

Mechanisms of immune regulation in allergic diseases: the role of regulatory T and B cells

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Summary

Allergy is a major public health problem with a high socio-economic impact. The number of allergic patients is expected to reach to four billion within two decades when the World's population reaches to 10 billion. Our knowledge on the molecular mechanisms underlying allergic diseases and allergen tolerance induction had significant advances during the last years. Nowadays, it is well accepted that the generation and maintenance of allergen-specific regulatory T cells (Tregs) and regulatory B cells (Bregs) and the involvement of their suppressive cytokines and surface molecules are essential for the induction of allergen tolerance. These mechanisms play essential roles for the restoration of healthy immune responses to allergens in allergen-specific immunotherapy (AIT) and healthy immune response during high-dose antigen exposure in beekeepers and cat owners. AIT remains as the only disease-modifying and curative treatment for allergic diseases and represents a perfect model to investigate the antigen-specific immune responses in humans. A large number of clinical trials demonstrated AIT as an effective treatment in many patients, but it still faces several drawbacks in relation to efficacy, safety, long duration, and patient adherence. Novel strategies to overcome these inconveniences, such as the development of novel adjuvants and alternative routes of administration are being developed. The better understanding of the molecular mechanism governing the generation of Treg and Breg cells during allergen tolerance might well open new avenues for alternative therapeutic interventions in allergic diseases and help better understanding of other immune-tolerance-related diseases.

KEYWORDS

allergen tolerance, allergen-specific immunotherapy, allergy, Breg cells, immunologic mechanisms, Treg cells

1 | INTRODUCTION

The immune system is a very complex interactive network of cells and molecules with the capacity to protect the host from a broad range of pathogens, while keeping a state of tolerance to self and external innocuous antigens. Immune-tolerance-related diseases such as

cancer, organ transplantation, autoimmunity successful pregnancy, asthma, and various allergic disease arise as a direct consequence of deregulated immune responses.^{1–5} The main allergic diseases include allergic rhinitis, allergic asthma, food allergy, atopic eczema/dermatitis, and anaphylaxis.^{6–8} Allergic diseases significantly impairs the patients' quality of life and lead to very high healthcare-associated costs, thus representing a major public health problem worldwide with a high socio-economic impact.⁹ The prevalence of allergic diseases has dramatically increased over the past decades, affecting up

to 30% of the population worldwide. Although the actual reasons for this phenomenon are not yet fully understood, modern-life style and exposome interactions have been postulated as an important factors contributing the burst of allergic diseases in genetically predispose and epigenetically regulated individuals.^{4,10,11} Type 2 immune-response-mediated mechanisms play a role in a major fraction of allergic diseases that involves the formation of specific IgE antibodies against innocuous environmental substances, so-called allergens.

Induction and maintenance of allergen tolerance is a hallmark of healthy immune responses to allergens, but the molecular mechanisms involved in these process are not yet completely elucidated. In vivo models of natural high-dose allergen exposure and AIT demonstrated that the generation of functional allergen-specific regulatory T cells (Tregs) and regulatory B cells (Bregs) is an essential component of allergen tolerance induction.^{4,5,12–16} The identification of human tissues in which tolerance induction takes place might be of great relevance for the designing of novel therapeutic interventions. At this regard, human tonsils have been shown as organs where allergen tolerance induction and immunologic mechanisms operating during sublingual immunotherapy (SLIT) might well occur.^{12,17,18} AIT remains as the single treatment for allergic diseases with potential curative capacity, but it still faces several drawbacks related to efficacy, security, long duration, and patient adherence.⁴ Novel strategies to overcome such inconveniences, including alternative adjuvants and routes of vaccine administration, are being developed and assayed in preclinical in vivo and in vitro models.^{19,20} Some of them have been already tested in humans, but clinical trials with larger cohorts of patients are still awaiting to firmly confirm their potential beneficial improvements.

Herein, we review the current knowledge and recent findings in our understanding on the molecular mechanisms underlying allergic diseases and allergen tolerance induction with special focus on the role played by Tregs and Bregs in such processes. We will also discuss recent findings related to the design and development of novel strategies to improve current allergen vaccines for AIT.

2 | IMMUNOLOGIC MECHANISMS UNDERLYING ALLERGIC DISEASES

The pathophysiology of allergic diseases consists of the interaction between resident tissue cell responses and a complex network of immunologic events that interplay for the development of inflammation, tissue injury, and loss of organ function. Several subtypes of inflammation and complex immune-regulatory networks have been described, which open the way for the better understanding of immune mechanisms that may lead to development of new diagnostic tools and innovative targeted treatments.²¹ Accordingly, the concept of disease endotypes emerged that particularly represent immune networks in allergic diseases.²² An endotype is a subtype of a disease condition, which is defined by a distinct pathophysiological mechanism, whereas a disease phenotype defines any observable characteristic of a disease without any implication of a mechanism. Within

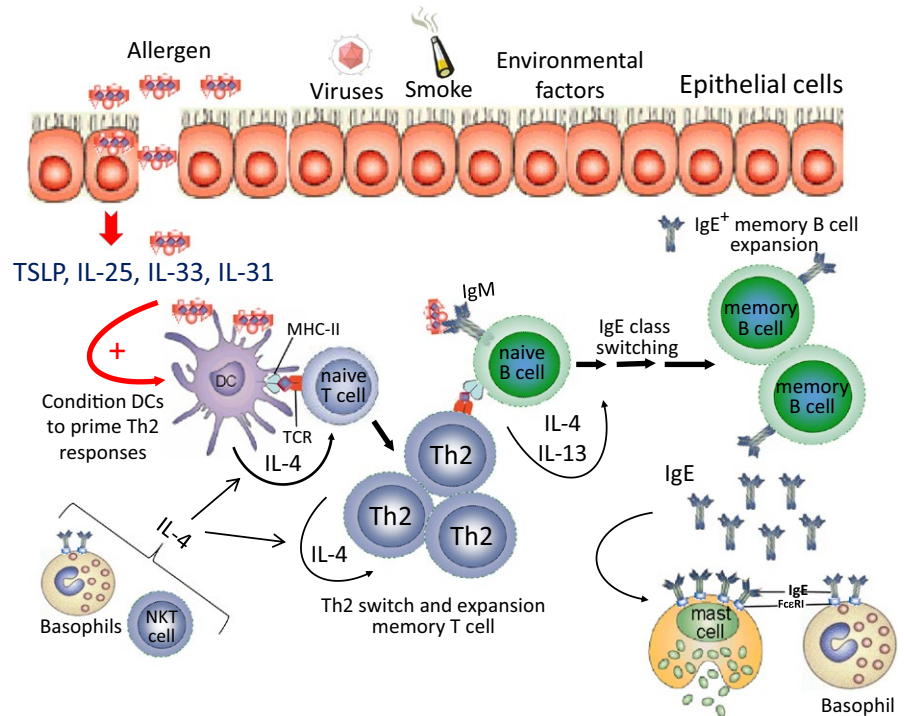
a complex network of immunologic mechanisms of allergic diseases, type 2 immune response represents the major and also clinically most relevant immune response in allergic diseases.

Generally, it is considered that a type 2 immune response underlines allergic asthma, atopic dermatitis, chronic rhinosinusitis with nasal polyps, and allergic rhinitis. Type 2 immune response involves Th2 cells, group 2 innate lymphoid cells (ILC2), B cells, a small fraction of IL-4-secreting NK cells, IL-4-secreting NK-T cells, basophils, eosinophils, and mast cells and their major cytokines.²³ From a complex network of cytokines, IL-4, IL-5, IL-9, and IL-13 are mainly secreted from the immune system cells and IL-25, IL-31, IL-33, and TSLP from tissue cells, particularly epithelial cells (EC) (Figure 1).²² It is now clear that both the innate and the acquired immune response contribute to type 2 immune response endotypes. In addition, type-2-driven inflammation is characterized by a high cellular plasticity that enables the cells to adapt to a specific inflammatory milieu. Several sub-endotypes might exist within the type 2 immune response complex endotype such as the IL-5-high, IL-13-high or IgE-high endotype and their predominance differs between allergic diseases.

Immune mechanisms operating in allergic diseases can be divided into two main phases: (i) sensitization and memory and (ii) effector phase.^{7,8,24} As shown in Figure 1, during patient's sensitization, allergens are captured in the airways, gut or skin by specific mucosal-resident dendritic cells (DCs) subsets. DCs internalize the antigen and transport it to drain lymph nodes where it is presented as processed peptides associated with MHC class II molecules to naïve CD4⁺ T cells, leading to the generation of allergen-specific CD4⁺ Th2 cells. Th2 cells produce high levels of IL-4 and IL-13 providing help to B cells for IgE isotype class-switching. IgE memory B cells can mature and differentiate into plasma cells producing large amounts of allergen-specific IgE antibodies that bind to the high-affinity FcεRI on the surface of mast cells and basophils, thus leading to the IgE sensitization of individuals against allergens. At this stage, patients do not suffer from clinical symptoms, but they are ready to react upon new allergen encounters. Remarkably, in this phase, populations of allergen-specific memory Th2 and B cells are generated after clonal expansion, which are ready to interact upon allergen encounters (Figure 1).

Recent findings have shown that resident tissue cells might also contribute to allergic sensitization. Epithelial cells form an impermeable barrier representing the first line of defense against environmental insults. Allergic patients display altered barrier function allowing the penetration of allergens, pathogens, and other particles into the submucosa, which may also favor allergic sensitization and chronic inflammation.^{25–27} ECs should not be considered as mere physical barrier, but also as important immune-regulatory cells able to produce different cytokines after stimulation, such as IL-25, IL-33, IL-31, or TSLP that act on DCs and ILC2 contributing to Th2 generation and allergic sensitization.^{28,29} Another important role of ECs is to control immune tolerance to allergens and recently it was shown that upon epithelial activation established airway tolerance may not be sustained in the presence of an innate immunologic stimulation, such as IL-33.³⁰ Other cells such as basophils and NKT cells might also contribute to allergic sensitization as an early source of IL-4.^{5,31}

FIGURE 1 Immunologic mechanisms underlying allergic sensitization. During allergic sensitization, mucosal dendritic cells (DCs) capture, process, and transport the allergens to closer lymph nodes to promote the generation of allergen-specific Th2 cells in a process partially depending on IL-4. ECs-derived Th2 cytokines produced after environmental stimulation condition and the Th2 polarization capacity of DCs. Clonal expansion and activation of allergen-specific Th2 cells producing IL-4 and IL-13 provide help favoring the B cell IgE class-switch. Allergen-specific IgE antibodies bind to the surface of effector cells (mast cells and basophils) through the high-affinity IgE receptor FcεRI, leading to patient's sensitization. A memory pool of allergen-specific Th2 cells and B cells is also generated in this phase. Basophils and NKT cells might also represent an early source of IL-4



The effector phase of allergic inflammation (Figure 2) is a type I hypersensitivity reaction initiated when an encounter with the allergen causes cross-linking of the IgE that is bound to FcεRI on sensitized basophils and mast cells. IgE cross-linking triggers their activation and release of anaphylactogenic preformed mediators such as histamine, heparin or proteases, de novo synthesis of lipid mediators such as prostaglandins, leukotrienes, and cytokines, responsible for the symptoms of the immediate phase and acute inflammation. Late-phase reactions occur in the tissues as shown in Figure 2 when submucosal tissues or dermis in the atopic dermatitis skin show infiltration and activation of memory T cells with the formation of tertiary lymphatic tissues. Recent findings demonstrated that the formation of inducible bronchus-associated lymphoid tissue within the lung containing a large number of memory Th2 cells play a very important role during chronic allergic inflammation. The maintenance of memory Th2 cells within these tertiary lymphoid tissues is sustained by Thy1⁺IL-7-producing lymphatic endothelial cells via mechanisms depending on the production of IL-33 and CCL21 and CCL19.³² The accumulation of effector cell-derived mediators and the activation of memory allergen-specific Th2 cells by antigen-presenting cells (APC) (DCs and B cells) through mechanisms depending on IgE-facilitated allergen presentation are key factors for the initiation and maintenance of late-phase reactions. Activated memory allergen-specific Th2 cells produce IL-4, IL-5, IL-9, and IL-13, which play a key role in the maintenance of allergen-specific IgE levels, eosinophilia and recruitment of inflammatory cells to inflamed tissues, production of mucus, and tissue inflammation and damage (Figure 2). Other cells may also contribute to the late-phase responses to a different extent depending on endotypes of the disease.

