴ TCR-activated posttranslational modification by O-linked N-acetylglucosamine (O-GlcNAc) stabilizes FOXP3 and activates STAT5. Treg cells with O-GlcNAc-deficiency usually develop but exhibit modestly decreased FOXP3 expression and subsequently ameliorate Treg cell dysfunction in O-GlcNAc deficient mice.¹¹⁵

Treg cells in atopic dermatitis

Atopic dermatitis (AD) occurs from the combination of an altered skin barrier and dysregulated immune reactions, mainly driven by Treg cell dysfunction.¹¹⁶ The term autoallergy describes autoimmunity in AD patients, with antigen-specific IgE raised against self-proteins as a hallmark. This phenomenon has been found in 23 to 91 % of the patients with AD.¹¹⁷ These skin autoallergens were able to induce T-cell proliferation in AD patients. Tregs constitute between 20-80% of CD4⁺ T cells in the skin at steady-state.¹¹⁸ Skin-homing Tregs also play a critical role in mitigating the reactivity of immune cells, secreting high levels of cytokines that promote tolerance.¹¹⁹ Study of AD patients in China revealed that the number of FOXP3⁺ Tregs before receiving treatment correlated with disease severity, whereas IL-10⁺ Tregs did not show related to disease severity.¹²⁰ The retinoic acid receptor-related orphan receptor α (RORα)-expressing in skin resident Treg was significant for suppressing type 2 cytokines that related allergic skin inflammation.¹²¹

Treg cells in food allergy

IgE-mediated food allergy is a type 2 immune response that the prevalence is increasing in the children as a direct consequence of reduced tolerance to food antigens. In ovalbumin-sensitized mice suggested that administration of high doses of allergens through the oral route predominantly induced deletion of antigen-specific-effector T cells, whereas administration of low doses favored the generation and expansion of antigen-specific Tregs.¹²² The frequency of Tregs is associated with the maintenance of tolerance in food allergy. Studies in young atopic children proposed that food-allergic children have lower percentages of FOXP3⁺ Tregs compared with healthy controls of similar age.¹²³ Moreover, age-related increases in CCR6 expression on Tregs were observed in healthy controls but not food-allergic children, which may be essential for Tregs migration to peripheral sites of inflammation in the maintenance of tolerance.¹²³ Likewise, the infant with cow milk allergy was related to decreased the number of Tregs and vitamin D levels.¹²⁴ The higher frequency of cow milk allergen-specific FOXP3 Tregs correlated with a phenotype of mild clinical disease and favorable prognosis.¹²⁵

Tregs play a major role in oral tolerance, and the large piece of evidence has demonstrated Tregs implicated in the success of oral immunotherapy (OIT).¹²⁶ Oral tolerance could suppress experimental food allergy through the development of antigen-specific FOXP3⁺ Tregs in mice.¹²⁷ Recent findings demonstrate that oral tolerance can be induced in the tonsils through the generation and maintenance of functional allergen-specific Tregs.¹²⁸ Tonsil pDCs able to generate functional FOXP3⁺ Tregs with suppressive properties from naïve T cells. Allergen-specific FOXP3⁺ Tregs were found to be high in human tonsils compared to peripheral blood.¹²⁸ Syed et al. reported that hypomethylation of FOXP3 and increased Treg function in patients with oral tolerance during peanut OIT.¹²⁹ It was associated with long-term protection from food anaphylaxis.

Patients with Wiskott-Aldrich syndrome (WAS) are deficient in WAS protein (WASP). These patients usually present with autoimmunity and elevated levels of serum IgE that is pronounced with food allergy. The study in WASP-deficient mice showed the deletion of WASP in FOXP3⁺ Tregs resulted in more severe Th2-type intestinal inflammation.¹³⁰ Loss of WASP was phenotypically associated with increased GATA3 expression in effector memory FOXP3⁺ Tregs.¹³⁰

Treg cells and microbiota

The hygiene hypothesis is linked between Tregs and microbiota. Many studies have revealed that growing up in rural areas protects from allergic disorders, possibly by a relative increase in bacterial or fungal diversity.¹³¹ The experiment in mice showed that low-dose exposure to lipopolysaccharide (LPS), a cell-wall component of gram-negative bacteria, has protective effects in a mouse model of house dust mite allergic lung inflammation.¹³² The mice raised under germ-free conditions that show drastically reduced frequencies of Tregs in the gut.¹³³ The bacteria in the gut have an impact on the immune system. In murine studies, identified individual bacterial strains such as *Bacteroides fragilis* and clostridium strains had been directed the development of FOXP3⁺ Tregs and Tr1 cells.^{134,135} The other mechanism is the fermentation of complex carbohydrate fibers by the microbiota leads to the production of short-chain fatty acids (SCFA). Butyrate is a SCFA that had been shown to enhance the generation of Tregs.¹³⁶ Butyrate supplementation enhanced OIT-induced desensitization of basophils and mast cells and Treg functionality in mice experiment.¹³⁷

The recent study in Finland and Estonia reported that an infant Treg phenotype characterized by high Treg frequency and increased the highly activated Tregs.¹³⁸ These changes in Treg population associated first with the relative abundance of *Bifidobacterium longum* followed by increasing colonization with butyrate-producing bacteria. High bifidobacteria and butyrate-producing bacteria abundance in the neonatal microbiota seems to be beneficial the protection of allergic disease.

