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REVIEW

Regulatory T cells and immune regulation of allergic diseases: roles of IL-10 and TGF-β

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The prevalence of allergic diseases has significantly increased in industrialized countries. Allergen-specific immunotherapy (AIT) remains as the only curative treatment. The knowledge about the mechanisms underlying healthy immune responses to allergens, the development of allergic reactions and restoration of appropriate immune responses to allergens has significantly improved over the last decades. It is now well-accepted that the generation and maintenance of functional allergen-specific regulatory T (Treg) cells and regulatory B (Breg) cells are essential for healthy immune responses to environmental proteins and successful AIT. Treg cells comprise different subsets of T cells with suppressive capacity, which control the development and maintenance of allergic diseases by various ways of action. Molecular mechanisms of generation of Treg cells, the identification of novel immunological organs, where this might occur *in vivo*, such as tonsils, and related epigenetic mechanisms are starting to be deciphered. The key role played by the suppressor cytokines interleukin (IL)-10 and transforming growth factor (TGF)-β produced by functional Treg cells during the generation of immune tolerance to allergens is now well established. Treg and Breg cells together have a role in suppression of IgE and induction of IgG4 isotype allergen-specific antibodies particularly mediated by IL-10. Other cell types such as subsets of dendritic cells, NK-T cells and natural killer cells producing high levels of IL-10 may also contribute to the generation of healthy immune responses to allergens. In conclusion, better understanding of the immune regulatory mechanisms operating at different stages of allergic diseases will significantly help the development of better diagnostic and predictive biomarkers and therapeutic interventions.

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INTRODUCTION

The immune system has evolved to distinguish and mount appropriate immune responses against potentially harmful pathogens and harmless exogenous- and self-antigens. For these vital functions of immune tolerance development, the immune system uses a large number of mechanisms, whose deregulation may lead to development of tumors, rejection of transplanted organs, graft-versus-host disease, autoimmune diseases, asthma and allergies. The prevalence of many of these immune regulation-related diseases have significantly increased over the last decades.[1,2] In the case of cancer, malignant cells are able to generate tolerant local microenvironments that promote the generation of tumor antigen-specific regulatory T (Treg) cells, thus blocking proper immune responses against uncontrolled proliferating cells. In contrast, in the case of autoimmune or allergic diseases, mechanisms of breaking of tolerance that impairs function of self-antigen or allergen-specific Treg cells occur. Although deregulation events leading to cancer or to autoimmune and allergic diseases are opposite, the molecular mechanisms involved in such processes share many common pathways.[3] The main clinical manifestations of allergic diseases include allergic rhinitis, allergic asthma, food allergy, atopic dermatitis and anaphylaxis. They are characterized by a Th2-type immune response together with the production of specific immunoglobulin (Ig) E antibodies against environmental proteins, called allergens.[1,4] Although there are many different ways of symptomatic treatment of allergic diseases, currently, allergen-specific immunotherapy (AIT) by the administration of increasing doses of the causative allergen that induces a state of allergen-specific immune tolerance remains as the single long-term curative treatment of allergic diseases.[5] Although many different clinical trials demonstrated the efficacy of AIT, the development of novel strategies with better therapeutic outcomes, enhanced safety and reduced treatment time are still highly demanded.[6]

The knowledge about the mechanisms underlying allergic disease has significantly increased over the last decades.[7–9] Recent findings in the field have contributed to elucidate key molecular and cellular events involved not only in healthy immune response to allergens but also in breaking-tolerance to allergens or restoration of appropriate immune responses to allergens after AIT.[10–17] In this context, it is now well accepted that the generation and preservation of functional allergen-specific Treg cells are essential for healthy immune responses to allergens and successful AIT.[18–20] Treg cells comprise different subsets with suppressive capacity able to inhibit the initiation and development of allergic diseases and allergic patients seem to exhibit a specific impairment in the generation of regulatory T cells.[21]

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Mouse immune tolerance models have been well studied and the role of Treg cells in allergen tolerance has been demonstrated in adaptive transfer models.[22] Obviously, in humans, immune tolerance studies are more difficult to obtain evidence at the level of mouse models, but none of the human reported studies have shown any similarity to anti-viral or anti-bacterial vaccines. There are three levels of evidence that prove this concept in humans. First, the relationship of clinical non-reactivity (allergen tolerance) to allergens and immune tolerance could be observed in two types of direct tissue analysis. One of them is investigation of skin late phase responses, and the second one is investigation of nasal mucosa biopsies in allergic rhinitis. These data show decrease in Th2 cells and eosinophils in both cases by AIT and a parallel increase in these tissue Treg cells and their related cytokines.[23,24] Similar data have been shown also in T-cell epitope peptide immunotherapy.[25] In addition, allergen tolerance in beekeepers is also associated with similar mechanisms and decreased skin late phase responses.[10] Second, direct analysis of human peripheral blood cells without any further culture has been obtained for mechanisms of allergen tolerance in healthy beekeepers, who are exposed to high dose of venom allergens and also during AIT. Allergen tetramer-positive $CD4^+$ antigen-specific T cells have been analyzed or cytokine-secreting cells have been purified in these studies, and the data demonstrate that allergen-specific Treg cells increase in these clinical allergen tolerance models.[10,26] Third, in both allergen extract and peptide immunotherapy, peripheral T-cell tolerance has been shown with the development of decreased T-cell reactivity to whole allergens and their T-cell epitope peptides at the cell culture level.[25,27]

The essential role played by the inhibitory cytokines interleukin (IL)-10 and transforming growth factor (TGF)-β produced by Treg cells in the immunosuppression of allergic reactions has been studied in detail during the last decades. In addition, a regulatory/suppressor role for IL-10-secreting B cells has been recently suggested.[13] Dendritic cells (DCs), natural killer cells, epithelial cells, macrophages and glial cells also express suppressor cytokines such as IL-10 and TGF-β.[28] Apparently, multiple cellular and molecular mechanisms have roles on allergen tolerance. The aim of this review is to discuss mechanisms of immune regulation of allergic diseases with special focus on the role played by the immunosuppressive cytokines IL-10 and TGF-β produced by Treg cells in the establishment of tolerance to allergens.

ROLE OF TREG CELLS IN THE INDUCTION OF IMMUNE TOLERANCE TO ALLERGENS

Treg cells comprise a group of different T-cell subsets with suppressive capacity being essential not only to avoid excessive immune responses to pathogens, but also for the induction of immune tolerance.[29] For long time, it has been accepted based on mouse experiments that exposure to high doses of antigens leads mainly to anergy or clonal deletion of antigen-specific T cells, whereas low doses induce preferentially Treg cells.[30,31] Recent findings in mice demonstrated that exposure to high doses of antigens through the oral route induced allergen-specific Treg cells, thus supporting the main role of Treg cells in the generation of oral tolerance to allergens.[32,33]

Two broad subsets of $CD3^+CD4^+$ Treg cells have been described (Figure 1). These are the thymus-derived naturally occurring $CD4^+CD25^+$ forkhead box protein 3 $(FOXP3)^+$ Treg cells, also called natural Treg (nTreg) cells, and the inducible Treg (iTreg) cells. The iTreg cells are generated in the periphery after antigenic stimulation[34] and three main subsets have been characterized: (i) induced $FOXP3^+$ Treg cells, (ii) $CD4^+FOXP3^-$ IL-10-producing type 1 Treg (Tr1) cells and (iii) TGF-β-expressing T_H3 cells.[35] In humans, these cells co-exist in many immune tolerance-related situations such as allergic or autoimmune diseases.[36] In addition, other Treg cell populations, such as $CD8^+$ Treg cells,[37] double negative $CD4^-CD8^-$ $TCR\alpha\beta^+$ Treg cells, which mediate tolerance in several experimental autoimmune diseases,[38] and $TCR\gamma\delta$ Treg cells, which can have a role in the inhibition of immune responses to tumors,[39] have been described.