Innate lymphoid cells (ILCs), particularly ILC2 represent one of the main players in allergic inflammation.²⁹ ILCs are defined as ILCs type 1, 2, and 3 cells. ILC1 mainly produce IFN-γ³³ and ILC3 produce IL-17

and IL-22.³⁴ ILCs control the mucosal environment through close interaction with ECs and other tissue cells, production of cytokines, and induction of chemokines that recruit suitable cell populations to initiate and promote distinct types of immune responses and tissue inflammation.³⁵ These cells can be detected in several body fluids and tissues such as, sputum, peripheral blood, nasal polyps, or esophagus for their characterization in allergy and asthma patients.^{33,36,37} For example, ILC2 activated by ECs-derived cytokines produce high levels of IL-13 and IL-5 enhancing mucus production and eosinophilia²⁹ and they can promote IgE production.³⁸ In addition, a transcription factor of a Th1 cells and ILC1, such as T-bet can inhibit type 2 allergic inflammation demonstrating the complexity of these molecular mechanisms.³⁹

Specific NKT cells subsets may also produce high levels of Th2 cytokines under certain conditions, thus cooperating with Th2 and ILC2 to enhance Type-2-mediated immune response and ongoing inflammation.^{5,34} Other effector T-cell subsets also participate in the ongoing allergic reactions. IFN-γ-producing Th1 and NKT cells can either exacerbate the effector phase by inducing apoptosis of the epithelium,^{40,41} or dampen allergic inflammation by counterbalancing Th2 responses.⁴² IL-17-producing Th17 cells in cooperation with NKT cells have also been shown to contribute the ongoing of allergic diseases by indirectly enhancing IL-8-mediated neutrophil recruitment (Figure 2).⁴³ In addition, different roles have been attributed to Th9 or Th22 cells. Th9 cells produce IL-9 and IL-10 that promote tissue inflammation due to the lack of suppressive capacity,⁴⁴ whereas Th22 cells have been shown to contribute to epidermal hyperplasia in inflammatory skin diseases.^{43,45} Recent studies with atopic dermatitis biopsies have identified Th2 and Th22 cells as well as the corresponding functional CD8⁺ T cell subsets TC2 and TC22, respectively, playing a role in severe forms of this disease⁴⁶ demonstrating that a combined Th2 Th22 pattern may play an important role in allergic inflammation.⁴⁷

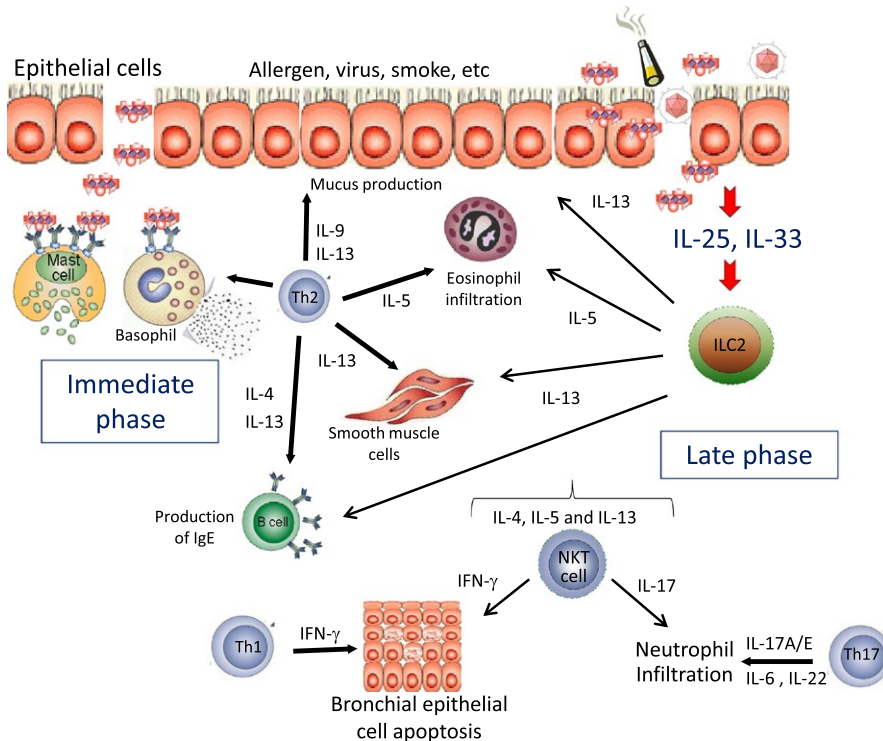


FIGURE 2 Immunologic mechanisms underlying allergic effector phases. In sensitized patients, the new encounters with the allergen cause IgE cross-linking on basophils and mast cells, leading to the release of anaphylactogenic mediators such as histamine, proteases, prostaglandins, leukotrienes, and cytokines, responsible for the symptoms of the immediate phase and acute inflammation. Accumulation of these mediators and activation of allergen-specific Th2 cells in cooperation with ILC2 activated by ECs-derived cytokines producing IL-4, IL-5, IL-9, and IL-13 play a key role in the maintenance of allergen-specific IgE levels, eosinophilia and recruitment of inflammatory cells to inflamed tissues, production of mucus, and tissue inflammation and damage, leading to the most severe clinical manifestations and chronic inflammation. Depending on the specific endotypes, NKT cell subsets, Th1 and/or Th17 cells might also contribute to neutrophil infiltration and tissue damage

In addition, different cell types can cooperate and contribute to keep allergen tolerance and tissue homeostasis. Among them, it is noteworthy to highlight the essential role played by Tregs and Bregs not only in healthy subjects, but also in the restoration of healthy immune responses to allergens after successful allergen-specific immunotherapy (AIT).^{5,31,48}

3 | THE ROLE OF TREG CELLS IN ALLERGIC DISEASES AND ALLERGEN TOLERANCE

Treg cells encompass a heterogeneous group of T-cell subsets with suppressive capacity that are indispensable to impair excessive immune responses to pathogens, for immune tolerance induction toward environmental proteins, cancer cells, and transplanted organs and to avoid and control the development of autoimmune and allergic diseases.^{49–51} Although there are some overlaps, CD3⁺ CD4⁺ Tregs can be classified into two main groups: the thymus-derived CD25⁺ FOXP3⁺ Tregs (tTregs),⁵² and the peripherally derived Tregs generated outside the thymus after antigenic stimulation (pTregs): (i) peripherally induced FOXP3⁺ T cells,⁵³ (ii) IL-10-producing Treg cells (Tregs1),⁵⁴ and (iii) TGF- β -producing Th3 cells.⁵⁵ It is nowadays shown that FOXP3, TGF- β , and IL-10 are expressed in different Tregs in different balances. In addition, other Tregs populations such as CD8⁺ Tregs,⁵⁶ double negative CD4[−] CD8[−] TCR $\alpha\beta$ ⁺ Tregs that mediate tolerance in several experimental autoimmune diseases,⁵⁷ and TCR $\gamma\delta$ Tregs, which can play a role in the inhibition of immune responses to tumors⁵⁸ have been described. Mucosal-associated invariant T cells are a new T-cell subset missing in severe asthma which show correlation with Treg cells.⁵⁹

Treg cells can prevent and inhibit the ongoing of allergic inflammation by acting at different levels via mechanisms that include cell contact and/or secretion of soluble molecules. There are four main groups of suppressive mechanisms used by Treg cells including suppressive cytokines (IL-10, TGF- β , and IL-35), metabolic disruption mechanisms (CD25, cAMP, adenosine receptor 2, histamine receptor 2, CD39, and CD73), suppression of DC activation by membrane-bound molecules (CTLA-4, PD-1), and cytolysis (granzymes A and B).^{5,31} Using these mechanisms, Tregs directly or indirectly suppress almost all cell types that contribute to the initiation and maintenance of allergic reactions including effector cells (mast cells and basophils), eosinophils, B cells, DCs, T cells as well as inflamed resident tissue cells (Figure 3).^{5,31} Tregs directly or indirectly inhibit allergen-induced activation and degranulation of effector mast cells and basophils.⁶⁰ For example, Tregs directly inhibit the degranulation of mast cells via mechanisms that require OX40–OX40L interactions. They can also downregulate the expression of Fc ϵ RI in mast cell, thus indirectly reducing the amount of IgE anchored to their membranes and consequently allergen-induced degranulation, by a mechanism independent of IL-10 and TGF- β .^{60–62} Another important feature of Treg cells to suppress allergic reactions is their ability to inhibit DC maturation promoting the generation of tolerogenic DC phenotypes, thus avoiding the progression of inflammatory responses.⁶³ At the B-cell level, Tregs induce allergen-specific IgG4 and inhibit allergen-specific IgE production.⁶⁴ At the T-cell level, Tregs not only directly inhibit the activation of allergen-specific Th2 cells, but also can suppress T-cell responses driven by Th1 and Th17 cells, thus impairing the contribution of all these T-cell subsets to allergic inflammation. Tregs can also directly and indirectly impair the infiltration of eosinophils and

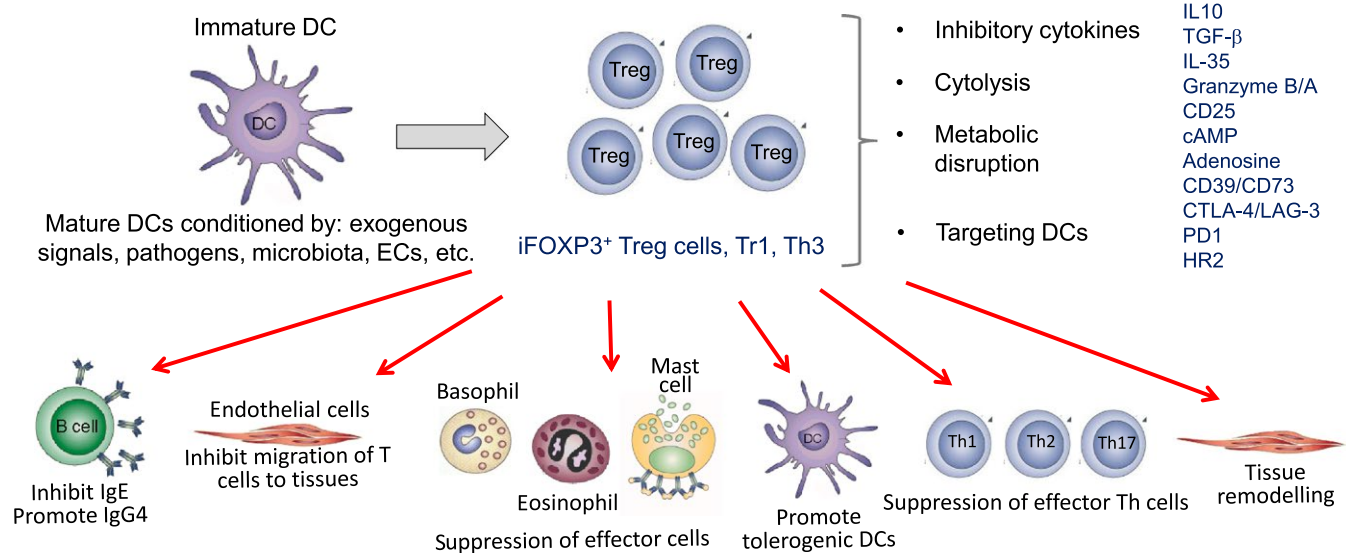


FIGURE 3 Treg cells suppress allergic reactions by inhibiting different key inflammatory events. Steady-state dendritic cell (DCs) or DCs conditioned by exogenous signals, ECs, pathogens, microbiota, etc., promote the generation of different types of functional Treg cells that employ four main mechanisms for suppression (inhibitory cytokines, cytolysis, metabolic disruption, and targeting DCs) using a plethora of soluble and surface-bound molecules. Treg cells prevent and inhibit ongoing inflammation by acting on many different cell types driving allergic inflammation including B cells, endothelial and smooth muscle cells, effector cells, DCs, T cells, and tissues

other effector T cells into inflamed tissues.⁶⁵ Importantly, Tregs may interact with resident tissue cells and contribute to tissue remodeling.^{61,62} For example, in asthma, Treg-derived TGF-β constitutes an important factor for lung healing with important immunomodulatory and fibrogenic activities that might aim to contribute to the repair of asthmatic airways but end up with incorrect remodeling with some fibrosis.⁶⁶ The role of TGF-β produced by Tregs in asthma is complex and needs to be further investigated as many other cells including eosinophils and tissues also produce TGF-β. Recently, it has been demonstrated that the numbers of Tregs in the asthmatic patients are significantly reduced during viral and non-viral exacerbations, supporting their essential role in lung homeostasis and possibility of breaking allergen tolerance by viral infections.^{17,47,67,68}

3.1 | Thymus-derived CD4⁺ CD25⁺ Foxp3⁺ Tregs

Thymus-derived Tregs (tTregs) cells represent <5% of the T-cell population in healthy mice and humans.⁶⁹ There is still some controversy regarding their *in vivo* generation, mainly due to the lack of definitive markers to distinguish between tTregs generated in the thymus or potentially induced in the periphery.^{53,70} Different studies suggest that tTregs specific for self-peptides arise from the thymus, whereas those required to avoid pathologies triggered by antigens/allergens may be generated in the periphery from naïve T cells after antigen encounter.^{71,72}

The forkhead-winged transcription factor FOXP3 (forkhead box P3) is the master switch transcription factor for the generation of functional tTregs.^{73,74} Although FOXP3 is exclusively expressed by tTreg cells in mice,^{75,76} in humans it might be also expressed in a proportion of activated T cells.⁷⁷ The role of FOXP3 for tTregs development was

initially demonstrated in mice with a deletion in the forkhead domain of FOXP3 (scurfy mice), showing an intense multiorgan inflammatory response associated with allergic airway inflammation, a striking hyper IgE, eosinophilia, and dysregulated Th1 and Th2 cytokine production.⁷⁸ More recently, essential *in vivo* functions of Tregs have been demonstrated in the DEpletion of REGulatory T cells (DEREG) mice,^{79–81} allowing selective depletion of FOXP3⁺ Tregs.

