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Regulatory T cells in Allergic Diseases

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Abstract

The pathogenesis of allergic diseases entails an ineffective tolerogenic immune response towards allergens. Regulatory T cells (T_{Reg}) cells play a key role in sustaining immune tolerance to allergens, yet mechanisms by which T_{Reg} cells fail to maintain tolerance in allergic diseases are not well understood. We review current concepts and established mechanisms regarding how T_{Reg} cells regulate different components of allergen-triggered immune responses to promote and maintain tolerance. We will also discuss more recent advances that emphasize the "dual" functionality of T_{Reg} cells in allergic diseases: how T_{Reg} cells are essential in promoting tolerance to allergens but also how a pro-allergic inflammatory environment can skew T_{Reg} cells towards a pathogenic phenotype that aggravates and perpetuates disease. These advances highlight opportunities for novel therapeutic strategies that aim to re-establish tolerance in chronic allergic diseases by promoting T_{Reg} cell and stability function.

Keywords

Asthma; Food Allergy; Regulatory T cells; FOXP3; Interleukin 4; T Helper cells type 2

Introduction

The increased prevalence in allergic diseases has become a major health problem in affluent and rapidly developing societies. Over the last 150 years, accelerated social and environmental changes augured by the industrial revolution, and which profoundly altered patterns of human activity, living arrangements, diet and infections, all came to influence the rise and severity of allergic disorders[1]. In the USA, food allergy prevalence reaches up to 8% among children and 5% of the adult population whereas 8.6% of children and 7.4% of adults are affected by asthma[2,3]. The dramatically increased burden of allergic diseases has exacted considerable morbidity on those suffering from those disorders and resulted in substantial financial costs incurred by affected individuals and their healthcare systems. While therapies for allergic diseases have improved over the years with the introduction of agents aimed at

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combating the inflammatory processes as well as providing symptomatic relief, those therapies have remained for the most non-curative.

Allergic diseases arise in response to normally innocuous environmental agents, including aeroallergens and foods. They involve the participation of components of the innate and adaptive immune responses, such innate lymphoid cells type 2 (ILC2) mast cells, basophils, eosinophils, as well as activated T helper type 2 (Th2) cells and B cells switched to the production of immunoglobulin type E (IgE)[4,5]. Immune regulatory mechanisms normally operative to maintain tolerance to allergens breakdown for reasons that still remain obscure. The dramatic increase in prevalence of allergic disease during the past decades indicates a strong influence of environmental factors acting on genetically susceptible hosts to promote disease[6,7]. Emerging studies emphasize the interaction of environmental factors, including diet, antibiotic usage and others, with components of the immune system, affecting their function and modify the outcome of the immune response[8]. They also support the idea that the commensal bacteria play a central role in the regulation of allergic diseases and that they dynamically interact with host genetic background and environmental factors to promote or disrupt oral tolerance[9-11]. Genetic and immunological evidence also reinforce the idea of a pivotal role for regulatory T (T_{Reg}) cells in promoting tolerance to allergens and preventing allergic disorder[12-16]. In this review, we will discuss recent advances demonstrating the "dual potential" of T_{Reg} cells in allergic diseases; how T_{Reg} cells are beneficial in promoting tolerance but also how the pro-allergic environment can derange the T_{Reg} cell response to aggravate and perpetuates disease.

Natural and induced Foxp3$^+$ T_{Reg} cells

T_{Reg} cells were initially described as a population of CD4$^+$ T cells expressing the IL-2 receptor α chain (CD25) and CD45RB, able to protect mice from developing autoimmune diseases[17,18]. Afterwards, the establishment of T_{Reg} cells as a distinct CD4$^+$ T cells sub-population was empowered by the identification of the Forkhead winged helix transcription factor FOXP3 (Forkhead Box 3) as a specific T_{Reg} cell maker essential to their function[19,20]. FOXP3 is required for the differentiation of T_{Reg} cells, evidenced by the generation of aberrant T_{Reg} cells lacking in regulatory function in mice with loss-of-function mutations in *Foxp3* [21,22]. FOXP3 deficiency results in the development of a multi-organ lymphoproliferative autoimmune disease, referred to as Immune Dysregulation, Polyendocrinopathy, Enteropathy X-linked (IPEX) in human subjects and *scurfy* in mice[12,23-26]. Expression of FOXP3 into human and murine conventional CD4$^+$ Foxp3$^-$ non-T_{Reg} cells by means of retroviral gene transfer, converts naïve T cells into T_{Reg} cells[19]. It is now well established that T_{Reg} cells enforce tolerance to both self-antigens and to the "extended-self", the latter encompassing commensal flora and innocuous environmental antigens such as allergens [Reviewed in [27-30]].