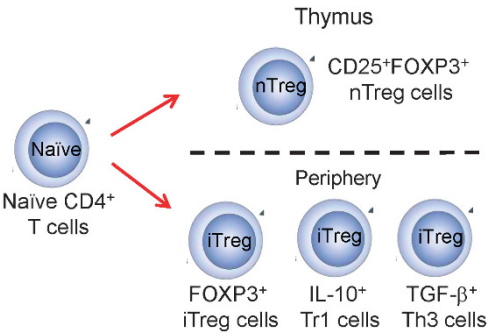

Figure 1. Different subsets of Treg cells. The naturally occurring $CD4^+CD25^+FOXP3^+$ Treg (nTreg) cells are generated in the thymus, whereas three different subsets of inducible Treg (iTreg) cells can be generated in the periphery: (i) $FOXP3^+$ Treg cells, (ii) $CD4^+FOXP3^-$ IL-10–producing Tr1 cells and (iii) TGF-β-expressing T_H3 cells.

There are several important tissues in the body, where the generation of tolerance to allergens through the generation of allergen-specific Treg cells and regulatory B cells take place including tonsils, gastrointestinal mucosa, respiratory tract, skin and oral mucosa.[11–13,40,41] The detailed mechanisms underlying immune tolerance in gastrointestinal tissues have not been completely understood yet. Interestingly, it has been recently shown that human tonsils have important roles in immune tolerance, where the generation of functional allergen-specific iTreg cells occurs by mechanisms partially depending on plasmacytoid DCs (pDCs).[11] In that study, tonsil allergen-specific Treg cells were detected using major histocompatibility complex class II tetramer molecules coupled to specific peptides of the allergen, Bet v 1, which showed allergen-specific suppressive effect on T cells. Supporting these data, two other studies demonstrated that extrathymic Treg cells might develop in tonsils.[42,43] In addition, T-cell cytokine profile of tonsils from allergic patients is characterized by low levels of interferon (IFN)-γ, T-bet and IL-10 with increased Th2/Th1 ratio in polyallergic atopic individuals an indicative of the atopic status.[12]

For a long time, it has been an open question how allergen tolerance is broken in sensitized individuals. It was recently demonstrated that triggering of Toll-like receptor (TLR)-4 or TLR8 and proinflammatory cytokines, such as IL-1β and IL-6, breaks allergen-specific T-cell tolerance in human tonsils and peripheral blood.[12] Particularly, myeloid DCs are essential in breaking allergen tolerance, whereas pDCs do not change the tolerance status. Considering that relatively large lingual tonsil is not removed by tonsillectomy and remains life-long intact, it is reasonable to conceive that tonsils represent the immunological sites, where immune tolerance induction during successful sublingual immunotherapy may take place, thus representing a potential novel target for future therapeutic interventions.[11,12]

NATURALLY OCCURRING $CD4^+CD25^+FOXP3^+$ TREG CELLS

Thymic-derived nTreg cells represent less than 5% of the T-cell population in healthy mice and humans. Concerning the generation of nTreg cells, there are two hypotheses. One suggest that nTreg cells emerge from the thymus as a distinct subset of mature T cells with defined functions.[44] Conversely, other studies demonstrated that Treg may arise from naive T cells in the periphery.[34] The relative contribution of the two pathways *in vivo*

in humans is still unclear. Although a number of studies suggest that thymic differentiation accounts for Treg cells that are specific for self-peptides, peripheral differentiation seems to be required for environmental antigen/allergen-specific T cells, for which an undesired immune response results in pathology.[21,45]

nTreg cells constitutively express high levels of CD25, the alpha chain of the IL-2 receptor and cytotoxic T-lymphocyte antigen-4, a molecule acting as a negative regulator of adaptive immune responses after ligation to CD80 and CD86.[46] They also express program death 1, an immunoreceptor tyrosine-based inhibitory motif-containing receptor,[47] the glucocorticoid-induced tumor-necrosis factor receptor family-related gene, CD103 ($\alpha E\beta 7$ integrin) and CD122 (β chain of IL-2 receptor), whose expression correlates with the suppressive activity of Treg cells (Table 1).[35] Two different functional subsets of nTreg cells in humans have been defined according to the expression of inducible costimulatory molecule.[48] Other proposed markers for Treg cells include certain chemokine receptors, TLRs, glycoprotein-A repetitions predominant, membrane-bound TGF-β, neuropilin-1, lymphocyte activation gene-3, latency-associated peptide and granzymes (Table 1). None of these markers are fully Treg cell specific and are also expressed by other effector T-cell populations.[49,50] Gene arrays identified additional markers, including G-protein coupled receptor 83 (Gpr83), Ecm1 and Helios.[51–53] The alpha-chain of the IL-7R (CD127) was proposed as a differentiation marker expressed on activated effector T cells, but not on Treg cells.[54]

FOXP3 AND MOLECULAR MECHANISMS OF TREG CELL GENERATION

The intracellular forkhead-winged transcription factor FOXP3 (forkhead box P3) has been suggested for long time, as the master switch transcription factor for the differentiation of functional nTreg cell development.[21] In mice, FOXP3 is specifically expressed by nTreg cells,[55,56] whereas in humans, it might be also expressed in a fraction of activated T cells.[49] In any case, human subjects affected by the immune dysregulation polyendocrinopathy enteropathy-X-linked syndrome or the X-linked autoimmune and allergic dysregulation syndrome, characterized by mutations in FOXP3, suffer from typical severe autoimmune and allergic phenotype starting on from infancy, including eczema, food allergy and eosinophilic inflammation.[57,58] Studies in the scurfy mouse with impaired capacity to generate functional Treg cells because of deletion in the forkhead domain of FOXP3 have demonstrated an intense multiorgan inflammatory response associated with allergic airway inflammation, a striking hyper IgE, eosinophilia, and dysregulated Th1 and Th2 cytokine production.[59] Recently, the generation of Depletion of Regulatory T cells mice, a bacterial artificial chromosome transgenic mouse line that express the diphtheria toxin receptor coupled to enhanced green fluorescence protein under the control of an additional Foxp3 promoter[60] has allowed the direct *in vivo* analysis and depletion of $FOXP3^+$ Treg cells, contributing to elucidate essential *in vivo* functions of Treg cells.[61,62]

It was demonstrated that the expression of FOXP3 was sufficient to convert non-regulatory $CD4^+CD25^-$ T cells into T cells with regulatory activity and this conversion can be induced by TGF-β.[34] In addition, allergen-specific human Treg cells generated by retroviral transduction of a transcription unit encoding FOXP3 and allergen-specific TCR $\alpha\beta$-chains displayed a classical phenotype of Treg cells and suppressive capacity.[63] FOXP3 exerts suppressive activity by inhibiting the expression of IL-2 and IFN-γ in nTreg cells after direct interaction with the Runt-related transcription factor1 (RUNX1).[64] In mice, RUNX transcription factors are essential for maintaining high FOXP3

Table 1. Molecular and functional features of Treg cells

Treg cell markers	*Factors driving Treg cell generation*	*Mechanisms of suppression employed by Treg cells*
CD25: alpha chain of the IL-2 receptor[141]	Specific probiotic strains[142]	Suppressive cytokines[36,143]
CTLA4: cytotoxic T lymphocyte antigen-4[91]	*B. animalis*	IL-10
PD1: program death 1[47]	*B. infantis*	TGF-β
	L. rhamnosus	IL-35
TGF-β[36]	Pathogen-derived molecules[145]	Metabolic disruption[10,147]
IL-10[109]	filamentous hemagglutinin	CD25
CD49b: integrin alpha subunit[76]	$FOXP3\pm$ Treg cells[146]	cAMP
LAG-: lymphocyte activation gene-3[76]		ADR2
LAP: latency-associated peptide[144]		HR2
		CD39
		CD73
Granzymes A and B[37]	Exogenous signals[85]	Targeted molecules DCs[146]
CD122; β chain of IL-2 receptor[148]	Vitamin D3	CTLA-4
CD103:$\alpha E\beta 7$ integrin[149]	Retinoic acid	PD-1
	Adenosine	TGF-β R
	Histamine	IL-10R
	Dexamethasone	
ICOS: inducible costimulatory molecule[48]	DC-derived molecules[35]	Cytolysis[37]
GARP: glycoprotein-A repetitions predominant[150]	TGF-β	Granzyme A and B perforin
Neuropilin-1: membrane-bound correceptor[151]	IL-10	
Gpr83: G-protein coupled receptor 83[51,52]	RA	
ECM1: extracellular matrix protein 1[51,52]	IDO	
HELIOS: ikaros transcription factor member[51,52]	RALDH	
GITR: glucocorticoid-induced TNFR gene[152]	ILT-3	
	ILT-4	
	CD80	
	CD86	
	ICOS-L	
	PD-L1	
	MHC-II	