The key role of FOXP3 for functional tTregs development in humans was definitely confirmed in subjects with typical severe autoimmune and allergic phenotype starting on from infancy, including eczema, food allergy, and eosinophilic inflammation. These patients suffer from the immune dysregulation polyendocrinopathy enteropathy-X-linked syndrome or the X-linked autoimmune and allergic dysregulation syndrome, characterized by mutations in FOXP3.^{82,83} The retroviral transduction of FOXP3 and allergen-specific TCR αβ-chains allowed the generation of allergen-specific Tregs with suppressive capacity.⁸⁴ The knowledge of the molecular mechanisms involved in the control of FOXP3 expression has also improved in the last years. TGF-β induces RUNX1 and RUNX3, which are essential for FOXP3 expression and for the generation of Tregs with suppressive capacity.⁸⁵ GATA-3 binds to the promoter region of FOXP3 inhibiting its expression, which constitutes a mechanism by which Type 2 immune responses might inhibit tolerance.⁸⁶ Treg-cell-specific epigenetic changes are also critical for the maintenance of FOXP3 expression and functional and stable Tregs generation.^{76,87,88} In mice, it was shown that FOXP3 partners with other transcription factors to control distinct biologic aspects of Treg cells.⁸⁹ Other transcription factors such as FOXO1 play important roles in the control of functional Tregs cells.^{76,90}

Different surface markers have been identified to be highly expressed by tTregs but none of them are fully specific and are also

expressed under certain conditions by other effector T cells.^{76,77,91} The alpha chain of IL-2R (CD25) is constitutively and highly expressed in tTregs and the alpha chain of the IL-7R (CD127) was proposed as a differentiation marker expressed on activated effector T cells, but not on tTregs.⁹² Other markers expressed by tTregs include cytotoxic T lymphocyte antigen-4 (CTLA4), a molecule that after ligation to CD80 and CD86 suppress DCs activation,⁹³ PD1, an immunoreceptor tyrosine-based inhibitory motif-containing receptor,⁹⁴ GITR, CD103 (α E β 7 integrin), and CD122 (β chain of IL-2 receptor). Monoclonal Abs targeting CTLA-4 and PD-1 are significantly extending the survival of cancer patients are now called check point inhibitors,⁹⁵ demonstrating that these essential suppressor mechanisms for allergen tolerance⁹⁶ are being used in tumor tolerance. Gene arrays and proteomic studies uncovered additional markers, including GPR83, Ecm1, Helios, certain chemokine receptors and toll-like receptors (TLR), GARP, neuropilin-1, LAG-3, LAP, granzymes, etc.^{97–99}

3.2 | IL-10-producing type 1 Treg cells (Treg1)

Type 1 T regulatory (Treg1) cells generated in the periphery play a very important role in the induction and maintenance of healthy immune response in autoimmunity, graft-versus-host disease, or allergy.^{15,100,101} Treg1 cells produce high levels of IL-10 production and display potent regulatory/suppressive capacity.^{15,102} Treg1 inhibits effector T cells through mechanisms depending on IL-10 and also TGF- β and may kill APC using perforin and granzymes (Figure 3).¹⁰² Functional Treg1 coexpress CD49b and LAG-3 both in human and mouse.¹⁰⁰ In addition to IL-10, Treg1 may also produce low/medium levels of TGF- β , IFN- γ , and IL-5, but not IL-4 nor IL-2.^{103,104} Stimulation of CD4⁺ T cells with glucocorticoids, alone or in combination with 1 α 25-dihydroxy vitamin D3, or with different cytokines cocktails (i.e. IL-10 and IFN- α or TGF- β and IL-27), or with anti-CD3 and anti-CD46 monoclonal antibodies, always in the absence of Th1 and Th2 polarizing cytokines have been shown as suitable in vitro strategies to generate IL-10-producing Treg1 with suppressive capacity in different in vivo models.^{105,106} For example, these induced Treg1 were able to impair proliferation and cytokine production of naïve and memory T cells in humans, including allergen-specific Th2 cell lines.¹⁰⁷ Transducing human CD4⁺ T cells with a lentiviral vector encoding for human IL-10 promotes the generation of stable T cells producing high levels of IL-10 and displaying equivalent phenotypic and functional properties to Treg1.¹⁰⁸ They were first demonstrated in colitis models in mice and AIT and high-dose bee venom exposed, but venom allergen-tolerant individuals in humans.^{109,110}

In the context of allergic diseases, IL-10 has been demonstrated as a key cytokine to impair allergic inflammation in many studies. For example, in the respiratory tract, DCs from healthy individuals constitutively produce high levels of IL-10, which is significantly reduced in allergic rhinitis and asthma patients.^{31,111} In the same way, clinically successful AIT is associated with the generation of local and peripheral IL-10-producing Tregs.^{109,112} The generated Tregs1 employ the above-described mechanisms to inhibit all the features associated with

allergic inflammation (Figure 3), thus contributing to impair clinical symptoms associated with allergy. Both healthy and allergic patients display all different subsets of allergen-specific T cells (Th1, Th2, and Tregs1), but at distinct proportions and depending on the predominance of these specific subsets the final outcome will be allergy development or recovery. In healthy individuals, allergen-specific Tregs1 are the predominant T-cell subset able to inhibit allergic responses to different allergenic sources such as birch pollen and related food allergens, house dust mite, or bee venom.^{5,31,96}

An important breakthrough in the understanding of the mechanisms underlying allergen tolerance induction in vivo in humans came from different models of high-dose exposure to allergens. It was demonstrated that exposure to high doses of bee venom allergen in professional beekeepers due to multiple stings during the beekeeping season was also demonstrated as a natural mechanism of immune tolerance induction to allergens.^{15,109} Similarly, exposure to high doses of cat allergen promotes the generation of functional IL-10-producing Tregs1 and high levels of allergen-specific IgG4, which is associated with tolerance induction without new sensitizations or asthma development.¹⁶ Under these conditions, a clonal differentiation and expansion of IL-10-producing Tregs1 from allergen-specific Th1 and Th2 cells is observed as long as venom exposure persists and returns back to previous levels within 2–3 months after the end of beekeeping season.¹⁵ Different molecules were identified as important regulators of these processes. Among them, histamine receptor 2 was shown to be upregulated on specific Th2 cells, which contributed to suppress allergen stimulated T cells and to enhance IL-10 production.¹⁵ The molecular mechanisms involved in the generation of functional Tregs during natural tolerance induction or successful AIT and the specific contribution of DCs in these processes are starting to be elucidated but are not yet fully understood.

3.3 | Mechanisms of generation of Treg cells: role of DCs

DCs are essential in the orchestration of immune responses by linking innate and adaptive immunity.^{113,114} They are equipped with a wide battery of receptors including pattern recognition receptors (PRRs), such as TLRs and other sensors of pathogen-associated molecular patterns; receptors that recognize danger signals (DAMPs) released after tissue damage; and many other receptors recognizing different surrounding substances including neurotransmitters.^{115–118} In humans, circulating DCs can be broadly divided into two groups: (i) myeloid DCs (mDCs) and (ii) plasmacytoid DCs (pDCs).^{114,119} mDCs can be further divided into type 1 mDCs (mDC1), expressing BDCA1, and type 2 mDCs (mDC2), expressing BDCA3.^{12,120,121} All these subsets display a different repertoire of receptors and cytokine signature after stimulation and they might play distinct roles in the context of allergy.

Several studies have demonstrated that immature or mature DCs conditioned by different stimulus induce functional Tregs using soluble (TGF- β , IL-10, IDO, RALDH, etc.) and costimulatory (ICOS-L, PD-L1, ILT-3, ILT-4, 4-1BB, CD80, CD86, MHC-II, etc.) molecules.³ The

tolerogenic capacity of DCs depends on certain maturation stages, subsets of different ontogeny and can be influenced by signals delivered by neighboring cells, such as ECs.^{5,12,31} The capacity of DCs to generate Tregs can be conditioned by FOXP3⁺ Treg, pathogen-derived molecules, and exogenous signals, such as histamine, adenosine, flavonoids, vitamin D3 metabolites, retinoic acid, mannan or neurotransmitters.^{5,31} For example, vitamin D acts on different DC subsets conditioning their capacity to generate Treg1 cells or FOXP3⁺ Treg cells.¹²² Similarly, retinoic acid, a metabolite derived from vitamin A, favors the maintenance of Treg cells by inhibiting the formation of inflammatory Th17 cells. Recently, it has been demonstrated that allergoids conjugated to mannan endorses human DCs with the capacity to generate functional FOXP3⁺ Tregs via mechanisms that depend on PD-L1.¹²³

4 | HISTAMINE AND IMMUNE-REGULATORY EFFECTS OF HISTAMINE RECEPTOR 2

The important roles of histamine in various physiopathology events and immune regulation have been well established.^{124,125} It is not only the major mediator of the acute inflammatory and immediate hypersensitivity responses, but has also been demonstrated to regulate essential events in the immune response. The diverse effects of histamine on immune regulation are due to the differential expression and regulation of four histamine receptors (HR) and their distinct intracellular signals. In particular, the discovery of reciprocal regulation of T-cell activity by H1 and H2 receptor activation indicating histamine receptor cross-talk with cytokines and their expression on numerous immune and inflammatory cells were demonstrated a mostly immune suppressive effects of HR2 on DC, T cells, and basophils.^{126–128} In contrast, immune activator roles of HR1 and HR4 have been shown on many cells.^{128,129} HR2 has been demonstrated to play an essential role in immune regulation by Treg cells and DCs. Gut microbiome has been suggested as one of the sources of histamine and recently histamine-secreting bacteria have been demonstrated.

5 | THE ROLE OF REGULATORY B CELLS IN ALLERGIC DISEASES

Regulatory B cells encompass a heterogeneous group of different immunosuppressive B-cell subsets with distinct phenotypic and functional properties that have been described both in mice and humans.¹³ Early experiments in guinea pig models of allergen sensitization showed that adoptively transferred splenocytes depleted of B cells lost their suppressive capacity on delayed-type hypersensitivity reactions in the skin, suggesting the existence of B cells with potential immunosuppressive capacity.^{130,131} However, at that time, mechanisms of suppression were not identified and immunosuppressive B cells did not receive significant attention for over 20 years. It was not until the discovery that mice deficient in B cells did not properly

recover from experimental autoimmune encephalomyelitis when immunosuppressive B cells recovered back the scientific community's interest.¹³² B cells switching to IL-10-producing B cells were demonstrated in humans in high-dose allergen exposure models and AIT.¹⁰⁹ In addition, anti-CD20 mAb Rituximab treatment, a biologic agent that depletes total B cells, causes exacerbation of colitis and new onset of psoriasis, suggesting that B-cell subsets with immunosuppressive capacity might well exist in humans.^{133,134} Different studies in autoimmune models convincingly demonstrated that IL-10-producing B cells were essential in the suppression of intestinal inflammation and arthritis.^{135–137} Although IL-10 has been demonstrated as the key cytokine involved in the immunosuppressive capacity of Bregs, other cytokines such as TGF- β and IL-35 as well as several surface-bound suppressive molecules have also been shown to contribute to their immune-regulatory capacity.^{138–140}

5.1 | Different subsets of Bregs in mouse and humans and suppressor molecules

Several subsets of Bregs with distinct phenotypic features and mechanisms of suppression have been described both in mice and humans.¹³ According to their specific features, mouse Bregs could be broadly divided into CD5⁺CD1d^{hi} B10 cells, CD21^{hi}CD23^{hi}CD24^{hi} transitional stage 2(T2)-like B cells, and CD138⁺ plasma cells and plasmablasts. Different subsets of human Bregs subsets have been also described including CD27⁺CD24^{high}CD148^{high}CD48^{high} B10/pro-B10 cells, CD24^{hi}CD38^{hi} immature transitional B cells, CD73⁺CD25⁺CD71⁺ Br1 cells, and a CD27^{int}CD38⁺ subset of plasma cells.¹³

Murine B1 cells are found in the peritoneal and pleural cavities and can be subdivided in CD5⁺ B1a, which have been shown to produce significant amounts of IL-10,^{141,142} and CD5⁺ B1b cells. Murine CD5⁺CD1d^{hi} B10 cells¹⁴³ share CD5 expression and many other features of B1a B cells. However, most of the other types of mouse Bregs described to date display phenotypes related to B2 B cells. B2 cells can be classified as follicular B cells and marginal zone (MZ) B cells. These immature B cells are called transitional B cells (T1 and T2 depending on their maturation stage).¹⁴⁴ In mice, both T2 MZ precursor B cells (CD21^{hi}CD23^{hi}CD24^{hi}IgM^{hi}IgD^{hi}CD1d^{hi})¹⁴⁵ and MZ B cells (CD21^{hi}CD23⁺CD24^{hi}IgM^{hi}IgD^{lo}CD1d^{hi})¹⁴⁶ have been associated with IL-10-mediated immunoregulatory functions. In addition, human Bregs have been also found among immature transitional B cells (CD19⁺CD24^{hi}CD38^{hi}).¹⁴⁷ Remarkable, Bregs are also reported to be contained among both mouse and human plasma cells.^{138,148,149}

It is noteworthy that Bregs can be found among B cells at different stages of maturation and differentiation including early transitional B cells¹⁴⁵ and highly differentiated plasmablasts¹⁴⁹ and plasma cells.^{138,150} These data suggest that there are either multiple distinct lineages of Breg cells, or that IL-10 can be induced by environmental stimuli at various stages of B-cell development. This is an emerging field of research and still many questions remain to be addressed regarding to the ontogeny, plasticity as well as mechanisms of suppression employed by Bregs.

Up to date, three main cytokines produced by Bregs have been reported to significantly contribute to suppressive properties: IL-10, TGF- β , and IL-35.¹⁵¹ IL-10 not only displays a very potent immunosuppressive capacity, but also positively regulates B-cell survival and class-switch recombination, as well as plasma cell differentiation.^{23,152} Many cell types, including monocytes, T cells, DCs, natural killer cells, macrophages, mast cells, and B cells, can produce IL-10 and potentially contribute to the reported effects on B cells.^{23,152} TGF- β is a pleiotropic cytokine that plays a key role wound healing, tissue remodeling, and immune regulation as above described. IL-35 contributes to immune suppression by suppressing Th17 responses while promoting Tregs proliferation.²³ Bregs also employ a plethora of surface-bound and intracellular molecules that contribute at different extend to the reported immunosuppression mechanisms, such as CD19,¹⁴³ CD62L and MHCII,¹⁵³ TIM1,^{154,155} and PD-L1^{14,148,156,157}; as well as signaling molecules STAT3¹⁴⁷ and Myd88.^{150,158}

5.2 | Breg cells in induction and maintenance of immune tolerance to allergens

Bregs may also play a key role in the induction of immune tolerance to allergens by suppressing allergen-mediated inflammation via IL-10 and TGF- β , thereby suppressing effector T-cell responses and favoring Tregs induction and by promoting the production of IgG4 antibodies upon differentiation to plasma cells (Figure 4).^{13,14} IL-10-producing Bregs were purified from humans using a specific IL-10-secretion assay after TLR9 stimulation, which allowed the isolation and detailed characterization of live IL-10⁺ and IL-10⁻ B cells. These inducible IL-10⁺ Bregs were enriched among CD19⁺CD73⁻CD25⁺CD71⁺ cells that also expressed PD-L1, a suppressor co-stimulatory molecule that impairs T-cell activation after binding to PD-1 on T cells.¹⁴ IL-10-producing Bregs potently suppressed in vitro antigen-specific T-cell proliferative responses and showed a substantial shift toward production of anti-inflammatory IgG4 antibodies.¹⁴ Remarkably, IL-10-producing Bregs produced >15-fold more IgG4 than IL-10⁻ B cells without significant differences in the other immunoglobulin isotypes. The specific phenotype of IL-10-producing Bregs is associated with their immunosuppressive properties. Elevated CD25 was shown in Breg cells. Its expression on human B cells correlates with enhanced in vitro antigen-presenting capacity,¹⁵⁹ as well as elevated B-cell IL-10 production in a murine model for intestinal inflammation.¹⁶⁰ CD71, the major transferrin receptor mediating iron uptake, is the activation marker of T and B cells. CD73 is a classical surface marker expressed by Tregs that converts adenosine monophosphate to adenosine, which has potent immunosuppressive effects. The lack of CD73 on IL-10-producing Bregs suggests that this molecule is not involved in the observed suppressive capacity in B cells. These inducible IL-10-producing Bregs were named as Br1 cells in analogy to inducible Tregs1.