A major population of T_{Reg} cells arises in the thymus and is known as CD4$^+$ FOXP3$^+$ "natural" T_{Reg} (nT_{Reg}, also known as thymus-derived or tT_{Reg}) cells, which chiefly mediates tolerance to self-antigens[31] (Fig 1). A second population of CD4$^+$ FOXP3$^+$ T_{Reg} cells arises extra-thymically in peripheral lymphoid tissues from a pool of naïve conventional CD4$^+$ FOXP3$^-$ T cells (T_{conv}) after exposure to antigens and in the presence of TGF-β [reviewed

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in[32]]. These induced T_{Reg} (iT_{Reg}, also known as peripheral or pT_{Reg}) cells are particularly enriched in the gastro-intestinal tract and in the lungs during chronic inflammation, with specificities directed against microbial antigens or environmental allergens[33-35] (Fig 1). The generation of iT_{Reg} cells at the intestinal mucosa is facilitated by the large abundance of TGF-β and retinoic acid (RA), a vitamin A metabolite, both secreted by the $CD103^+$ $CD11c^+$ dendritic cells (DCs)[36-38]. In lung tissues, resident macrophages ($CD45^+$ $CD11c^+$ MHC class-II^{low} $F4/80^+$) constitutively expressing TGF-β and RA are the main subset of cells driving iT_{Reg} cells induction from naïve $CD4^+$ T_{conv} cells[39] (Fig 1). Both $FOXP3^+$ nT_{Reg} and iT_{Reg} cells subsets play a key function in the maintenance of peripheral tolerance by suppressing reactivity to self-antigens and by containing the amplitude of immune responses to foreign antigens.

Because of their different origins, the TCR repertoires of thymic nT_{Reg} and peripheral iT_{Reg} cells are largely non-overlapping and biased towards self and non-self antigens, respectively [40]. However, iT_{Reg} cells are known to be less stable than nT_{Reg} cells and under inflammatory conditions can lose FOXP3 expression (ex-T_{Reg}) and produce cytokines such as IFN-γ and IL-17[41,42]. This lack of stability can be explained by the methylation status of the conserved non-coding region 2 (CNS2) of the *Foxp3* gene. The *FOXP3* CNS2 locus, which acts to maintain T_{Reg} cell lineage identity under inflammatory conditions, is known to be stably hypomethylated in nT_{Reg} whereas it is incompletely demethylated in iT_{Reg} cells[43-46] .One difficulty for the functional and genetic study of iT_{Reg} and nT_{Reg} cells is the lack of unique and specific markers allowing the distinction between those two populations and their identification *in vivo*. nT_{Reg} and iT_{Reg} cells express similar levels of shared T_{Reg} cell markers such FOXP3, CTLA-4, GITR, ICOS, CD103 and CD25. However, many of those markers are also up-regulated by activated $CD4^+$ T cells under inflammatory conditions and their level of expression do not allow the distinction between nT_{Reg} and iT_{Reg} cells[47]. The use of Helios and Neuropilin-1 (Nrp-1) has been proposed to specifically discriminate nT_{Reg} from iT_{Reg} cells, since the expression of those markers is higher in nT_{Reg} compared to iT_{Reg} cells[48-50]. While Nrp1 may be upregulated in the context of an inflammatory environment, $Helios^{low}$ expression has been extensively used as an *in vivo* marker that distinguishes iT_{Reg} from nT_{Reg} cells[50-52].