Abbreviations: DC, dendritic cell; IDO, indoleamine deoxygenase; IL, interleukin; ILT, immunoglobulin-like transcript; MHC, major histocompatibility complex; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; TGF, transforming growth factor; TNFR, tumor necrosis factor receptor; Treg, regulatory T.

expression, thus ensuring Treg cell lineage identity.[65] A molecular mechanism linking TGF-β and FOXP3 expression in humans was reported demonstrating that the induction of RUNX1 and RUNX3 by TGF-β is essential for the generation and suppressive function of induced Treg cells.[66] Although TGF-β drives the conversion of naïve T cells into Treg cells,[67] it mainly promotes the generation of Th17 cells in the presence of IL-6.[68] Other metabolites such as retinoic acid contribute to the balance of Th17 and Treg cells by enhancing the expression of FOXP3 through a STAT3/STAT5-independent mechanism.[69] It was also demonstrated that the inhibition of FOXP3 expression by direct GATA3-binding to the promoter region constitutes a mechanism for the inhibition of tolerance by Th2-type immune response.[70] Recent findings indicated that the expression of FOXP3 alone is not sufficient for conferring Treg cell function and phenotype. It was demonstrated that Treg-cell-specific epigenetic changes are also critical for the maintenance of FOXP3 expression, Treg cell differentiation and to establish a stable lineage.[71,72] In addition, detailed biochemical and mass spectrometry analysis revealed that FOXP3 associates to other transcription factor partners to control distinct aspects of Treg cell biology.[73] Novel findings have also shown that alternative transcriptional programs, including those regulated by FOXO1 control Treg cell function.[74] The study of the epigenetic changes leading to FOXP3 expression and maintenance as well as the role played by other transcriptional programs is a new exciting area of research that may contribute to determine the pathways involved in the establishment of functional nTreg cells.

IL-10-PRODUCING TYPE 1 TREG (TR1) CELLS

In addition to naturally occurring $CD4^+CD25^+Foxp3^+$ Treg cells, Tr1 cells represent a key subset of iTreg cells generated in the periphery after antigenic stimulation characterized by high levels of IL-10 production and regulatory/suppressive properties.[10,75] Different studies demonstrated that the generation of IL-10-producing Tr1 cells has a very important role in the maintenance of healthy immune response in different diseases, such as allergy, autoimmunity or graft-versus-host disease.[10,76,77] Tr1 cells are generated after constant exposure to peripheral antigens in the presence of IL-10. Although the main cytokine produced by Tr1 cells is IL-10, they also produce low/medium levels of TGF-β, IFN-γ and IL-5, but not IL-4 nor IL-2, which could be a limitation for therapeutic interventions.[78,79] Tr1 cells suppress effector T cell responses by mechanisms that depend on IL-10 and also TGF-β and produce perforin and granzymes to kill antigen-presenting cells.[75] A strategy designed to generate Tr1 cell-secreting IL-10, in the absence of Th1- and Th2-associated cytokines, has utilized *in vitro* stimulation of $CD4^+$ cells in the presence of glucocorticoids, either alone or more effectively, together with the active form of vitamin D, 1α25-dihydroxyvitamin D3, cytokines (IL-10 and IFN-α or TGF-β and IL-27) or anti-CD3 in combination with anti-CD46 monoclonal antibodies. Similarly, a wide range of antigen administration protocols has been used to induce Tr1 cells, not only *in vitro*, but also *in vivo*. These cells were able to regulate autoimmunity *in vivo* (experimental allergic encephalomyelitis or EAE) in an IL-10-dependent manner.[80] Interestingly, in mice, these drug-induced Tr1 cells were shown to have similar suppressive properties toward naturally occurring Treg cells *in vitro*. In addition, they were able to inhibit naïve T cells via cell contact-dependent pathways, even though they did not express Foxp3.[81] In humans, these drug-induced Tr1 cells inhibited the proliferation and cytokine responses of naïve as well as established Th1 and Th2 cells, including allergen-specific Th2 cell lines.[82] Interestingly, the enforced expression of IL-10 by transducing human $CD4^+$ T cells with a bidirectional lentiviral vector encoding for human IL-10 leads to a stable T-cell population that recapitulated the phenotype and function of Tr1 cells.[83] Recent findings demonstrated that the coexpression of CD49b and lymphocyte activation gene--3 identifies human and mouse functional Tr1 cells.[76] The authors proposed the combination of these two markers to track Tr1 cells *in vivo* and to purify them for cellular therapy aiming at restoration of immune tolerance.

MECHANISMS OF GENERATION OF TREG CELLS: ROLE OF DCs

DCs have an essential role in the initiation of adequate immune responses and directly participate in the generation of functional

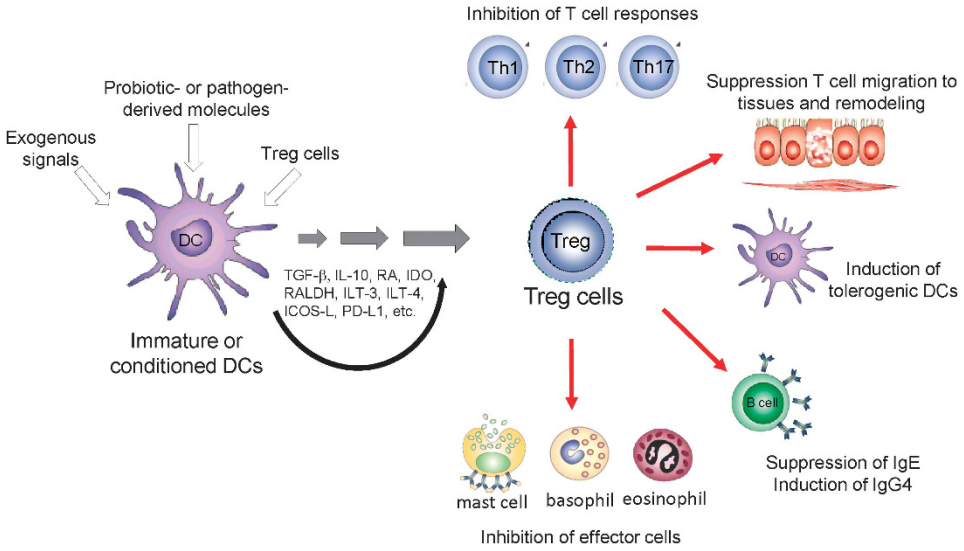

Figure 2. Treg cells inhibit allergic inflammation by different modes of action. Immature DCs or mature DCs conditioned by different stimulus such as Treg cell-derived molecules, exogenous signals or specific molecules produced by probiotics or pathogens promote the generation of functional Treg cells able to inhibit the ongoing allergic diseases by acting on different cells and tissues. Treg cells directly or indirectly inhibit the activation of effector T cells (Th1, Th2 and Th17), basophils, mast cells and eosinophils, inhibit inflammatory DCs promoting the generation of tolerogenic DCs, suppress the production of IgE, induce IgG4 and suppress the infiltration of T cells to inflamed tissues and remodeling. ICOS, inducible costimulatory molecule; IDO, indoleamine 2,3-dioxygenase; ILT, immunoglobulin-like transcripts; PD-1, program death 1; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase.