The important role played by Br1 cells in tolerance to allergens was initially observed in healthy beekeepers and bee venom allergic patients undergoing AIT. Phospholipase A2 (PLA) is the major

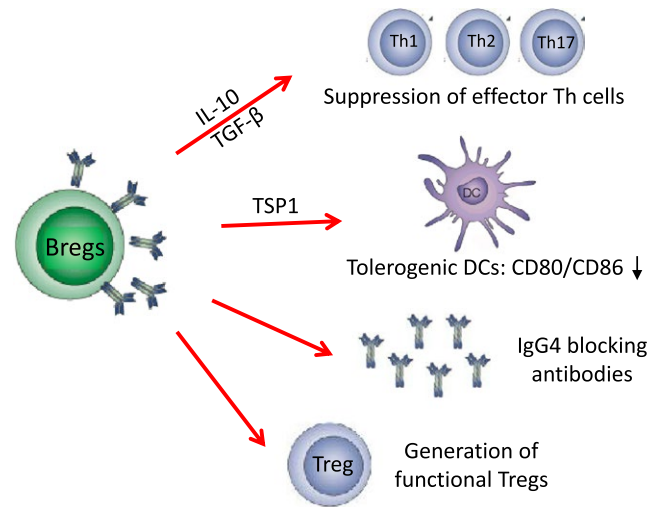


FIGURE 4 Role of Breg cells in the regulation of allergic inflammation and allergen tolerance induction. Different mechanisms have been shown to be employed by Breg cells to promote proper responses to allergens in healthy subjects and after successful AIT. Breg cells suppress effector Th cells, promote the generation of tolerogenic DCs, produce blocking IgG4 antibodies, and promote the generation of Treg cells

bee venom allergen. PLA-specific B cells isolated from healthy beekeepers displayed high levels of IL-10 and mature IgG4 mRNA. Interestingly, in bee venom allergic patients undergoing AIT, the frequency of IL-10-producing PLA-specific B cells increased two- to fivefold after four months of venom immunotherapy, reaching levels comparable to those observed in healthy beekeepers.¹⁴ These data clearly indicate that high doses of allergen exposure promote PLA-specific Bregs expansion both in healthy beekeepers and bee venom allergic patients during AIT, suggesting the IL-10-producing Bregs might well play important functional roles in the induction and maintenance of allergen tolerance. In addition, it has been recently shown that PLA-specific Bregs upregulate CCR5 expression,¹⁶¹ a marker expressed by Tregs that is needed for migration to inflamed tissues, in both healthy beekeepers and patients undergoing AIT after venom exposure.¹⁶² The overexpression of solely IL-10 on human B cells demonstrated the importance of IL-10 in Breg cells¹⁶³ with the induction of a prominent immunoregulatory profile comprising upregulation of suppressor of cytokine signaling 3 (SOCS3), glycoprotein A repetitions predominant (GARP), the IL-2 receptor α chain (CD25), and programmed cell death 1 ligand 1 (PD-L1). Their cytokine secretion profile was characterized by a significant reduction in proinflammatory TNF- α , IL-8, and macrophage inflammatory protein 1 α and increased anti-inflammatory IL-1 receptor antagonist and vascular endothelial growth factor in addition to IL-10. IL-10-overexpressing B cells secreted less IgE and potently suppressed proinflammatory cytokines in PBMCs, maturation of monocyte-derived DCs (rendering their profile to regulatory phenotype).¹⁶³

Mouse models of allergic airway inflammation have also delineated the important immunoregulatory role played by

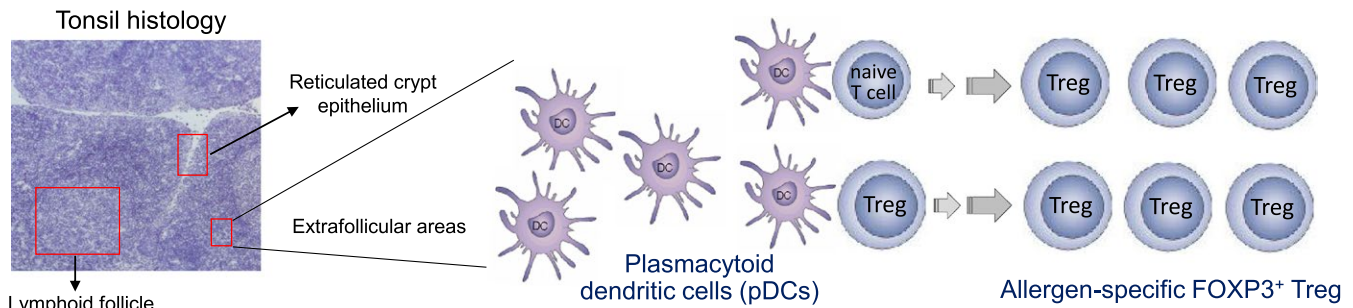


FIGURE 5 Lingual and palatine tonsils are organs of allergen tolerance induction. Large numbers of pDCs and Treg cells colocalize in the extrafollicular areas of human tonsils. pDCs promotes the generation of allergen-specific FOXP3⁺ Tregs from naive CD4⁺ T cells and expand already generated Treg cells, which proliferate in vivo in human tonsils

IL-10-producing Bregs. It has been demonstrated by adoptive transfer experiments that CD9⁺ B cells were able to suppress Th2 and Th17-mediated airway inflammation by mechanisms dependent on IL-10.¹⁶⁴ TGF- β -producing B cells may also contribute to allergen tolerance as demonstrated in mouse models of allergic airway inflammation. Mice subjected to chronic exposure of aerosolized OVA develop tolerance without any sign of eosinophilia, T-cell inflammation, or airway hyperresponsiveness. This model resembles high-dose allergen tolerance induction. B cells isolated from hilar lymph nodes of tolerant mice suppress allergic airway sensitization as demonstrated by adoptive transfer experiments. This suppression was not mediated by IL-10 as demonstrated using B cells from tolerant IL-10^{-/-} mice.¹⁶⁵ Further studies demonstrated that TGF- β -producing CD5⁺CD1d^{hi} B cells able to induce Foxp3 in CD4⁺ T cells in vitro mediated this suppressive effect. TGF- β -producing CD5⁺CD1d^{hi} B cells were primarily localized in the regional lymph nodes and not in the spleen where IL-10-producing CD5⁺CD1d^{hi} B10 cells are mainly found.¹⁶⁶ Supporting the role of TGF- β -producing Bregs in allergen tolerance induction, murine models for food allergy and food allergy immunotherapy also showed that their generation and expansion are essential for tolerance induction to food allergens.^{167,168} Adoptive transfer of activated TGF- β -producing CD5⁺CX3CR1⁺ Bregs able to promote in vitro induction of CD4⁺CD25⁺Foxp3⁺ cells and suppressed T-cell proliferation, leading to suppression of food allergy-related T_H2-mediated intestinal inflammation.¹⁶⁷ OVA sensitized mice receiving AIT displayed a significant increase in lamina propria CD35⁺ B cells producing thrombospondin 1, a protein that binds to latent TGF- β to generate active TGF- β .¹⁶⁸

Collectively, these data demonstrated that IL-10- and TGF- β -producing Bregs are expanded in response to high-dose allergen exposure both in humans and mice. These responses appear to be, at least in part, antigen-specific as frequencies of allergen-specific IL-10-producing B cells specifically increased after AIT. Importantly, Breg cells promote the generation of functional Tregs and suppress effector T-cell activation. The phenotypes of the Breg cells involved in these responses and their mechanisms of generation and expansion are starting to be elucidated but they still remain poorly defined.

6 | TONSILS AS AN ORGAN OF ALLERGEN TOLERANCE

Induction and maintenance of immune tolerance to self-antigens and innocuous environmental antigens are essential to prevent autoimmune and allergic diseases. A fine balance between pro-inflammatory responses needed to clear pathogens, and immunosuppressive responses to prevent chronic inflammation and tissue damage is preserved in healthy subjects. Under normal conditions, the default immune responses against innocuous environmental antigens such as allergens should be ignorance or tolerance induction. Based on mouse models, it was accepted for long time that exposure to high doses of innocuous antigens leads mainly to anergy or clonal deletion of antigen-specific T cells, whereas low doses induces preferentially Tregs as a main mechanism of tolerance induction.^{169,170} Neither high nor low allergen doses nor even T-cell peptide treatment caused clonal deletion in humans. In addition, more recent findings demonstrated that administration through the oral route of high doses of antigens may also induce allergen-specific Tregs, thus supporting the main role of Tregs in the generation of oral tolerance to allergens.^{171,172} Similarly, studies in murine models of food allergens revealed an important role of allergen-specific Bregs in tolerance induction to food allergens.^{167,168}

In humans, it has been shown that the generation of tolerance to allergens might well occur in several important tissues in the body such as tonsils, gastrointestinal mucosa, respiratory tract, skin, and oral mucosa via different immune mechanisms leading to the generation of allergen-specific Treg and Bregs.^{12,14,17,173,174} Tonsils represent first-line immunologic organs against food and aeroallergens. They are above gastric acid and digestive proteases. Swallowing squeezes food antigens into tonsils and aeroallergens reach to a high speed during inspiration and hit the walls of pharyngeal tonsils. Tonsils have only one level ECs, which is much thinner compared to oral mucosa with non-cornified but squamous epithelium. Tonsillectomy removes palatine tonsils and adenoids but lingual tonsil remains intact. Human tonsils have been demonstrated as organs where allergen tolerance induction takes place (Figure 5).¹² Functional allergen-specific Tregs are generated in palatine and lingual tonsils by mechanisms partially depending on pDCs.¹² Human tonsils are organs containing a large number

of pDCs that co-localize *in vivo* with Tregs at the T-cell extrafollicular areas in close proximity to high endothelial venules. Tonsils also contain a large number of allergen-specific Tregs, which can proliferate *in vivo* as demonstrated by ki-67 staining. *In vitro* studies uncovered that purified tonsil pDCs are able to generate functional Tregs from allogeneic naïve CD4⁺ T cells. Interestingly, tonsil Bet v 1-specific T cells and Tregs were identified in human tonsils using major histocompatibility complex class II tetramer molecules coupled to specific Bet v 1 peptides.¹² Different studies also demonstrated that extrathymic T cells and Tregs might develop in human tonsils.^{175,176} All these data support the notion that human tonsils are organs where immune regulation takes place, which may have important implications for the development of future therapeutic interventions. In addition, human tonsils resemble the classical peripheral cytokine profile features ascribed to allergic patients.¹⁷ It was demonstrated that palatine tonsils from allergic patients display low levels of IFN- γ , T-bet, and IL-10 and increased Th2/Th1 ratio, which are typical features of the atopic status.¹⁷ The immunologic mechanisms by which allergen tolerance might be broken in humans leading to allergic diseases is still an open question that remains not fully understood, but recent studies have contributed to shed light into this exciting topic. Compelling experimental evidence have demonstrated that viral and bacterial infections might be important risk factors for allergy development and severity of symptoms due to their capacity to alter immune responses, leading to breaking tolerance to allergens.^{177–179} In this context, early studies demonstrated that¹⁸⁰ triggering of TLR4 or TLR8 and proinflammatory cytokines, such as IL-1 β and IL-6 break allergen-specific T-cell tolerance in human tonsils and peripheral blood.¹⁷ In contrast, activation with TLR7 or TLR9 and other assayed cytokines such as IL-10 or IL-17A did not show such effects. In this study, it was also demonstrated that mDCs represented the DC subset involved in breaking allergen tolerance, whereas pDCs did not change the tolerance status under the assayed conditions.¹⁷ Supporting the notion that tonsils as organs where immune regulation takes place, it has been recently shown that tonsillar cytokine expression is closely related to existing viral infections, age, and allergic illnesses.¹⁸ Network analyses uncovered strongly polarized clusters of immune-regulatory (IL-10, IL-17, TGF- β , FOXP3, GATA3, RORC2, and Tbet) and antiviral (IFN- α , IFN- β , IL-28, and IL-29) genes. These two clusters became more distinctive in the presence of viral infection or allergy.¹⁸ In addition, it was demonstrated that the mRNA expression levels of the cannabinoid receptor CB1 are upregulated in peripheral blood and tonsils of allergic patients compared to control subjects.¹⁸¹ Using validated chemical probes for the cannabinoid receptor CB1,¹⁸⁰ these findings were confirmed at the protein levels and CB1 expression demonstrated in purified tonsil T cells, B cells, mDCs, and pDCs.¹⁸¹ More recently, it has been also demonstrated a direct relationship of serum vitamins A, D, and E and LL-37 levels with allergic status, tonsillar virus detection, and immune response.¹⁸² In this study, it was demonstrated that an antiviral activity was linked to serum levels of vitamin A, anti-inflammatory activity was linked to vitamin D, and Tregs was linked to LL-37. Interestingly, vitamin D and E levels were inversely associated with allergic status, which might be associated with allergen tolerance induction. Collectively, these data

indicate that tonsils are immunologically active mucosa-associated lymphoid tissues located in the main entrance of the respiratory and gastrointestinal tracts as a first line of defense where immune regulation and allergen tolerance induction take place (Figure 5). Therefore, it could be hypothesized that human tonsils may represent immunologic relevant tissues, where immune tolerance induction during successful SLIT occurs.^{12,17,18} Considering that the relatively large lingual tonsil is not removed by tonsillectomy and remains life-long intact, it is reasonable to conceive that tonsils are organs involved in tolerance induction that might well represent a potential novel target for future therapeutic interventions. Therapeutic strategies specifically targeting main cell components located in human tonsils to promote allergen tolerance induction through the generation of allergen-specific Tregs and Bregs could be developed in the future as a novel promising strategy not only for allergic diseases, but also for other immune tolerance-related diseases.