Probiotic is live microorganisms that can act as promoters of an adequate balance in the gut microbiota to improve allergic symptoms and prevent the development of allergies.¹³⁹ Administration of probiotics include *L. acidophilus*, *L. casei*, *Lactobacillus reuteri*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus* which associated with upregulated Tregs population and increase the suppressor activity of Treg function.¹⁴⁰ Moreover, probiotic supplement with *Bifidobacterium infantis* 35624 has been reported to increase the percentage of peripheral FOXP3⁺ Tregs in the human study.¹⁴¹ Probiotics are used as an adjuvant in AIT to improve clinical efficacy.¹⁴² In clinical studies, the addition of probiotics with *Lactobacillus rhamnosus* GG to sublingual immunotherapy (SLIT) improved symptom scores and the induction of Tregs after 5 months of treatment.¹⁴³ The use of probiotics in the treatment of allergic diseases is very promising but needs more studies to support the data.

Treg-based therapy in allergic disease

Treg-based therapy are a various study in oncology, transplantation and autoimmune disease. A cure for the allergic disease is imagined that increasing allergen-specific Tregs to restrain IgE-producing allergen-specific memory B cells and plasma cells. The study in the murine model showed a low dose IL-2/anti-IL-2Ab complex combined with AIT could augment more Tr1 cells than AIT alone.¹⁴⁴ Also, nasal allergen-CpG immunotherapy enhanced Tr1 in mice model.¹⁴⁵ In atopic dermatitis, Treg-based therapy might contribute to repair of the skin barrier, with a subsequent decrease in the local availability of autoallergens.¹⁴⁶ The chimeric antigen receptor (CAR) approach is engineered as a target protein antigen. The engineered allergen-specific T-regulatory cells to target B cell antibody receptor can provide clinical protection against severe allergic reactions in individuals already IgE-sensitized to ovalbumin in mice study.¹⁴⁷

Update on Tregs in allergic disease

IL-35-inducible Tregs (iTr35) have been reported as a new subset of inducible Tregs. IL-35 can suppress IL-5 and IL-13 production by ILC2 and Th2 cytokines-producing T effector cells.¹⁴⁸ IL-35 also inhibited CD40L-, IL-4-, and IL-21-mediated IgE⁺ B cells.¹⁴⁸ Moreover, iTr35 cells can suppress Th2 cell proliferation and cytokine production.¹⁴⁸ Notably, IL-35 levels and iTr35 cell counts were increased in patients receiving SLIT and healthy individuals compared to patients with allergic rhinitis.¹⁴⁸ IL-21 controls effector CD4⁺ T cell responses and Tregs homeostasis. In a mice study, IL-21 directly promotes apoptosis of Tregs by interfering with the expression of Bcl-2 family genes and therefore indirectly sustains generation of inflammatory T effector cell responses.¹⁴⁹

MicroRNA (miR) regulates protein expression post-transcriptionally through mRNA destabilization or translational silencing and implicates for adoptive cellular therapy. The study in allergic rhinitis children revealed miR 188a transfected into Tregs, which promoted IL-10 and TGF- β . The miR-155 also promoted Treg proliferation directly through suppressor of cytokine signaling 1 (SOCS1) and sirtuin1 (SIRT1) signaling pathway.¹⁵⁰

Conclusion

In the last two decades, it has been discovered that various functional Treg cell subsets exist in the immune system. Dysregulated or irregular Treg cell function is one of the current hypotheses for the development and the immunopathology of allergic diseases. Further evidence has demonstrated that both FOXP3⁺ Tregs and Tr1 cells play a vital role in peripheral T-cell tolerance to allergens in healthy individuals. Tregs regulate allergic immune responses in type 2 immune cells through the suppressive mechanism and contribute to the control of allergic diseases in several pathways. AIT is an induction therapy and maintains allergen immune tolerance in patients with allergic diseases. In a successful AIT, significant increase in functional Tregs and a decrease in dysfunctional Tregs are associated with improvement in allergic symptoms. Curative therapeutic strategies specifically targeting Tregs are expected to develop in the future and may be promising as a treatment option in the management of allergic diseases.

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