Treg cells by using different mechanisms.[35] For a long time, the dogma has been that immature or partially mature DCs retain the capacity to generate Treg cells, whereas mature DCs, depending on the stimuli and the specific environments, are able to polarize different effector T-cell subsets.[40] Recent studies demonstrated that fully matured DCs are also able to generate functional Treg cells under certain conditions.[11,84] The capacity of mature DCs to polarize Treg cell responses is conditioned by specific probiotic or pathogen-derived molecules, by FOXP3$^+$ Treg cells and by exogenous signals (for example, vitamin D3 metabolites, retinoic acid, adenosine and histamine;[85–87] Figure 2). For example, vitamin D was demonstrated to act on different DC subsets conditioning their capacity to generated Tr1 cells or FOXP3$^+$ Treg cells.[85] In the same way, retinoic acid, a metabolite derived from vitamin A, favors the maintenance of Treg cells by inhibiting the formation of inflammatory Th17 cells. DCs use a large number of soluble molecules such as cytokines with tolerogenic capacity (for example, TGF-β and IL-10), distinct metabolites (for example, retinoic acid) and specific enzymes (for example, indoleamine 2,3-dioxygenase or retinaldehyde dehydrogenase to polarize Treg cells (Table 1). In addition, different costimulatory molecules expressed by DCs, such as ILT-3, ILT-4, CD80, CD86, inducible costimulator-ligand, programmed death-ligand 1 and major histocompatibility complex-II, contribute to the generation and expansion of functional Treg cells (Table 1). Myeloid DCs and pDCs constitute two complementary and specialized DC subsets exhibiting different functional roles. Myeloid DCs express TLR2–6 and -8 and respond to bacterial and viral infections by producing large amounts of IL-12, whereas pDCs express TLR-7 and -9, and represent the main producers of type I IFNs after viral infections.[84,88]

MECHANISMS OF IMMUNE SUPPRESSION BY TREG CELLS

Treg cells use four main mechanisms of suppression that are mediated by many soluble and membrane-bound suppressor factors (Table 1): suppressive cytokines (IL-10, TGF-β and IL-35), metabolic disruption mechanisms (CD25, cAMP, adenosine receptor 2, histamine receptor 2, CD39 and CD73), mechanisms that utilize surface molecules that may target DCs (cytotoxic T-lymphocyte antigen-4, program death-1) and cytolysis (granzymes A and B).[10,36,37,89] In allergic diseases, Treg cells are able to suppress both the sensitization and effector phases through different modes of action, which include cell contact-dependent mechanisms as well as secreted cytokine-dependent ones.

Treg cells directly or indirectly are able to suppress almost all cell types contributing to the ongoing allergic reactions including B cells, DCs, T cells, effector cells (mast cells and basophils), eosinophils as well as specific inflamed tissue cells (Figure 2).[35] Treg cells act on B cells to promote the production of allergen-specific IgG4, whereas inhibiting IgE[90] and directly inhibiting the activation of allergen-specific Th2, Th1 and Th17 cells, thus blocking all the specific effects mediated by these cells during allergic reactions. Treg cells also induce the generation of tolerogenic DC phenotypes and also inhibit DC maturation.[91] In addition, Treg cells are directly or indirectly able to inhibit allergen-induced activation and degranulation of mast cells and bashophils[92] and impair the infiltration of eosinophils and other effector T cells into inflamed tissues by cytokine-dependent rather than cell–cell contact-dependent mechanisms.[93] Treg cells were are able to suppress mast cell FcεRI expression by a mechanism independent of IL-10 and TGF-β as well as mast cell degranulation through OX40–OX40L interactions.[92,94,95] Furthermore, Treg cells interact with resident tissue cells and contribute to tissue remodeling.[94,95] TGF-β produced by Treg cells in the lung of asthmatic patients represents an important factor in healing with important immunomodulatory and fibrogenic activities that might contribute to the repair of asthmatic airways.[96] The role of TGF-β in asthma is complex and many other cells including eosinophils and tissue resident cells also produce TGF-β. The actual contribution of Treg-derived TGF-β to these processes in asthmatic patients needs to be further investigated.

THE ROLE OF IL-10 IN THE IMMUNE REGULATION OF ALLERGIC DISEASES

IL-10 has a potent immunosuppressive capacity that has an essential role not only in the establishment of peripheral tolerance to allergens, but also in protecting the host from exaggerated inflammatory responses to pathogens as well as autoimmune diseases.[85,97,98] In humans, IL-10 is mainly produced by

Table 2. Comparison of IL-10 and TGF-β functions on target cells

Cell type	*IL-10*	*TGF-β*
APCs	Inhibits APC maturation and pro-inflammatory cytokine secretion Inhibits antigen presentation, induced T-cell proliferation and cytokine production (Th1 and Th2)	Promotes Langerhans cell development and downregulates FcεRI expression Inhibits APC maturation and antigen presentation and T-cell proliferation Inhibits scavenger and effector functions on monocytes and macrophages
T cells	Suppresses allergen-specific Th1 and Th2 cells Blocks B7/CD28 co-stimulatory pathway on T cells	Promotes T-cell survival Inhibits proliferation, differentiation and effector function, including allergen-specific Th1 and Th2 cells Promotes the Th17 lineage
Treg cells	Promotes the generation of Tr1 cells	Promotes generation of FOXP3$^+$ Treg cells
B cells	Enhances survival Promotes Ig production, including IgG4 Suppresses allergen-specific IgE	Inhibits proliferation Apoptosis of immature or naïve B cells Inhibits most Ig class switching Switch factor for IgA Suppresses allergen-specific IgE
Eosinophils, mast cells and neutrophils	Inhibits survival and cytokine production Inhibits mast cell activation, including cytokine production Inhibits chemokine and pro-inflammatory cytokine production	Promotes chemotaxis Variable effects on other functions; that is, inhibit expression of FcεR

Abbreviations: APC, antigen-presenting cell; IL, interleukin; TGF, transforming growth factor; Treg cell, regulatory T cell.

monocytes, T cells, B cells, macrophages, DCs and mast cells.[99,100] IL-10 is secreted as a homodimer consisting of two subunits of 178 amino acids (18 kDa).[101,102] IL-10 binds to a receptor complex composed of two IL-10R1 chains and two IL-10R2.[103] The IL-10R2 is ubiquitously expressed and the IL-10R1 chain is only expressed on target cells including T cells, B cells, natural killer cells, monocytes, mast cells and DCs.[99,104] The primary function of IL-10 as an immunosuppressive cytokine is to inhibit the production of proinflammatory cytokines, chemokines and chemokine receptors as well as the expression of class II major histocompatibility complex and costimulatory molecules CD80/CD86 on monocyte/macrophages and DCs, which in turn leads to indirect inhibition of proliferation of $CD4^+$ T cells (Table 2).[105,106] In addition, IL-10 inhibits T-cell proliferation and cytokine production through direct suppression of T-cell costimulation depending on CD28, inducible costimulatory molecule and CD2.[107,108]