7 | ALLERGEN-SPECIFIC IMMUNOTHERAPY

Currently, AIT is the single disease-modifying treatment of allergic diseases with potential curative capacity.^{4,183–186} AIT consists of repeated administration of high doses of the causative allergen, usually by subcutaneous injection (SCIT) or sublingual application (SLIT), to induce a state of permanent tolerance after treatment discontinuation.¹⁸⁶ Our understanding on the immunologic mechanisms operating during successful AIT has significantly improved over the last decades.^{4,183,186,187}

7.1 | Immunologic mechanisms operating during AIT

Nowadays, it is very well established that during AIT a very rapid desensitization of mast cells and basophils occurs, which impairs systemic anaphylaxis. This desensitization occurs immediately after the first administration. Although the involved mechanisms are not yet fully understood, it is thought that similar events to those observed during rapid drug desensitization might be working during AIT.^{4,126,183} Different mechanisms of action and molecules such as histamine receptor 2 have been shown to contribute to the rapid desensitization of effector cells.¹²⁶ The cellular immunologic changes associated with successful AIT encompasses the generation of allergen-specific Treg and/or Th1 cells, allergen-specific Breg cells, inhibition of Th2 responses as well as reduction of infiltrating inflammatory cells.^{4,183} The humoral response that marks allergen tolerance induction during AIT is characterized by a transient rise in allergen-specific IgE followed by a decrease in IgE and a gradual increase in allergen-specific IgG antibodies. IgG4 particularly increases during the course of AIT linked to continuous administration of the allergen, which results in a 10- to 100-fold decreased ratio of allergen-specific IgE:IgG4.^{183,188} IgG4 production has been linked to Breg cells¹⁴ and they are considered as classical non-inflammatory due to their specific structural and functional features: (i) they do not activate complement, (ii) they display low affinity for Fc γ receptors, and (iii) they are able to exchange Fab arms leading

to unique functional bi-specific monovalent antibodies.¹⁸⁹ Therefore, anti-inflammatory allergen-specific IgG4 may protect against allergic responses by competing with IgE for allergen binding.^{190,191} All these cellular and humoral events cooperate together to keep allergen tolerance and need maintenance during time, which correlate with the reductions in late-phase responses (decrease in tissue mast cells and eosinophils and release of their mediators) and skin test reactivity.

Efficacy in the restoration of healthy immune responses to allergens after AIT for allergic rhinitis and venom allergy has been convincingly demonstrated.^{192,193} In addition, randomized controlled clinical trials have shown AIT as an effective treatment in reducing symptom scores and medication use, improving quality of life and inducing favorable changes in specific immunologic markers in allergic moderate asthma.^{184,185,194} Subcutaneous administration routes combined with aluminum salts as adjuvants or sublingual administration routes were employed.¹⁹⁴ Despite these reported clinical benefits, AIT still faces several major drawbacks related to safety, efficacy, long treatment duration, and low patient adherence.^{19,184,195} Over the past few years, intensive research has been performed to develop different strategies to overcome such inconveniences, including alternative routes of administrations, the use of different hypoallergenic variants, or the use of novel types of adjuvants.^{19,195,196}

7.2 | Immunologic effects of novel adjuvants and novel routes of administration to improve AIT

The development of novel adjuvants for AIT is a promising strategy that might well help to improve efficacy, safety, and reduce treatment duration.^{19,20} In general, adjuvants could be defined as substances with the capacity to stimulate or potentiate the therapeutic effect of an allergen vaccine through different mechanisms without being an allergen per se. Adjuvants need to fulfill different properties so that they can be considered as potential candidates for AIT and be employed and formulated in allergen vaccines. Apart from the expected beneficial features, adjuvants must be safe without any toxic effects or risk to potentiate other diseases such as autoimmune disorders, cancer, etc. and they should show stability within the vaccine formulation. Importantly, according to the above-described immunologic mechanisms operating during AIT, adjuvants should be synthetic or biologic compounds able to stimulate potent allergen-specific Th1 and/or Treg cells by directly targeting different immune system cells or act as vector systems able to enhance allergen uptake by APCs, mainly tolerogenic DCs. Taking all of the above aspects into account, adjuvants are broadly classified into two main groups: (i) immunopotentiators and (ii) vector systems.

7.3 | Immunopotentiators

Among immunopotentiators, mineral adjuvant compounds such as aluminum hydroxide or calcium phosphate have been broadly used in humans for AIT.¹⁹ Although mechanisms of action are not completely understood yet, mice models demonstrated that aluminum hydroxide might exert adjuvant capacity by promoting the release of uric acid,

which in turn contributes to the recruitment of inflammatory cells and activate NALP3 inflammasome in inflammatory DCs.¹⁹⁷ Alum promotes the production of PGE2 in macrophages via inflammasome-independent mechanisms favoring IgE production.¹⁹⁸ More recently, it has been shown that aluminum salts kill alveolar macrophages, which subsequently release IL-1 α promoting the formation of tertiary lymphoid tissues in the lung that sustain type-2 allergic immune responses after allergen exposure.¹⁹⁹ Many different immunopotentiators have been analyzed in mouse models of allergy. For example, agonists targeting TLRs such as TLR-4 (monophosphoryl lipid A, MPL), TLR5 (flagellin), TLR-7/-8 (imidazoquinolines or resiquimod, R848), TLR-9 (unmethylated CpGs), living or heat-inactivated bacteria (*Lactobacillus plantarum*, *Lactococcus lactis*, or *Mycobacterium vaccae*); or small molecules such as 1,25-dihydroxy vitamin D3 (vitD3) metabolite.^{19,200} In humans, a beneficial effect and safety profile have only been reported for some of these adjuvants such as TLR agonists (TLR4, 7/8, and 9 agonists), virus-like particles (VLPs), 1,25-dihydroxy vitamin D3 (vitamin D).

Monophosphoryl lipid A (MPL) is a detoxified derivative from *Salmonella Minnesota* LPS acting on TLR4.²⁰¹ MPL was shown to act as an effective adjuvant in both SCIT and SLIT. Recently, the efficacy of MPL has been demonstrated in combination with L-tyrosine in ultra-short subcutaneous SCIT (only four injections) in grass or ragweed pollen allergic patients with rhinoconjunctivitis.^{200,201} MPL-adjuvanted pollen allergoids induced FOXP3⁺ Tregs and IgG4 blocking antibodies in asthmatic patients after 2 years of short-term SCIT.²⁰²

Unmethylated CpG oligonucleotides (CpG-ODN) sequences from bacterial DNA are TLR9 agonists that are able to activate B cells and plasmacytoid dendritic cells promote Th1 and Treg cells. Mouse models of allergic asthma demonstrated that CpG-ODN alone or in combination with nanoparticles inhibits classical asthma features after allergen challenge.^{203–206} In humans, initial studies using the major allergen of ragweed pollen, Amb a 1, conjugated to CpGs demonstrated efficacy, but subsequent larger phase III clinical studies did not show significant differences between placebo and treated groups after 1 year.²⁰⁷ Senti et al.²⁰⁸ showed that QbG10, type-A CpG-ODNs encapsulated in VLPs from the capsid of Q β phage, subcutaneously administrated with HDM extract was safe and significantly reduced asthma symptoms in mite allergic patients. Similarly, SCIT with QbG10 alone improved different asthma outcomes including asthma control during steroid reduction in patients on moderate or high-dose inhaled steroids.²⁰⁹ These promising results need to be further confirmed in larger clinical trials.

A TLR8 agonist could directly stimulate monocytes and DCs expressing this receptor by promoting their capacity to polarize Th1 immune responses. A study demonstrated that patients receiving weekly local administration of a TLR8 agonist for a total of 4 weeks followed by a grass pollen allergen challenge in an allergen exposure chamber experience significant improvements in nasal symptom scores compared to placebo receiving patients.^{200,210} The main advantage of this treatment is that it might work independently from the sensitizing allergen and that it is not needed to be co-administered with the allergen, thus avoiding undesired side effects. This represents another exciting approach that not needs to be investigated in larger clinical trials.

Mouse models of allergic asthma indicated that vitamin D significantly potentiates the beneficial effects of SCIT and SLIT through IL-10 and/or TGF- β inducing mechanisms.^{211–214} The use of vitamin D in combination with corticosteroids did not improve the clinical efficacy of SCIT in HDM asthmatic children.²¹⁵ Combined administration of corticosteroids and HDM extract suppressed the early clinical and immunologic effects of SCIT, which was prevented by the parallel administration of Vitamin D.²¹⁵ Vitamin D together with HDM SCIT increased the number of FOXP3⁺ Treg cells and corticosteroid-sparing effect in asthmatic children.²¹⁶

7.4 | Vector systems

Different vector systems have been tested as potential adjuvants administered via parenteral routes in mouse models of allergy, but human data are still scarce.^{19,217–219} For example, virosomes, polylactic-co-glycolic acid microparticles, immunostimulating complexes, or plasmid DNA absorbed to microparticles. Preclinical studies were also reported for several novel immunomodulators. TLR7 ligands coupled to allergens, probiotics, microbial products, heat-killed bacterial strains, or polybacterial preparations showed efficacy in mouse models of allergic airway inflammation.^{220–223}

Different types of fusion proteins that conjugate allergens to different carriers such as Fc γ fragments,²²⁴ bacterial S layer proteins,²²⁵ TLR5-ligand flagellin,²²⁶ or grass or cat pollen peptides fused to an immunogenic carrier from hepatitis B virus (BM32)²²⁷ have been developed and assessed in different preclinical models. In humans, until present, only few vectors administered via parenteral route have been studied. It was shown that coupling of non-replicating VLPs to peptides derived from different major allergens (Der p 1 or Fel d 1) might be good candidates for AIT.^{228,229} Recent studies demonstrated that a fusion protein linking the major cat allergen Fel d 1 to a modular antigen transporter (MAT) is a novel vaccine able to induce tolerance after three intra-lymph node injections.²³⁰ This construction was efficiently internalized by DCs, which induce activation of the inflammasome pathway leading to the release of IL-1 β . MAT-Fel d 1 vaccine promoted the generation of IL-10⁺ and IFN- γ T-cell responses with decreased Th2 cytokines.²³¹ Early Fel d 1-specific T-cell activation was followed by full T-cell unresponsiveness to allergen after 1 year in the MAT-Fel d 1 group, characterized by increased allergen-specific T regulatory cells, decreased circulating Fel d 1 tetramer-positive cells, increased IL-10 and FOXP3 expression, and change in the HR2/HR1 ratio toward HR2.²³¹ T-cell epitope peptide immunotherapy has also significantly improved over the last years and the underlying mechanisms have been better understood.^{196,232–234}

Very recently, it has been shown that the conjugation of mannan to grass pollen allergoids enhances immunogenicity and imprint DCs with the capacity to generate FOXP3⁺ Tregs via PD-L1.¹²³ Allergoid-mannan glycoconjugates were produced in a single step by treating with glutaraldehyde, a defined mixture of allergens derived from *Phleum pratense* grass pollen and native mannan (non-oxidized) from *Saccharomyces cerevisiae*. Analytical and structural studies, using nuclear magnetic resonance spectra, demonstrated the stability

and reproducibility of this conjugation method and showed that the obtained glycoconjugates were polymers of high molecular weight with a higher stability compared to native allergen or the conventional allergoid without mannan.²³⁵ Allergoids conjugated to mannan display in vivo hypoallergenicity, induce potent blocking antibodies, and are captured by human DCs much more efficiently than native allergens or polymers without mannan by mechanisms depending on mannose receptor- and DC-SIGN-mediated internalization. Allergoids conjugated with mannan endorse DCs with the capacity to generate functional FOXP3⁺ Tregs via PD-L1 and also promote Th1 responses with high levels of IL-10, thus representing suitable candidates for AIT. Supporting these data, in vivo immunization of mice with allergoids conjugated to mannan induces a shift to non-allergic responses and increases the frequency of splenic FOXP3⁺ Treg cells. Mild oxidation impairs these effects in human and mice, demonstrating the essential role of preserving the carbohydrate structure of mannan.¹²³

Different types of substances (immunopotentiators and vector systems) have been tested as potential mucosal adjuvants in mice models. For example, different types of toxins, TLR2- or TLR4-agonists, dexamethasone combined with vitD3, different probiotics strains, mucoadhesive polymers formed by maltodextrin nanoparticles or chitosan microparticles or fusion proteins composed of allergens coupled to distinct microbial-derived proteins. In humans, apart from MPL in SLIT trials, only some probiotic strains have been tested as adjuvants targeting the mucosal immune systems. The combination of specific probiotic strains administered through the oral route showed efficacy for atopic dermatitis.¹⁹

Alternative routes of administration such as the intralymphatic (ILIT) and epidermal (EIT) are suitable for reducing treatment duration while keeping efficacy and security in allergic rhinitis,^{195,236} clinical trials employing these alternative routes for AIT in asthma are being undertaken and provide encouraging results. Peanut EIT administration is currently being focused and associated with a modest treatment response with the highest responses among younger children. High adherence and significant changes in immune pathways support further investigation.²³⁷ In an adjuvant-free mouse model of food allergy, EIT induced sustained protection with the generation of a population of gastrointestinal-homing LAP⁺ Foxp3⁺ Treg cells. The mechanism of protection was found to be a novel pathway of direct TGF- β -dependent Treg-cell suppression of mast cell activation.²³⁸

8 | CONCLUSION

Our understanding on the complex immunologic mechanisms underlying allergic diseases has experienced a significant improvement over the last decades. Nowadays, it is very well known that various immune cells predominate in allergy within the type 2 immunity and different disease endotypes can be identified depending on the specific contribution of these cells and dominant cytokine profile. The generation and maintenance of allergen tolerance is a hallmark of healthy immune responses to allergens. In this context, human in vivo models of high-doses allergen exposure and AIT demonstrated that allergen-specific Treg and Breg cells are essential players in these processes. Human

tonsils have been demonstrated as key organs where allergen tolerance induction takes place, thus representing ideal candidates for novel therapeutic interventions. Up to date, AIT remains as the single disease-modifying treatment for allergic diseases with potential curative capacity. The advances experienced in biotechnology²³⁹ over the last decades have allowed the design and study of many different adjuvants for their potential use in AIT. Most of these adjuvants have been already validated in mouse models of allergy and some of them also in humans. Further clinical trials in humans with large number of patients will help to elucidate how these novel adjuvants can contribute to the improvement of the current treatments of allergy. Finally, the better understanding of the immunologic mechanisms underlying allergic diseases and allergen tolerance might well open avenues for the development of new preventive and therapeutic interventions for allergic diseases.