In the context of allergic diseases, the protective role of IL-10 is very well-established. DCs from the respiratory tract of healthy individuals constitutively express high levels of IL-10 and this expression is impaired in patients suffering from rhinitis and allergic asthma.[35,97] In addition, many different clinical trials demonstrated that the generation of IL-10-producing Tr1 cells correlates with the restoration of healthy immune responses to allergens after allergen SIT.[109–111] Although healthy and allergic individuals display all different subsets of allergen-specific T cells (Th1, Th2 and Tr1 cells), it is the imbalance between Th2 and Tr1 cells what leads to allergy development or recovery. In healthy individuals, allergen-specific Tr1 cells are the predominant T-cell subset and different studies showed that these cells are able to inhibit allergic responses to different allergenic sources such as birch pollen or related food allergens, house dust mite or bee venom.[19,35] IL-10-secreting Tr1 cells not only suppress Th2 immune responses, but also utilize all of the above-described mechanisms of suppression to overcome and inhibit allergic inflammation. Different models of high-dose exposure to allergens showed that the generation of functional IL-10-producing Tr1 cells correlates with the generation of tolerance to allergens.[10,112] In this regard, a cross-sectional study demonstrated that exposure to high doses of cat allergen leads to tolerance induction, which is associated with elevated levels of allergen-specific IgG4 levels and generation of IL-10-secreting Tr1 cells without new sensitizations or asthma development.[112] Exposure to high doses of bee venom allergen in professional beekeepers during beekeeping season was also demonstrated as a natural mechanism of immune tolerance induction to allergens in humans.[10] This mechanism involves the clonal differentiation and expansion of IL-10-secreting Tr1 cells from allergen-specific Th1 and Th2 cells. Histamine receptor 2, which is also upregulated on specific Th2 cells, suppresses allergen stimulated T cells and enhances IL-10 production. These processes persist as long as venom exposure and returns to previous levels within 2–3 months after the end of beekeeping season.[10]

Interestingly, very recent findings demonstrated that IL10-secreting B regulatory (Br1) cells essentially contribute to these mechanisms.[13] Human IL-10-secreting Br1 cells are able to suppress antigen-specific $CD4^+$ T-cell proliferation. In addition, the major bee venom allergen phospholipase A-specific B cells from non-allergic beekeepers showed increased expression of IL-10 and IgG4, and the frequency of IL-10-secreting phospholipase A-specific B cells increased in allergic patients receiving AIT. These data provide novel information on IL-10-secreting Br1 cells in allergic inflammation in humans. Supporting these findings, other studies in humans reported that the levels of IL-10-secreting $CD5^+$ B cells in peripheral blood are higher in healthy individuals compared with milk allergic patients after challenge with casein.[113] In mouse models, helminth-induced Br1 cells inhibit Th2 cells and promote the generation Treg cells, which also contribute to the suppression of Th2-mediated allergic inflammation. It was previously shown that in contrast to the inhibitory effects on many cell types, IL-10 enhances the survival of human B cells, their proliferation, isotype switching and differentiation into antibody-secreting cells.[114] Despite this, serum immunoglobulin levels are normal in *il-10*$^{-/-}$.[115]

THE ROLE OF TGF-β IN THE IMMUNE REGULATION OF ALLERGIC DISEASES

TGF-β is a pleiotropic cytokine with potent regulatory capacity that has a very important role in suppression of immune responses, especially in induction and maintenance of peripheral tolerance to allergens. Treg cells are a major source of TGF-β and in the particular case of Th3 cells, TGF-β is the main effector molecule involved in the generation of oral tolerance.[116,117] TGF-β1 is the prototypic member of the complex TGF-β superfamily consisting of more than 35 members.[118,119] TGF-β1 is synthesized and produced as a homodimer propeptide that is constitutively associated to latency-associated peptide to prevent the binding to its receptors. To be functional, mature TGF-β1 requires the degradation or conformational alteration of latency-associated peptide, which is mediated by proteases, including plasmin, matrix metalloproteinases, thrombospondin 1 and the integrins αvβ6 or αvβ8.[120] There are three types of TGF-β receptors (type I, II and III) with different structural and functional properties that can act as homo- or heterodimers.[121] TGF-β receptors are single pass receptors with serine/threonine kinase activity. Type I and II TGF-β receptors display high affinity for TGF-β1, but not for TGF-β2, whereas type III receptor also binds TGF-β2. The TGF-β superfamily can act on virtually all mammalian cell types expressing different combinations of TGF-β receptors. Ligand-induced activation of TGF-β receptors starts an intracellular signaling cascade leading to the activation and nucleus accumulation of SMAD transcription factors that coordinate the transcriptional initiation of target genes.[121,122] The activation of SMAD-independent pathways, including mitogen-activated protein kinases and phosphoinositide 3-kinase, and other transcription factors such as RUNX-1 and -3 can be also induced contributing to different types of responses. TGF-β inhibits both B- and T-lymphocyte proliferation, Th1 and Th2 responses, their differentiation and survival.[123–125] Although TGF-β inhibits most Ig isotype switching, it can drive the differentiation of IgA-secreting plasma cells in mouse models.[126] TGF-β promotes immature and inhibitory phenotypes on Langerhans cells, DCs and macrophages.[127,128]

The effect of TGF-β on effector cells of allergy is complex and diverse (Table 2). For example, TGF-β is able to induce chemotaxis, whereas blocking FcεRI expression in mast cells.[28] On the other hand, TGF-β is associated with the conversion of naïve $CD4^+CD25^-$ T cells into functional Treg cells that contribute to the resolution of immune responses.[34,129] As discussed above, TGF-β induces the expression of FOXP3 expression in naïve $CD4^+$ T cells and it is essential for *in vivo* expansion and immunosuppressive capacity of iTreg cells, which might well have very important implications for immune tolerance as well as for the design of alternative mucosal vaccination strategies.

Considering all these diverse immune regulatory properties of TGF-β, the effects of this cytokine in allergic diseases are complex and there is evidence for both promoting and inhibitory effects. It has been suggested that TGF-β expressed by eosinophils and resident tissue cells may have an important role in airway remodeling in asthmatic patients.[130] Airway epithelial cells produce TGF-β that induces the synthesis of collagen-I[131] and inhibits collagenase production[132] in an autocrine manner, thus contributing to particularly airway remodeling and fibrosis in the pathogenesis of asthma. Supporting these data, experiments using anti-TGF-β antibodies or neutralization of Smad-3 demonstrated significant reduction of airway smooth muscle

proliferation, peribronchial fibrosis and mucus production without influencing airway inflammation.[133,134] In contrast, TGF-β1-deficient mice die by the age of 3 or 4 weeks under germ-free conditions because of wasting syndrome characterized by excessive activation of autoreactive $CD4^+$ T cells, indicating the predominant role of TGF-β1 in controlling inflammation.[135,136]

In this context, the overexpression of TGF-β1 in ovalbumin-specific $CD4^+$ T cells abolished airway hyperresponsiveness and airway inflammation induced by ovalbumin-specific Th2 cells in a murine model of allergic asthma.[137] Involvement of TGF-β in the regulation of allergic airway disease by naturally occurring Treg cells has also been reported.[138,139] The exact contribution of TGF-β in the regulation of human asthma remains to be established. Initial studies demonstrated that atopic patients suffering from asthma showed higher basal levels of TGF-β1 and that these levels were further increased after allergen provocation, which is in line with the role of TGF-β1 in airway wall remodeling.[140] On the other hand, compelling experimental evidence demonstrated the important role of TGF-β, especially after activation of Treg cells, in the control of airway inflammation and restoration of healthy immune responses to allergens.[120] In summary, TGF-β1 has a dual role in the pathogenesis of allergic diseases. From one side, it might act as a negative feedback mechanism to control airway inflammation and inducing T-cell tolerance (protective effect), whereas at the same time, and because of its important role in healing, it is able to induce airway remodeling and fibrosis (promoting effect). Further research will be needed to clarify the paradoxical effect of TGF-β in the regulation of allergic diseases.

CONCLUSIONS

The generation and preservation of allergen-specific Treg cells are essential to maintain healthy immune responses to allergens as well as for the restoration of normal responses after AIT. Our knowledge in the mechanisms operating during Treg cell generation as well as their mode of suppression to inhibit the development and continuation of allergic diseases has significantly increased over the past years. In particular, compelling experimental evidence demonstrated the key role played by the inhibitory cytokines IL-10 and TGF-β produced by Treg cells for the generation of immune tolerance to allergens. Other cell types such as regulatory B cells or DCs producing high levels of IL-10 seem to have also key roles in the initiation and maintenance of immune tolerance as appropriate healthy immune response to allergens. Still little is known regarding the actual capacity and importance of IL-10-producing Tr1 and Br1 cell types in modulating the allergic response in humans. In addition, further research is also required to better understand the paradoxical capacity of TGF-β to prevent and/or promote the development of allergic diseases. The better understanding of the molecular and cellular events implicated in the pathogenesis of the allergic diseases will significantly help the development of better prevention strategies and therapeutic options.

CONFLICT OF INTEREST

C Akdis has consulted for Actellion, Aventis, Stallergenes and Allergopharma; is a pastpresident of the European Academy of Allergology and Clinical Immunology; is a fellow and interest group member of the American Academy of Allergy, Asthma and Immunology; is an ex-committee member of the Global Allergy and Asthma European Network; and is director of Christine Kühne-Center for Allergy Research and Education. The remaing authors declare no conflict of interest.

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REFERENCES

1 Akdis CA. Therapies for allergic inflammation: refining strategies to induce tolerance. *Nat Med* 2012; **18**: 736–749.
2 El-Gabalawy H, Guenther LC, Bernstein CN. Epidemiology of immune-mediated inflammatory diseases: incidence, prevalence, natural history, and comorbidities. *J Rheumatol Suppl* 2010; **85**: 2–10.
3 Belkaid Y, Oldenhove G. Tuning microenvironments: induction of regulatory T cells by dendritic cells. *Immunity* 2008; **29**: 362–371.
4 Ring J, Akdis C, Behrendt H, Lauener RP, Schappi G, Akdis M *et al.* Davos declaration: allergy as a global problem. *Allergy* 2012; **67**: 141–143.
5 Burks AW, Calderon MA, Casale T, Cox L, Demoly P, Jutel M *et al.* Update on allergy immunotherapy: American Academy of Allergy, Asthma & Immunology/European Academy of Allergy and Clinical Immunology/PRACTALL consensus report. *J Allergy Clin Immunol* 2013; **131**: 1288–1296 e3.
6 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.
7 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; quiz1450-1.
8 Sackesen C, van de Veen W, Akdis M, Soyer O, Zumkehr J, Ruckert B *et al.* Suppression of B-cell activation and IgE, IgA, IgG1 and IgG4 production by mammalian telomeric oligonucleotides. *Allergy* 2013; **68**: 593–603.
9 Novak N, Peng WM, Bieber T, Akdis C. FcepsilonRI stimulation promotes the differentiation of histamine receptor 1-expressing inflammatory macrophages. *Allergy* 2013; **68**: 454–461.
10 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.
11 Palomares O, Ruckert B, Jartti T, Kucuksezer UC, Puhakka T, Gomez E *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, 520 e1–9.
12 Kucuksezer UC, Palomares O, Ruckert B, Jartti T, Puhakka T, Nandy A *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.
13 van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG *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.
14 Rebane A, Zimmermann M, Aab A, Baurecht H, Koreck A, Karelson M *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.
15 Meyer N, Christoph J, Makrinioti H, Indermitte P, Rhyner C, Soyka M *et al.* Inhibition of angiogenesis by IL-32: possible role in asthma. *J Allergy Clin Immunol* 2012; **129**: 964–973 e7.
16 Janzi M, Melen E, Kull I, Wickman M, Hammarstrom L. Rare mutations in TNFRSF13B increase the risk of asthma symptoms in Swedish children. *Genes Immun* 2012; **13**: 59–65.
17 Early SB, Huyett P, Brown-Steinke K, Borish L, Steinke JW. Functional analysis of -351 interleukin-9 promoter polymorphism reveals an activator controlled by NF-kappaB. *Genes Immun* 2009; **10**: 341–349.
18 Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E *et al.* Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J Allergy Clin Immunol* 2011; **127**: 701–721 e70.
19 Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; **199**: 1567–1575.
20 Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4 + CD25 + cells in the nasal mucosa. *J Allergy Clin Immunol* 2008; **121**: 1467–1472, 1472 e1.

21 Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S *et al.* Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol* 2013; **14**: 307–308.
22 Cottrez F, Hurst SD, Coffman RL, Groux H. T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol* 2000; **165**: 4848–4853.
23 Durham SR, Till SJ. Immunological changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998; **102**: 157–164.
24 Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002; **360**: 47–53.
25 Larche M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat Med* 2005; **11**(4 Suppl): S69–S76.
26 Van Overtvelt L, Wambre E, Maillere B, von Hofe E, Louise A, Balazuc AM *et al.* Assessment of Bet v 1-specific CD4+ T cell responses in allergic and nonallergic individuals using MHC class II peptide tetramers. *J Immunol* 2008; **180**: 4514–4522.
27 Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Muller U *et al.* Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. *J Clin Invest* 1996; **98**: 1676–1683.
28 Soyer OU, Akdis M, Ring J, Behrendt H, Crameri R, Lauener R *et al.* Mechanisms of peripheral tolerance to allergens. *Allergy* 2013; **68**: 161–170.
29 Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2011; **127**: 18–27; quiz 28-9.
30 Berin MC, Mayer L. Can we produce true tolerance in patients with food allergy? *J Allergy Clin Immunol* 2013; **131**: 14–22.
31 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.
32 Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N *et al.* Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity* 2011; **34**: 237–246.
33 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.
34 Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N *et al.* Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003; **198**: 1875–1886.
35 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.
36 Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K *et al.* IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; **33**: 1205–1214.
37 Siegmund K, Ruckert B, Ouaked N, Burgler S, Speiser A, Akdis CA *et al.* Unique phenotype of human tonsillar and in vitro-induced FOXP3+CD8+ T cells. *J Immunol* 2009; **182**: 2124–2130.
38 Strober S, Cheng L, Zeng D, Palathumpat R, Dejbakhsh-Jones S, Huie P *et al.* Double negative (CD4-CD8- alpha beta+) T cells which promote tolerance induction and regulate autoimmunity. *Immunol Rev* 1996; **149**: 217–230.
39 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.
40 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.
41 Mascarell L, Saint-Lu N, Moussu H, Zimmer A, Louise A, Lone Y *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.
42 McClory S, Hughes T, Freud AG, Briercheck EL, Martin C, Trimboli AJ *et al.* Evidence for a stepwise program of extrathymic T cell development within the human tonsil. *J Clin Invest* 2012; **122**: 1403–1415.
43 Leavy O. T cell development: tonsils turn out T cells too. *Nat Rev Immunol* 2012; **12**: 232.
44 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.
45 Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y *et al.* Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 2012; **482**: 395–399.
46 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 63-6, 106-14.
47 Carter L, Fouser LA, Jussif J, Fitz L, Deng B, Wood CR *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.
48 Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L *et al.* Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. *Immunity* 2008; **28**: 870–880.
49 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.
50 Gao Y, Lin F, Su J, Gao Z, Li Y, Yang J *et al.* Molecular mechanisms underlying the regulation and functional plasticity of FOXP3(+) regulatory T cells. *Genes Immun* 2012; **13**: 1–13.
51 Pfoertner S, Jeron A, Probst-Kepper M, Guzman CA, Hansen W, Westendorf AM *et al.* Signatures of human regulatory T cells: an encounter with old friends and new players. *Genome Biol* 2006; **7**: R54.
52 Sugimoto N, Oida T, Hirota K, Nakamura K, Nomura T, Uchiyama T *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.
53 Hansen W, Westendorf AM, Toepfer T, Mauel S, Geffers R, Gruber AD *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.
54 Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A *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.
55 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; **299**: 1057–1061.
56 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; **4**: 330–336.
57 McMurchy AN, Gillies J, Allan SE, Passerini L, Gambineri E, Roncarolo MG *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.
58 Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C *et al.* JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J Clin Invest* 2000; **106**: R75–R81.
59 Lin W, Truong N, Grossman WJ, Haribhai D, Williams CB, Wang J *et al.* Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J Allergy Clin Immunol* 2005; **116**: 1106–1115.
60 Lahl K, Sparwasser T. *In vivo* depletion of FoxP3+ Tregs using the DEREG mouse model. *Methods Mol Biol* 2011; **707**: 157–172.
61 Baru AM, Untucht C, Ganesh V, Hesse C, Mayer CT, Sparwasser T. Optimal isolation of functional Foxp3+ induced regulatory T cells using DEREG mice. *PLoS ONE* 2012; **7**: e44760.
62 Klingenberg R, Gerdes N, Badeau RM, Gistera A, Strodthoff D, Ketelhuth DF *et al.* Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis. *J Clin Invest* 2013; **123**: 1323–1334.
63 Schmetterer KG, Haiderer D, Leb-Reichl VM, Neunkirchner A, Jahn-Schmid B, Kung HJ *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; **1271**: 238–245, 245 e1-3.
64 Ono M, Yaguchi H, Ohkura N, Kitabayashi I, Nagamura Y, Nomura T *et al.* Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. *Nature* 2007; **446**: 685–689.
65 Rudra D, Egawa T, Chong MM, Treuting P, Littman DR, Rudensky AY. Runx-CBFbeta complexes control expression of the transcription factor Foxp3 in regulatory T cells. *Nat Immunol* 2009; **10**: 1170–1177.
66 Klunker S, Chong MM, Mantel PY, Palomares O, Bassin C, Ziegler M *et al.* Transcription factors RUNX1 and RUNX3 in the induction and suppressive function of Foxp3+ inducible regulatory T cells. *J Exp Med* 2009; **206**: 2701–2715.
67 Zhang L, Zhao Y. The regulation of Foxp3 expression in regulatory CD4(+)CD25(+)T cells: multiple pathways on the road. *J Cell Physiol* 2007; **211**: 590–597.
68 Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO *et al.* Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* 2006; **441**: 231–234.
69 Elias KM, Laurence A, Davidson TS, Stephens G, Kanno Y, Shevach EM *et al.* Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway. *Blood* 2008; **111**: 1013–1020.
70 Mantel PY, Kuipers H, Boyman O, Rhyner C, Ouaked N, Ruckert B *et al.* GATA3-driven Th2 responses inhibit TGF-beta1-induced FOXP3 expression and the formation of regulatory T cells. *PLoS Biol* 2007; **5**: e329.
71 Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. *Immunity* 2013; **38**: 414–423.
72 Mackey-Cushman SL, Gao J, Holmes DA, Nunoya JI, Wang R, Unutmaz D *et al.* FoxP3 interacts with linker histone H1.5 to modulate gene expression and program Treg cell activity. *Genes Immun* 2011; **12**: 559–567.