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CONFLICT OF INTERESTS

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REFERENCES

1. Fuchs O, Bahmer T, Rabe KF, von Mutius E. Asthma transition from childhood into adulthood. *Lancet Respir Med*. 2017;5:224-234.
2. Werfel T, Allam JP, Biedermann T, et al. Cellular and molecular immunologic mechanisms in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2016;138:336-349.
3. Chinthrajah RS, Hernandez JD, Boyd SD, Galli SJ, Nadeau KC. Molecular and cellular mechanisms of food allergy and food tolerance. *J Allergy Clin Immunol*. 2016;137:984-997.
4. Akdis CA, Akdis M. Advances in allergen immunotherapy: aiming for complete tolerance to allergens. *Sci Transl Med* 2015;7:280 ps6.
5. Palomares O, Martin-Fontecha M, Lauener R, et al. Regulatory T cells and immune regulation of allergic diseases: roles of IL-10 and TGF-beta. *Genes Immun*. 2014;15:511-520.
6. Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. *Nat Rev Drug Discov*. 2009;8:645-660.
7. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med*. 2012;18:693-704.
8. Akdis CA. Therapies for allergic inflammation: refining strategies to induce tolerance. *Nat Med*. 2012;18:736-749.

9. Bousquet J, Hellings PW, Agache I, et al. ARIA 2016: Care pathways implementing emerging technologies for predictive medicine in rhinitis and asthma across the life cycle. *Clin Transl Allergy*. 2016;6:47.
10. Tan HT, Sugita K, Akdis CA. Novel biologicals for the treatment of allergic diseases and asthma. *Curr Allergy Asthma Rep*. 2016;16:70.
11. Smits HH, Hiemstra PS, Prazeres da Costa C, et al. Microbes and asthma: opportunities for intervention. *J Allergy Clin Immunol*. 2016;137:690-697.
12. Palomares O, Ruckert B, Jartti T, et al. Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance. *J Allergy Clin Immunol*. 2012;129:510-520.
13. van de Veen W, Stanic B, Wirz OF, Jansen K, Globinska A, Akdis M. Role of regulatory B cells in immune tolerance to allergens and beyond. *J Allergy Clin Immunol*. 2016;138:654-665.
14. van de Veen W, Stanic B, Yaman G, et al. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. *J Allergy Clin Immunol*. 2013;131:1204-1212.
15. Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med*. 2008;205:2887-2898.
16. 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;357:752-756.
17. Kucuksezer UC, Palomares O, Ruckert B, et al. Triggering of specific Toll-like receptors and proinflammatory cytokines breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood. *J Allergy Clin Immunol*. 2013;131:875-885.
18. Jartti T, Palomares O, Waris M, et al. Distinct regulation of tonsillar immune response in virus infection. *Allergy*. 2014;69:658-667.
19. Moingeon P. Adjuvants for allergy vaccines. *Hum Vaccin Immunother*. 2012;8:1492-1498.
20. Moingeon P. Update on immune mechanisms associated with sublingual immunotherapy: practical implications for the clinician. *J Allergy Clin Immunol Pract*. 2013;1:228-241.
21. Galli S. Toward precision medicine and health: promises, opportunities and challenges in allergic diseases. *J Allergy Clin Immunol*. 2016;137:1289-1300.
22. Agache I, Akdis CA. Endotypes of allergic diseases and asthma: an important step in building blocks for the future of precision medicine. *Allergol Int*. 2016;65:243-252.
23. Akdis M, Aab A, Altunbulakli C, et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor beta, and TNF-alpha: receptors, functions, and roles in diseases. *J Allergy Clin Immunol*. 2016;138:984-1010.
24. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol*. 2006;6:761-771.
25. Xiao C, Puddicombe SM, Field S, et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol*. 2011;128:549-556.
26. Wawrzyniak P, Akdis CA, Finkelman FD, Rothenberg ME. Advances and highlights in mechanisms of allergic disease in 2015. *J Allergy Clin Immunol*. 2016;137:1681-1696.
27. Wawrzyniak P, Wawrzyniak M, Wanke K, et al. Regulation of bronchial epithelial barrier integrity by type 2 cytokines and histone deacetylases in asthmatic patients. *J Allergy Clin Immunol*. 2017;139:93-103.
28. Hammad H, Lambrecht BN. Barrier epithelial cells and the control of type 2 immunity. *Immunity*. 2015;43:29-40.
29. Morita H, Moro K, Koyasu S. Innate lymphoid cells in allergic and non-allergic inflammation. *J Allergy Clin Immunol*. 2016;138:1253-1264.
30. Chen CC, Kobayashi T, Iijima K, Hsu FC, Kita H. IL-33 dysregulates regulatory T (Treg) cells and impairs established immunological tolerance in the lungs. *J Allergy Clin Immunol*. 2017; <https://doi.org/10.1016/j.jaci.2017.01.015>.

31. Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. Role of Treg in immune regulation of allergic diseases. *Eur J Immunol*. 2010;40:1232-1240.
32. Shinoda K, Hirahara K, Iinuma T, et al. Thy1+IL-7+ lymphatic endothelial cells in iBALT provide a survival niche for memory T-helper cells in allergic airway inflammation. *Proc Natl Acad Sci USA*. 2016;113:E2842-E2851.
33. Doherty TA, Baum R, Newbury RO, et al. Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis. *J Allergy Clin Immunol*. 2015;136:792-794.
34. Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. *J Allergy Clin Immunol*. 2015;135:626-635.
35. Morita H, Arae K, Unno H, et al. An interleukin-33-mast cell-interleukin-2 axis suppresses papain-induced allergic inflammation by promoting regulatory T cell numbers. *Immunity*. 2015;43:175-186.
36. Mjösberg J, Spits H. Human innate lymphoid cells. *J Allergy Clin Immunol*. 2016;138:1265-1276.
37. Nagakumar P, Denney L, Fleming L, Bush A, Lloyd CM, Saglani S. Type 2 innate lymphoid cells in induced sputum from children with severe asthma. *J Allergy Clin Immunol*. 2016;137:624-626.
38. Maggi E, Montaini G, Mazzoni A, et al. Human circulating group 2 innate lymphoid cells can express CD154 and promote IgE production. *J Allergy Clin Immunol*. 2017;139:964-976.
39. Matsuki A, Takatori H, Makita S, et al. T-bet inhibits innate lymphoid cell-mediated eosinophilic airway inflammation by suppressing IL-9 production. *J Allergy Clin Immunol*. 2017;139:1355-1367.
40. Basinski TM, Holzmann D, Eiwegger T, et al. Dual nature of T cell-epithelium interaction in chronic rhinosinusitis. *J Allergy Clin Immunol*. 2009;124:74-80.
41. Rebane A, Zimmermann M, Aab A, et al. Mechanisms of IFN-gamma-induced apoptosis of human skin keratinocytes in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2012;129:1297-1306.
42. Finotto S, Neurath MF, Glickman JN, et al. Development of spontaneous airway changes consistent with human asthma in mice lacking T-bet. *Science*. 2002;295:336-338.
43. Akdis M, Palomares O, van de Veen W, van Splunter M, Akdis CA. TH17 and TH22 cells: a confusion of antimicrobial response with tissue inflammation versus protection. *J Allergy Clin Immunol*. 2012;129:1438-1449.
44. Veldhoen M, Uytendhove C, van Snick 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;9:1341-1346.
45. Eyerich S, Eyerich K, Pennino D, et al. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest*. 2009;119:3573-3585.
46. Czarnecki T, Gonzalez J, Shemer A, et al. Severe atopic dermatitis is characterized by selective expansion of circulating TH2/TC2 and TH22/TC22, but not TH17/TC17, cells within the skin-homing T-cell population. *J Allergy Clin Immunol*. 2015;136:104-115.
47. Wegrzyn AS, Jakiela B, Ruckert B, et al. T-cell regulation during viral and nonviral asthma exacerbations. *J Allergy Clin Immunol*. 2015;136:194-197.
48. Noval Rivas M, Chatila TA. Regulatory T cells in allergic diseases. *J Allergy Clin Immunol*. 2016;138:639-652.
49. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2011;127:18-27.
50. Li MO, Rudensky AY. T cell receptor signalling in the control of regulatory T cell differentiation and function. *Nat Rev Immunol*. 2016;16:220-233.
51. Plitas G, Rudensky AY. Regulatory T cells: differentiation and function. *Cancer Immunol Res*. 2016;4:721-725.
52. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol*. 1995;155:1151-1164.
53. Chen W, Jin W, Hardegen 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;198:1875-1886.
54. Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev*. 2006;212:28-50.
55. Faria AM, Weiner HL. Oral tolerance. *Immunol Rev*. 2005;206:232-259.
56. Siegmund K, Ruckert B, Ouaked N, et al. Unique phenotype of human tonsillar and in vitro-induced FOXP3+CD8+ T cells. *J Immunol*. 2009;182:2124-2130.
57. Strober S, Cheng L, Zeng D, et al. Double negative (CD4-CD8- alpha beta+) T cells which promote tolerance induction and regulate autoimmunity. *Immunol Rev*. 1996;149:217-230.
58. 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;163:242-249.
59. Hinks TS, Zhou X, Staples KJ, et al. Innate and adaptive T cells in asthmatic patients: relationship to severity and disease mechanisms. *J Allergy Clin Immunol*. 2015;136:323-333.
60. Kashyap M, Thornton AM, Norton SK, et al. Cutting edge: CD4 T cell-mast cell interactions alter IgE receptor expression and signaling. *J Immunol*. 2008;180:2039-2043.
61. Gri G, Piconese S, Frossi B, et al. CD4+CD25+ regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. *Immunity*. 2008;29:771-781.
62. Nonaka M, Pawankar R, Fukumoto A, Yagi T. Heterogeneous response of nasal and lung fibroblasts to transforming growth factor-beta 1. *Clin Exp Allergy*. 2008;38:812-821.
63. Wing K, Onishi Y, Prieto-Martin P, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008;322:271-275.
64. 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;63:1455-1463.
65. Ring S, Schafer SC, Mahnke K, Lehr HA, Enk AH. CD4+ CD25+ regulatory T cells suppress contact hypersensitivity reactions by blocking influx of effector T cells into inflamed tissue. *Eur J Immunol*. 2006;36:2981-2992.
66. Yang YC, Zhang N, Van Crombruggen K, Hu GH, Hong SL, Bachert C. Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. *Allergy*. 2012;67:1193-1202.
67. Aab A, Wirz O, van de Veen W, et al. Human rhinoviruses enter and induce proliferation of B lymphocytes. *Allergy*. 2017;72:232-243.
68. Mehta AK, Duan W, Doerner AM, et al. Rhinovirus infection interferes with induction of tolerance to aeroantigens through OX40 ligand, thymic stromal lymphopoietin, and IL-33. *J Allergy Clin Immunol*. 2016;137:278-288.
69. Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. *Nat Rev Immunol*. 2003;3:253-257.
70. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol*. 2004;22:531-562.
71. Josefowicz SZ, Niec RE, Kim HY, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature*. 2012;482:395-399.
72. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell*. 2008;133:775-787.
73. Abbas AK, Benoist C, Bluestone JA, et al. Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol*. 2013;14:307-308.