73 Rudra D, deRoos P, Chaudhry A, Niec RE, Arvey A, Samstein RM *et al.* Transcription factor Foxp3 and its protein partners form a complex regulatory network. *Nat Immunol* 2012; **13**: 1010–1019.
74 Ouyang W, Liao W, Luo CT, Yin N, Huse M, Kim MV *et al.* Novel Foxo1-dependent transcriptional programs control T(reg) cell function. *Nature* 2012; **491**: 554–559.
75 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.
76 Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P *et al.* Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. *Nat Med* 2013; **19**: 739–746.
77 Guichelaar T, ten Brink CB, van Kooten PJ, Berlo SE, Broeren CP, van Eden W *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.
78 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.
79 Allan SE, Broady R, Gregori S, Himmel ME, Locke N, Roncarolo MG *et al.* CD4 + T-regulatory cells: toward therapy for human diseases. *Immunol Rev* 2008; **223**: 391–421.
80 Barrat FJ, Cua DJ, Boonstra A, Richards DF, Crain C, Savelkoul HF *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.
81 Vieira PL, Christensen JR, Minaee S, O'Neill EJ, Barrat FJ, Boonstra A *et al.* IL-10-secreting regulatory T cells do not express Foxp3 but have comparable regulatory function to naturally occurring CD4 + CD25 + regulatory T cells. *J Immunol* 2004; **172**: 5986–5993.
82 Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF *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.
83 Andolfi G, Fousteri G, Rossetti M, Magnani CF, Jofra T, Locafaro G *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.
84 Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA *et al.* Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *J Exp Med* 2007; **204**: 105–115.
85 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.
86 Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA *et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 2011; **332**: 974–977.
87 Darrasse-Jeze G, Deroubaix S, Mouquet H, Victora GD, Eisenreich T, Yao KH *et al.* Feedback control of regulatory T cell homeostasis by dendritic cells in vivo. *J Exp Med* 2009; **206**: 1853–1862.
88 McCartney SA, Colonna M. Viral sensors: diversity in pathogen recognition. *Immunol Rev* 2009; **227**: 87–94.
89 Novak N, Mete N, Bussmann C, Maintz L, Bieber T, Akdis M *et al.* Early suppression of basophil activation during allergen-specific immunotherapy by histamine receptor 2. *J Allergy Clin Immunol* 2012; **130**: 1153–1158 e2.
90 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.
91 Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z *et al.* CTLA-4 control over Foxp3 + regulatory T cell function. *Science* 2008; **322**: 271–275.
92 Kashyap M, Thornton AM, Norton SK, Barnstein B, Macey M, Brenzovich J *et al.* Cutting edge: CD4 T cell-mast cell interactions alter IgE receptor expression and signaling. *J Immunol* 2008; **180**: 2039–2043.
93 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.
94 Gri G, Piconese S, Frossi B, Manfroi V, Merluzzi S, Tripodo C *et al.* CD4 + CD25 + regulatory T cells suppress mast cell degranulation and allergic responses through OX40-OX40L interaction. *Immunity* 2008; **29**: 771–781.
95 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.
96 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.
97 Pacciani V, Gregori S, Chini L, Corrente S, Chianca M, Moschese V *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.
98 Grant AV, Araujo MI, Ponte EV, Oliveira RR, Cruz AA, Barnes KC *et al.* Polymorphisms in IL10 are associated with total Immunoglobulin E levels and Schistosoma mansoni infection intensity in a Brazilian population. *Genes Immun* 2011; **12**: 46–50.
99 Nagalakshmi ML, Murphy E, McClanahan T, de Waal Malefyt R. Expression patterns of IL-10 ligand and receptor gene families provide leads for biological characterization. *Int Immunopharmacol* 2004; **4**: 577–592.
100 Grimbaldeston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nat Immunol* 2007; **8**: 1095–1104.
101 Vieira P, de Waal-Malefyt R, Dang MN, Johnson KE, Kastelein R, Fiorentino DF *et al.* Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. *Proc Natl Acad Sci USA* 1991; **88**: 1172–1176.
102 Zdanov A, Schalk-Hihi C, Wlodawer A. Crystal structure of human interleukin-10 at 1.6 A resolution and a model of a complex with its soluble receptor. *Protein Sci* 1996; **5**: 1955–1962.
103 Liu Y, Wei SH-Y, Ho AS-Y, De Waal Malefyt R, Moore KW. Expression cloning and characterization of a human IL-10 receptor. *J Immunol* 1994; **152**: 1821–1829.
104 Tan JC, Braun S, Rong H, DiGiacomo R, Dolphin E, Baldwin S *et al.* Characterization of recombinant extracellular domain of human interleukin-10 receptor. *J Biol Chem* 1995; **270**: 12906–12911.
105 De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, De Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J. Exp. Med.* 1991; **174**: 1209–1220.
106 Timmann C, Fuchs S, Thoma C, Lepping B, Brattig NW, Sievertsen J *et al.* Promoter haplotypes of the interleukin-10 gene influence proliferation of peripheral blood cells in response to helminth antigen. *Genes Immun* 2004; **5**: 256–260.
107 Taylor A, Akdis M, Joss A, Akkoc T, Wenig R, Colonna M *et al.* IL-10 inhibits CD28 and ICOS costimulations of T cells via src homology 2 domain-containing protein tyrosine phosphatase 1. *J Allergy Clin Immunol* 2007; **120**: 76–83.
108 Oral HB, Kotenko SV, Yilmaz M, Mani O, Zumkehr J, Blaser K *et al.* Regulation of T cells and cytokines by the interleukin-10 (IL-10)-family cytokines IL-19, IL-20, IL-22, IL-24 and IL-26. *Eur J Immunol* 2006; **36**: 380–388.
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 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.
111 Suarez-Fueyo A, Ramos T, Galan A, Jimeno L, Wurtzen PA, Marin A *et al.* Grass tablet sublingual immunotherapy downregulates the TH2 cytokine response followed by regulatory T-cell generation. *J Allergy Clin Immunol* 2014; **133**: 130–138 e1-2.
112 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.
113 Noh J, Lee JH, Noh G, Bang SY, Kim HS, Choi WS *et al.* Characterisation of allergen-specific responses of IL-10-producing regulatory B cells (Br1) in Cow Milk Allergy. *Cell Immunol* 2010; **264**: 143–149.
114 Rousset F, Garcia E, Banchereau J. Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. *J Exp Med* 1991; **173**: 705–710.
115 Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; **75**: 263–274.
116 Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Immunol* 1998; **16**: 137–161.
117 Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by TGF-beta. *J Biochem* 2010; **147**: 781–792.
118 Li MO, Flavell RA. Contextual regulation of inflammation: a duet by transforming growth factor-beta and interleukin-10. *Immunity* 2008; **28**: 468–476.
119 Alansari A, Hajeer AH, Bayat A, Eyre S, Carthy D, Ollier WE. Two novel polymorphisms in the human transforming growth factor beta 2 gene. *Genes Immun* 2001; **2**: 295–296.
120 Ozdemir C, Akdis M, Akdis CA. T regulatory cells and their counterparts: masters of immune regulation. *Clin Exp Allergy* 2009; **39**: 626–639.
121 Wakefield LM, Hill CS. Beyond TGFbeta: roles of other TGFbeta superfamily members in cancer. *Nat Rev Cancer* 2013; **13**: 328–341.
122 Delisle JS, Giroux M, Boucher G, Landry JR, Hardy MP, Lemieux S *et al.* The TGF-beta-Smad3 pathway inhibits CD28-dependent cell growth and proliferation of CD4 T cells. *Genes Immun* 2013; **14**: 115–126.
123 Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* 2002; **195**: 1499–1505.

124 Gorelik L, Fields PE, Flavell RA. Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. *J Immunol* 2000; **165**: 4773–4777.
125 Heath VL, Kurata H, Lee HJ, Arai N, O'Garra A. Checkpoints in the regulation of T helper 1 responses. *Curr Top Microbiol Immunol* 2002; **266**: 23–39.
126 Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012; **336**: 1268–1273.
127 Moingeon P, Mascarell L. Induction of tolerance via the sublingual route: mechanisms and applications. *Clin Dev Immunol* 2012; **2012**: 623474.
128 Scadding GW, Shamji MH, Jacobson MR, Lee DI, Wilson D, Lima MT *et al.* Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. *Clin Exp Allergy* 2010; **40**: 598–606.
129 Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y *et al.* A functionally specialized population of mucosal CD103 + DCs induces Foxp3 + regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007; **204**: 1757–1764.
130 Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS *et al.* Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003; **112**: 1029–1036.
131 Coutts A, Chen G, Stephens N, Hirst S, Douglas D, Eichholtz T *et al.* Release of biologically active TGF-beta from airway smooth muscle cells induces autocrine synthesis of collagen. *Am J Physiol Lung Cell Mol Physiol* 2001; **280**: L999–1008.
132 Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007; **117**: 524–529.
133 Le AV, Cho JY, Miller M, McElwain S, Golgotiu K, Broide DH. Inhibition of allergen-induced airway remodeling in Smad 3-deficient mice. *J Immunol* 2007; **178**: 7310–7316.
134 McMillan SJ, Xanthou G, Lloyd CM. Manipulation of allergen-induced airway remodeling by treatment with anti-TGF-{beta} antibody: effect on the Smad signaling pathway. *J Immunol* 2005; **174**: 5774–5780.
135 Boivin GP, Ormsby I, Jones-Carson J, O'Toole BA, Doetschman T. Germ-free and barrier-raised TGF beta 1-deficient mice have similar inflammatory lesions. *Transgenic Res* 1997; **6**: 197–202.
136 Diebold RJ, Eis MJ, Yin M, Ormsby I, Boivin GP, Darrow BJ *et al.* Early-onset multifocal inflammation in the transforming growth factor beta 1-null mouse is lymphocyte mediated. *Proc Natl Acad Sci USA* 1995; **92**: 12215–12219.
137 Hansen G, McIntire JJ, Yeung VP, Berry G, Thorbecke GJ, Chen L *et al.* CD4(+) T helper cells engineered to produce latent TGF-beta1 reverse allergen-induced airway hyperreactivity and inflammation. *J Clin Invest* 2000; **105**: 61–70.
138 Joetham A, Takeda K, Taube C, Miyahara N, Matsubara S, Koya T *et al.* Naturally occurring lung CD4(+)CD25(+) T cell regulation of airway allergic responses depends on IL-10 induction of TGF-beta. *J Immunol* 2007; **178**: 1433–1442.
139 Ostroukhova M, Seguin-Devaux C, Oriss TB, Dixon-McCarthy B, Yang L, Ameredes BT *et al.* Tolerance induced by inhaled antigen involves CD4(+) T cells expressing membrane-bound TGF-beta and FOXP3. *J Clin Invest* 2004; **114**: 28–38.
140 Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST *et al.* Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. *Am J Respir Crit Care Med* 1997; **156**(2 Pt 1): 642–647.
141 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.
142 Konieczna P, Ferstl R, Ziegler M, Frei R, Nehrbass D, Lauener RP *et al.* Immunomodulation by Bifidobacterium infantis 35624 in the murine lamina propria requires retinoic acid-dependent and independent mechanisms. *PLoS ONE* 2013; **8**: e62617.
143 Seyerl M, Kirchberger S, Majdic O, Seipelt J, Jindra C, Schrauf C *et al.* Human rhinoviruses induce IL-35-producing Treg via induction of B7-H1 (CD274) and sialoadhesin (CD169) on DC. *Eur J Immunol* 2010; **40**: 321–329.
144 Tran DQ, Andersson J, Hardwick D, Bebris L, Illei GG, Shevach EM. Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/CD121b) on activated human FOXP3 + regulatory T cells allows for their purification from expansion cultures. *Blood* 2009; **113**: 5125–5133.
145 McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis. *J Exp Med* 2002; **195**: 221–231.
146 Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3 + natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. *Proc Natl Acad Sci USA* 2008; **105**: 10113–10118.
147 Yamaguchi T, Wing JB, Sakaguchi S. Two modes of immune suppression by Foxp3(+) regulatory T cells under inflammatory or non-inflammatory conditions. *Semin Immunol* 2011; **23**: 424–430.
148 Soper DM, Kasprowicz DJ, Ziegler SF. IL-2Rbeta links IL-2 R signaling with Foxp3 expression. *Eur J Immunol* 2007; **37**: 1817–1826.
149 Zhu J, Davidson TS, Wei G, Jankovic D, Cui K, Schones DE *et al.* Down-regulation of Gfi-1 expression by TGF-beta is important for differentiation of Th17 and CD103 + inducible regulatory T cells. *J Exp Med* 2009; **206**: 329–341.
150 Wang R, Kozhaya L, Mercer F, Khaitan A, Fujii H, Unutmaz D. Expression of GARP selectively identifies activated human FOXP3 + regulatory T cells. *Proc Natl Acad Sci USA* 2009; **106**: 13439–13444.
151 Battaglia A, Buzzonetti A, Monego G, Peri L, Ferrandina G, Fanfani F *et al.* Neuropilin-1 expression identifies a subset of regulatory T cells in human lymph nodes that is modulated by preoperative chemoradiation therapy in cervical cancer. *Immunology* 2008; **123**: 129–138.
152 Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol* 2002; **3**: 135–142.