74. Kitagawa Y, Wing JB, Sakaguchi S. Transcriptional and epigenetic control of regulatory T cell development. *Prog Mol Biol Transl Sci*. 2015;136:1-33.
75. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science*. 2003;299:1057-1061.
76. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4⁺CD25⁺ regulatory T cells. *Nat Immunol*. 2003;4:330-336.
77. Sakaguchi S, Vignali DA, Rudensky AY, Niec RE, Waldmann H. The plasticity and stability of regulatory T cells. *Nat Rev Immunol*. 2013;13:461-467.
78. Lin W, Truong N, Grossman WJ, et al. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J Allergy Clin Immunol*. 2005;116:1106-1115.
79. Lahl K, Sparwasser T. In vivo depletion of FoxP3⁺ Tregs using the DEREK mouse model. *Methods Mol Biol*. 2011;707:157-172.
80. Baru AM, Untucht C, Ganesh V, Hesse C, Mayer CT, Sparwasser T. Optimal isolation of functional Foxp3⁺ induced regulatory T cells using DEREK mice. *PLoS ONE*. 2012;7:e44760.
81. Klingenberg R, Gerdes N, Badeau RM, et al. Depletion of FOXP3⁺ regulatory T cells promotes hypercholesterolemia and atherosclerosis. *J Clin Invest*. 2013;123:1323-1334.
82. McMurchy AN, Gillies J, Allan SE, et al. Point mutants of forkhead box P3 that cause immune dysregulation, polyendocrinopathy, enteropathy, X-linked have diverse abilities to reprogram T cells into regulatory T cells. *J Allergy Clin Immunol*. 2010;126:1242-1251.
83. Chatila TA, Blaese F, Ho N, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest*. 2000;106:R75-R81.
84. Schmetterer KG, Haiderer D, Leb-Reichl VM, et al. Bet v 1-specific T-cell receptor/forkhead box protein 3 transgenic T cells suppress Bet v 1-specific T-cell effector function in an activation-dependent manner. *J Allergy Clin Immunol*. 2011;127:238-245.
85. Klunker S, Chong MM, Mantel PY, et al. Transcription factors RUNX1 and RUNX3 in the induction and suppressive function of Foxp3⁺ inducible regulatory T cells. *J Exp Med*. 2009;206:2701-2715.
86. Mantel PY, Kuipers H, Boyman O, et al. GATA3-driven Th2 responses inhibit TGF- β 1-induced FOXP3 expression and the formation of regulatory T cells. *PLoS Biol*. 2007;5:e329.
87. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. *Immunity*. 2013;38:414-423.
88. Begin P, Schulze J, Baron U, et al. Human in vitro induced T regulatory cells and memory T cells share common demethylation of specific FOXP3 promoter region. *Clin Transl Allergy*. 2015;5:35.
89. Rudra D, deRoos P, Chaudhry A, et al. Transcription factor Foxp3 and its protein partners form a complex regulatory network. *Nat Immunol*. 2012;13:1010-1019.
90. Ouyang W, Liao W, Luo CT, et al. Novel Foxo1-dependent transcriptional programs control T(reg) cell function. *Nature*. 2012;491:554-559.
91. Gao Y, Lin F, Su J, et al. Molecular mechanisms underlying the regulation and functional plasticity of FOXP3⁺ regulatory T cells. *Genes Immun*. 2012;13:1-13.
92. Seddiki N, Santner-Nanan B, Martinson J, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med*. 2006;203:1693-1700.
93. Boden E, Tang Q, Bour-Jordan H, Bluestone JA. The role of CD28 and CTLA4 in the function and homeostasis of CD4⁺CD25⁺ regulatory T cells. *Novartis Found Symp*. 2003;252:55-63; discussion 63-66, 106-114.
94. Carter L, Fouser LA, Jussif J, et al. PD-1:PD-L inhibitory pathway affects both CD4⁺ and CD8⁺ T cells and is overcome by IL-2. *Eur J Immunol*. 2002;32:634-643.
95. Perez-Gracia JL, Labiano S, Rodriguez-Ruiz ME, Sanmamed MF, Melero I. Orchestrating immune check-point blockade for cancer immunotherapy in combinations. *Curr Opin Immunol*. 2014;27:89-97.
96. Akdis M, Verhagen J, Taylor A, 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;199:1567-1575.
97. Pfoertner S, Jeron A, Probst-Kepper M, et al. Signatures of human regulatory T cells: an encounter with old friends and new players. *Genome Biol*. 2006;7:R54.
98. Sugimoto N, Oida T, Hirota K, et al. Foxp3-dependent and -independent molecules specific for CD25⁺CD4⁺ natural regulatory T cells revealed by DNA microarray analysis. *Int Immunol*. 2006;18:1197-1209.
99. Hansen W, Westendorf AM, Toepfer T, et al. Inflammation in vivo is modulated by GPR83 isoform-4 but not GPR83 isoform-1 expression in regulatory T cells. *Genes Immun*. 2010;11:357-361.
100. Gagliani N, Magnani CF, Huber S, et al. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. *Nat Med*. 2013;19:739-746.
101. Guichelaar T, ten Brink CB, van Kooten PJ, et al. Autoantigen-specific IL-10-transduced T cells suppress chronic arthritis by promoting the endogenous regulatory IL-10 response. *J Immunol*. 2008;180:1373-1381.
102. Gregori S, Magnani CF, Roncarolo MG. Role of human leukocyte antigen-G in the induction of adaptive type 1 regulatory T cells. *Hum Immunol*. 2009;70:966-969.
103. Bacchetta R, Sartirana C, Levings MK, Bordignon C, Narula S, Roncarolo MG. Growth and expansion of human T regulatory type 1 cells are independent from TCR activation but require exogenous cytokines. *Eur J Immunol*. 2002;32:2237-2245.
104. Allan SE, Broady R, Gregori S, et al. CD4⁺ T-regulatory cells: toward therapy for human diseases. *Immunol Rev*. 2008;223:391-421.
105. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. *Nat Med*. 2004;10:801-805.
106. Barrat FJ, Cua DJ, Boonstra A, et al. In vitro generation of interleukin 10-producing regulatory CD4⁺ T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J Exp Med*. 2002;195:603-616.
107. Xystrakis E, Kusumakar S, Boswell S, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest*. 2006;116:146-155.
108. Andolfi G, Foustieri G, Rossetti M, et al. Enforced IL-10 expression confers type 1 regulatory T cell (Tr1) phenotype and function to human CD4⁺ T cells. *Mol Ther*. 2012;20:1778-1790.
109. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest*. 1998;102:98-106.
110. Groux H, O'Garra A, Bigler M, et al. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature*. 1997;389:737-742.
111. Pacciani V, Gregori S, Chini L, et al. Induction of anergic allergen-specific suppressor T cells using tolerogenic dendritic cells derived from children with allergies to house dust mites. *J Allergy Clin Immunol*. 2010;125:727-736.
112. Francis JN, Till SJ, Durham SR. Induction of IL-10⁺CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol*. 2003;111:1255-1261.
113. Steinman RM. Decisions about dendritic cells: past, present, and future. *Annu Rev Immunol*. 2012;30:1-22.
114. Durand M, Segura E. The known unknowns of the human dendritic cell network. *Front Immunol*. 2015;6:129.
115. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol*. 2010;11:373-384.
116. Iborra S, Sancho D. Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors. *Immunobiology*. 2015;220:175-184.
117. Jones JD, Vance RE, Dangl JL. Intracellular innate immune surveillance devices in plants and animals. *Science*. 2016;354:aaf6395.

118. Sancho D, Reis e Sousa C. Sensing of cell death by myeloid C-type lectin receptors. *Curr Opin Immunol.* 2013;25:46-52.
119. Cohn L, Delamarre L. Dendritic cell-targeted vaccines. *Front Immunol.* 2014;5:255.
120. Dua B, Smith S, Kinoshita T, Imaoka H, Gauvreau G, O'Byrne P. Myeloid dendritic cells type 2 in allergic asthma. *Allergy.* 2013;68:1322-1326.
121. Segura E, Amigorena S. Cross-presentation in mouse and human dendritic cells. *Adv Immunol.* 2015;127:1-31.
122. Palomares O, O'Mahony L, Akdis CA. The many routes of dendritic cells to ensure immune regulation. *J Allergy Clin Immunol.* 2011;127:1541-1542.
123. Sirvent S, Soria I, Cirauqui C, et al. Novel vaccines targeting dendritic cells by coupling allergoids to nonoxidized mannan enhance allergen uptake and induce functional regulatory T cells through programmed death ligand 1. *J Allergy Clin Immunol.* 2016;138:558-567.
124. Jutel M, Watanabe T, Klunker S, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature.* 2001;413:420-425.
125. O'Mahony L, Akdis M, Akdis CA. Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol.* 2011;128:1153-1162.
126. Novak N, Mete N, Bussmann C, et al. Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. *J Allergy Clin Immunol.* 2012;130:1153-1158 e1152.
127. Smolinska S, Groeger D, Perez NR, et al. Histamine receptor 2 is required to suppress innate immune responses to bacterial ligands in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* 2016;22:1575-1586.
128. Ferstl R, Frei R, Schiavi E, et al. Histamine receptor 2 is a key influence in immune responses to intestinal histamine-secreting microbes. *J Allergy Clin Immunol.* 2014;134:744-746.
129. Barcik W, Pugin B, Westermann P, et al. Histamine-secreting microbes are increased in the gut of adult asthma patients. *J Allergy Clin Immunol.* 2016;138:1491-1494.
130. Katz SI, Parker D, Turk JL. B-cell suppression of delayed hypersensitivity reactions. *Nature.* 1974;251:550-551.
131. Neta R, Salvin SB. Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. *J Immunol.* 1974;113:1716-1725.
132. Wolf SD, Dittel BN, Hardardottir F, Janeway CA Jr. Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. *J Exp Med.* 1996;184:2271-2278.
133. Goetz M, Atreya R, Ghalibafian M, Galle PR, Neurath MF. Exacerbation of ulcerative colitis after rituximab salvage therapy. *Inflamm Bowel Dis.* 2007;13:1365-1368.
134. Dass S, Vital EM, Emery P. Development of psoriasis after B cell depletion with rituximab. *Arthritis Rheum.* 2007;56:2715-2718.
135. Fillatreau S, Sweeney CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol.* 2002;3:944-950.
136. Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity.* 2002;16:219-230.
137. Mauri C, Gray D, Mushtaq N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med.* 2003;197:489-501.
138. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature.* 2014;507:366-370.
139. Parekh VV, Prasad DV, Banerjee PP, Joshi BN, Kumar A, Mishra GC. B cells activated by lipopolysaccharide, but not by anti-Ig and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-beta 1. *J Immunol.* 2003;170:5897-5911.
140. Tian J, Zekzer D, Hanssen L, Lu Y, Olcott A, Kaufman DL. Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J Immunol.* 2001;167:1081-1089.
141. O'Garra A, Chang R, Go N, Hastings R, Haughton G, Howard M. Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. *Eur J Immunol.* 1992;22:711-717.
142. Zhang X, Deriaud E, Jiao X, Braun D, Leclerc C, Lo-Man R. Type I interferons protect neonates from acute inflammation through interleukin 10-producing B cells. *J Exp Med.* 2007;204:1107-1118.
143. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity.* 2008;28:639-650.
144. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nat Rev Immunol.* 2013;13:118-132.
145. Evans JG, Chavez-Rueda KA, Eddaoudi A, et al. Novel suppressive function of transitional 2 B cells in experimental arthritis. *J Immunol.* 2007;178:7868-7878.
146. Gray M, Miles K, Salter D, Gray D, Savill J. Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. *Proc Natl Acad Sci USA.* 2007;104:14080-14085.
147. Blair PA, Norena LY, Flores-Borja F, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity.* 2010;32:129-140.
148. Shalapour S, Font-Burgada J, Di Caro G, et al. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature.* 2015;521:94-98.
149. Matsumoto M, Baba A, Yokota T, et al. Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. *Immunity.* 2014;41:1040-1051.
150. Neves P, Lampropoulou V, Calderon-Gomez E, et al. Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during *Salmonella typhimurium* infection. *Immunity.* 2010;33:777-790.
151. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. *Immunity.* 2015;42:607-612.
152. Palomares O, Martin-Fontecha M, Lauener R, et al. Regulatory T cells and immune regulation of allergic diseases: roles of IL-10 and TGF-beta. *Genes Immun.* 2014;15:511-520.
153. Yoshizaki A, Miyagaki T, DiLillo DJ, et al. Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. *Nature.* 2012;491:264-268.
154. Ding Q, Yeung M, Camirand G, et al. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J Clin Invest.* 2011;121:3645-3656.
155. Lee KM, Kim JI, Stott R, et al. Anti-CD45RB/anti-TIM-1-induced tolerance requires regulatory B cells. *Am J Transplant.* 2012;12:2072-2078.
156. Iwata Y, Matsushita T, Horikawa M, et al. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood.* 2011;117:530-541.
157. Khan AR, Hams E, Floudas A, Sparwasser T, Weaver CT, Fallon PG. PD-L1hi B cells are critical regulators of humoral immunity. *Nat Commun.* 2015;6:5997.
158. Lampropoulou V, Hoehlig K, Roch T, et al. TLR-activated B cells suppress T cell-mediated autoimmunity. *J Immunol.* 2008;180:4763-4773.
159. Brisslert M, Bokarewa M, Larsson P, Wing K, Collins LV, Tarkowski A. Phenotypic and functional characterization of human CD25+ B cells. *Immunology.* 2006;117:548-557.
160. Sattler S, Ling GS, Xu D, et al. IL-10-producing regulatory B cells induced by IL-33 (Breg(IL-33)) effectively attenuate mucosal inflammatory responses in the gut. *J Autoimmun.* 2014;50:107-122.

161. Wysocki CA, Jiang Q, Panoskaltsis-Mortari A, et al. Critical role for CCR5 in the function of donor CD4+CD25+ regulatory T cells during acute graft-versus-host disease. *Blood*. 2005;106:3300-3307.
162. Boonpiyathad T, Meyer N, Moniuszko M, et al. High-dose bee venom exposure induces similar tolerogenic B-cell responses in allergic patients and healthy beekeepers. *Allergy*. 2017;72:407-415.
163. Stanic B, van de Veen W, Wirz OF, et al. IL-10-overexpressing B cells regulate innate and adaptive immune responses. *J Allergy Clin Immunol*. 2015;135:771-780.
164. Braza F, Chesne J, Durand M, et al. A regulatory CD9(+) B-cell subset inhibits HDM-induced allergic airway inflammation. *Allergy*. 2015;70:1421-1431.
165. Singh A, Carson WFT, Secor ER Jr. et al. Regulatory role of B cells in a murine model of allergic airway disease. *J Immunol*. 2008;180:7318-7326.
166. Natarajan P, Singh A, McNamara JT, et al. Regulatory B cells from hilar lymph nodes of tolerant mice in a murine model of allergic airway disease are CD5+, express TGF-beta, and co-localize with CD4+Foxp3+ T cells. *Mucosal Immunol*. 2012;5:691-701.
167. Liu ZQ, Wu Y, Song JP, et al. Tolerogenic CX3CR1+ B cells suppress food allergy-induced intestinal inflammation in mice. *Allergy*. 2013;68:1241-1248.
168. Zhang HP, Wu Y, Liu J, et al. TSP1-producing B cells show immune regulatory property and suppress allergy-related mucosal inflammation. *Sci Rep*. 2013;3:3345.
169. Berin MC, Mayer L. Can we produce true tolerance in patients with food allergy? *J Allergy Clin Immunol*. 2013;131:14-22.
170. Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature*. 1995;376:177-180.
171. Hadis U, Wahl B, Schulz O, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity*. 2011;34:237-246.
172. Gilbert RS, Kobayashi R, Sekine S, Fujihashi K. Functional transforming growth factor-beta receptor type II expression by CD4+ T cells in Peyer's patches is essential for oral tolerance induction. *PLoS ONE*. 2011;6:e27501.
173. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol*. 2001;2:725-731.
174. Mascarell L, Saint-Lu N, Moussu H, et al. Oral macrophage-like cells play a key role in tolerance induction following sublingual immunotherapy of asthmatic mice. *Mucosal Immunol*. 2011;4:638-647.
175. McClory S, Hughes T, Freud AG, et al. Evidence for a stepwise program of extrathymic T cell development within the human tonsil. *J Clin Invest*. 2012;122:1403-1415.
176. Leavy O. T cell development: tonsils turn out T cells too. *Nat Rev Immunol*. 2012;12:232.
177. Jackson DJ, Gern JE, Lemanske RF Jr. The contributions of allergic sensitization and respiratory pathogens to asthma inception. *J Allergy Clin Immunol*. 2016;137:659-665.
178. Teach SJ, Gill MA, Togias A, et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. *J Allergy Clin Immunol*. 2015;136:1476-1485.
179. Busse WW, Morgan WJ, Gergen PJ, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med*. 2011;364:1005-1015.
180. Martin-Couce L, Martin-Fontecha M, Palomares O, et al. Chemical probes for the recognition of cannabinoid receptors in native systems. *Angew Chem Int Ed Engl*. 2012;51:6896-6899.
181. Martin-Fontecha M, Eiwegger T, Jartti T, et al. The expression of cannabinoid receptor 1 is significantly increased in atopic patients. *J Allergy Clin Immunol*. 2014;133:926-929.
182. Elenius V, Palomares O, Waris M, et al. The relationship of serum vitamins A, D, E and LL-37 levels with allergic status, tonsillar virus detection and immune response. *PLoS ONE*. 2017;12:e0172350.
183. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*. 2014;133:621-631.
184. Jutel M, Agache I, Bonini S, et al. International consensus on allergen immunotherapy II: Mechanisms, standardization, and pharmacoeconomics. *J Allergy Clin Immunol*. 2016;137:358-368.
185. Jutel M, Agache I, Bonini S, et al. International consensus on allergy immunotherapy. *J Allergy Clin Immunol*. 2015;136:556-568.
186. Durham SR, Penagos M. Sublingual or subcutaneous immunotherapy for allergic rhinitis? *J Allergy Clin Immunol*. 2016;137:339-349.
187. Gepp B, Lengger N, Mobs C, et al. Monitoring the epitope recognition profiles of IgE, IgG1, and IgG4 during birch pollen immunotherapy. *J Allergy Clin Immunol*. 2016;137:1600-1603.
188. Schmid JM, Wurtzen PA, Dahl R, Hoffmann HJ. Pretreatment IgE sensitization patterns determine the molecular profile of the IgG4 response during up dosing of subcutaneous immunotherapy with timothy grass pollen extract. *J Allergy Clin Immunol*. 2016;137:562-570.
189. van der Neut Kolfschoten M, Schuurman J, Losen M, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. *Science*. 2007;317:1554-1557.
190. Santos AF, James LK, Bahnson HT, et al. IgG4 inhibits peanut-induced basophil and mast cell activation in peanut-tolerant children sensitized to peanut major allergens. *J Allergy Clin Immunol*. 2015;135:1249-1256.
191. Holt PG, Strickland D, Bosco A, et al. Distinguishing benign from pathologic TH2 immunity in atopic children. *J Allergy Clin Immunol*. 2016;137:379-387.
192. Caimmi D, Calderon MA, Bousquet J, Demoly P. Allergen immunotherapy outcomes and unmet needs: a critical review. *Immunol Allergy Clin North Am*. 2016;36:181-189.
193. Dhami S, Zaman H, Varga EM, et al. Allergen immunotherapy for insect venom allergy: a systematic review and meta-analysis. *Allergy*. 2017;72:342-365.
194. Jutel M. Allergen-specific immunotherapy in asthma. *Curr Treat Options Allergy*. 2014;1:213-219.
195. Senti G, Kundig TM. Novel delivery routes for allergy immunotherapy: intralymphatic, epicutaneous, and intradermal. *Immunol Allergy Clin North Am*. 2016;36:25-37.
196. Valenta R, Campana R, Focke-Tejkl M, Niederberger V. Vaccine development for allergen-specific immunotherapy based on recombinant allergens and synthetic allergen peptides: lessons from the past and novel mechanisms of action for the future. *J Allergy Clin Immunol*. 2016;137:351-357.
197. Kool M, Fierens K, Lambrecht BN. Alum adjuvant: some of the tricks of the oldest adjuvant. *J Med Microbiol*. 2012;61:927-934.
198. Kuroda E, Ishii KJ, Uematsu S, et al. Silica crystals and aluminum salts regulate the production of prostaglandin in macrophages via NALP3 inflammasome-independent mechanisms. *Immunity*. 2011;34:514-526.
199. Kuroda E, Ozasa K, Temizoz B, et al. Inhaled fine particles induce alveolar macrophage death and interleukin-1alpha release to promote inducible bronchus-associated lymphoid tissue formation. *Immunity*. 2016;45:1299-1310.
200. Casale TB, Stokes JR. Immunotherapy: what lies beyond. *J Allergy Clin Immunol*. 2014;133:612-619.
201. Patel P, Holdich T, von Fischer Weikersthal-Drachenberg KJ, Huber B. Efficacy of a short course of specific immunotherapy in patients with allergic rhinoconjunctivitis to ragweed pollen. *J Allergy Clin Immunol*. 2014;133:121-129.
202. Rosewich M, Schulze J, Eickmeier O, et al. Tolerance induction after specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A in children. *Clin Exp Immunol*. 2010;160:403-410.
203. Santeliz JV, Van Nest G, Traquina P, Larsen E, Wills-Karp M. A 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol*. 2002;109:455-462.

204. Campbell JD, Kell SA, Kozy HM, et al. A limited CpG-containing oligodeoxynucleotide therapy regimen induces sustained suppression of allergic airway inflammation in mice. *Thorax*. 2014;69:565-573.
205. Ballester M, Jeanbart L, de Titta A, et al. Nanoparticle conjugation enhances the immunomodulatory effects of intranasally delivered CpG in house dust mite-allergic mice. *Sci Rep*. 2015;5:14274.
206. Kim DH, Sohn JH, Park HJ, Lee JH, Park JW, Choi JM. CpG Oligodeoxynucleotide inhibits cockroach-induced asthma via induction of IFN- γ (+) Th1 cells or Foxp3(+) regulatory T cells in the lung. *Allergy Asthma Immunol Res*. 2016;8:264-275.
207. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med*. 2006;355:1445-1455.
208. Senti G, Johansen P, Haug S, et al. Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial. *Clin Exp Allergy*. 2009;39:562-570.
209. Beeh KM, Kannies F, Wagner F, et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. *J Allergy Clin Immunol*. 2013;131:866-874.
210. Horak F. VTX-1463, a novel TLR8 agonist for the treatment of allergic rhinitis. *Expert Opin Investig Drugs*. 2011;20:981-986.
211. Taher YA, van Esch BC, Hofman GA, Henricks PA, van Oosterhout AJ. 1 α ,25-dihydroxyvitamin D₃ potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF- β . *J Immunol*. 2008;180:5211-5221.
212. Petrarca C, Clemente E, Amato V, et al. Vitamin D3 improves the effects of low dose Der p 2 allergoid treatment in Der p 2 sensitized BALB/c mice. *Clin Mol Allergy*. 2016;14:7.
213. Lombardi V, Van Overtvelt L, Horiot S, et al. Toll-like receptor 2 agonist Pam3CSK4 enhances the induction of antigen-specific tolerance via the sublingual route. *Clin Exp Allergy*. 2008;38:1819-1829.
214. Grundstrom J, Neimert-Andersson T, Kemi C, et al. Covalent coupling of vitamin D3 to the major cat allergen Fel d 1 improves the effects of allergen-specific immunotherapy in a mouse model for cat allergy. *Int Arch Allergy Immunol*. 2012;157:136-146.
215. Majak P, Rychlik B, Stelmach I. The effect of oral steroids with and without vitamin D3 on early efficacy of immunotherapy in asthmatic children. *Clin Exp Allergy*. 2009;39:1830-1841.
216. Baris S, Kiykim A, Ozen A, Tulunay A, Karakoc-Aydiner E, Barlan IB. Vitamin D as an adjunct to subcutaneous allergen immunotherapy in asthmatic children sensitized to house dust mite. *Allergy*. 2014;69:246-253.
217. Scholl I, Weissenbock A, Forster-Waldl E, et al. Allergen-loaded biodegradable poly(D, L-lactic-co-glycolic) acid nanoparticles down-regulate an ongoing Th2 response in the BALB/c mouse model. *Clin Exp Allergy*. 2004;34:315-321.
218. Nouri HR, Varasteh A, Jaafari MR, Davies JM, Sankian M. Induction of a Th1 immune response and suppression of IgE via immunotherapy with a recombinant hybrid molecule encapsulated in liposome-protamine-DNA nanoparticles in a model of experimental allergy. *Immunol Res*. 2015;62:280-291.
219. Saint-Lu N, Tourdot S, Razafindratsita A, et al. Targeting the allergen to oral dendritic cells with mucoadhesive chitosan particles enhances tolerance induction. *Allergy*. 2009;64:1003-1013.
220. Pratesi S, Nencini F, Fili L, et al. Dermatophagoides pteronyssinus group 2 allergen bound to 8-OH modified adenine reduces the Th2-mediated airway inflammation without inducing a Th17 response and autoimmunity. *Mol Immunol*. 2016;77:60-70.
221. Adakal H, Stachurski F, Konkobo M, et al. Efficiency of inactivated vaccines against heartwater in Burkina Faso: impact of *Ehrlichia ruminantium* genetic diversity. *Vaccine*. 2010;28:4573-4580.
222. Navarro S, Cossalter G, Chiavaroli C, et al. The oral administration of bacterial extracts prevents asthma via the recruitment of regulatory T cells to the airways. *Mucosal Immunol*. 2011;4:53-65.
223. Tejera-Alhambra M, Palomares O, de Diego RP, Diaz-Lezcano I, Sanchez-Ramon S. New biological insights in the immunomodulatory effects of mucosal polybacterial vaccines in clinical practice. *Curr Pharm Des*. 2016;22:6283-6293.
224. Terada T, Zhang K, Belperio J, Londhe V, Saxon A. A chimeric human-cat Fc γ 1b-Fc γ 1d1 fusion protein inhibits systemic, pulmonary, and cutaneous allergic reactivity to intratracheal challenge in mice sensitized to Fel d1, the major cat allergen. *Clin Immunol*. 2006;120:45-56.
225. Gerstmayr M, Ilk N, Jahn-Schmid B, Sleytr UB, Bohle B. Natural self-assembly of allergen-S-layer fusion proteins is no prerequisite for reduced allergenicity and T cell stimulatory capacity. *Int Arch Allergy Immunol*. 2009;149:231-238.
226. Schulke S, Burggraf M, Waibler Z, et al. A fusion protein of flagellin and ovalbumin suppresses the TH2 response and prevents murine intestinal allergy. *J Allergy Clin Immunol*. 2011;128:1340-1348.
227. Niederberger V, Marth K, Eckl-Dorna J, et al. Skin test evaluation of a novel peptide carrier-based vaccine, BM32, in grass pollen-allergic patients. *J Allergy Clin Immunol*. 2015;136:1101-1103.
228. Kundig TM, Senti G, Schnetzler G, et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. *J Allergy Clin Immunol*. 2006;117:1470-1476.
229. Schmitz N, Dietmeier K, Bauer M, et al. Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. *J Exp Med*. 2009;206:1941-1955.
230. Senti G, Cramer R, Kuster D, et al. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. *J Allergy Clin Immunol*. 2012;129:1290-1296.
231. Zaleska A, Eiwegger T, Soyer O, et al. Immune regulation by intralymphatic immunotherapy with modular allergen translocation MAT vaccine. *Allergy*. 2014;69:1162-1170.
232. Larche M. Mechanisms of peptide immunotherapy in allergic airways disease. *Ann Am Thorac Soc*. 2014;11(Suppl 5):S292-S296.
233. Ellis AK, Frankish CW, O'Hehir RE, et al. Treatment with grass allergen peptides improves symptoms of grass pollen-induced allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2017; <https://doi.org/10.1016/j.jaci.2016.11.043>.
234. Spertini F, DellaCorte G, Kettner A, et al. Efficacy of 2 months of allergen-specific immunotherapy with Bet v 1-derived contiguous overlapping peptides in patients with allergic rhinoconjunctivitis: Results of a phase IIb study. *J Allergy Clin Immunol*. 2016;138:162-168.
235. Manzano AI, Javier Canada F, Cases B, et al. Structural studies of novel glycoconjugates from polymerized allergens (allergoids) and mannans as allergy vaccines. *Glycoconj J*. 2016;33:93-101.
236. Kundig TM, Johansen P, Bachmann MF, Cardell LO, Senti G. Intralymphatic immunotherapy: time interval between injections is essential. *J Allergy Clin Immunol*. 2014;133:930-931.
237. Jones SM, Sicherer SH, Burks AW, et al. Epicutaneous immunotherapy for the treatment of peanut allergy in children and young adults. *J Allergy Clin Immunol*. 2017;139:1242-1252.
238. Tordesillas L, Mondoulet L, Blazquez AB, Benhamou PH, Sampson HA, Berin MC. Epicutaneous immunotherapy induces gastrointestinal LAP+ regulatory T cells and prevents food-induced anaphylaxis. *J Allergy Clin Immunol*. 2017;139:189-201.
239. Palomares O, Cramer R, Rhyner C. The contribution of biotechnology toward progress in diagnosis, management, and treatment of allergic diseases. *Allergy*. 2014;69:1588-1601.

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