In addition to $FOXP3^+$ T_{Reg} cells, $CD4^+$ type 1 T regulatory cells (Tr1) represent another subset of T_{Reg} cells defined by the expression of IL-10 and the surface marker LAG-3 and CD49b in the face of absent FOXP3 and CD25 expression[53]. The relationship between $FOXP3^+$ T_{Reg} cells and Tr1 cells remains obscure, with both subsets employing common effector pathways including IL-10, TGF-β and CTLA-4[54]. Unlike $FOXP3^+$ T_{Reg} cells, Tr1 cells are not uniquely defined by one transcription factor such as FOXP3, but express a number of transcription factors common to other T cell populations including c-MAF, Ahr (Aryl hydrocarbon receptor), and others[54] . Many studies that have referred to IL-10 producing T_{Reg} cells as Tr1 cells did not discriminate between the two populations by appropriate staining for differentiating markers including FOXP3. In this review, we will focus on $FOXP3^+$ T_{Reg} cells as their role in the regulation of allergic disease is far more well defined.

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Mechanisms of T_{Reg} cells suppression

The suppressive functions of T_{Reg} cells are essential to control autoimmunity, allergic and inflammatory reactions and responses to infectious agents and tumors. $Foxp3^+$ nT_{Reg} and iT_{Reg} cells are characterized by a non-overlapping TCR repertoire, resulting in a division of labour where nT_{Reg} and iT_{Reg} cells regulate immune responses targeting "self" antigens and "non-self" infectious or innocuous agents respectively[40,55]. T_{Reg} cells suppressive function are mediated by multiple mechanisms that involve either the release of inhibitory cytokines (IL-10, TGF-β and IL-35)[56-59] and cytolytic molecules (granzymes A and B)[60-62], or the down-modulation of antigen presenting cells (Cytotoxic T Lymphocyte Antigen 4 (CTLA-4) and Lymphocyte-activation Gene 3 (LAG-3)[63,64], deprivation of trophic cytokines (IL-2 via CD25)[65] and modulation metabolic pathways (CD73 and CD39)[66] (Fig 2). Expression of select transcription factors and receptors enables T_{Reg} cells suppressive functions under inflammatory conditions. GATA-3 expression by T_{Reg} cells is triggered by TCR activation and is required to maintain FOXP3 expression and to allow accumulation of T_{Reg} cells at inflamed sites[67]. More recently, it has been demonstrated that Helios expression by T_{Reg} cells is key to support their suppressive functions and phenotypic stability during inflammation[68]. T_{Reg} cells functions can also be regulated by endogenous danger signals, or alarmins, released by epithelial cells at the mucosal barrier. Colonic T_{Reg} cells express the IL-33 receptor (ST2), allowing them to respond to epithelial cell IL-33 production resulting from tissue damage by amplifying their regulatory functions and restraining intestinal inflammation[69].

T_{Reg} cell role in tissue repair

In addition to their immunosuppressive functions and their capacities to restrict the intensity of immune responses, T_{Reg} cells can also control non-immunological process such as tissue repair resulting from extensive inflammation. Studies have identified the presence of T_{Reg} cells in a wide variety of non-lymphoid organs such as the skin, the intestinal mucosa, the lungs and visceral adipose tissues[70]. In mice, T_{Reg} cells accumulate and remain in skeletal muscles after acute injury and their depletion results in increased muscle damage[71]. T_{Reg} cell production of Amphiregulin (Areg), an epidermal growth factor family member known to promote healing and tissue regeneration, in injured lung and muscle tissues appears to be prevent the tissue damages[71,72].

T_{Reg} cells and allergic diseases

Allergic diseases reflect a failure to develop tolerance toward a specific allergen, provoking the emergence of an allergen-specific $CD4^+$ T helper type 2 (Th2) cell response, the generation of allergen-specific immunoglobulin E (IgE) and the recruitment of effector cells to the GI tract or lung tissues[4,73]. In humans, loss-of-function mutations affecting *FOXP3* result in the development of IPEX, characterized not only by autoimmunity but also severe allergic inflammation including atopic dermatitis, food allergy, asthma, elevated serum IgE levels and peripheral eosinophilia[12,13,74]. *Foxp3* mutant mice spontaneously exhibit allergic airways inflammation, atopic-dermatitis skin-like disease and elevated serum IgE levels independently of their genetic background[75]. By using another genetic murine model, the 'DEREG' mice which express the diphtheria toxin (DT) receptor under the control of the

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Foxp3 gene, Hadis *et al.* have demonstrated that T_{Reg} cells depletion in OVA-tolerant DEREG mice was sufficient to break oral tolerance[76]. Furthermore, *in vivo* depletion of $CD4^+$ $CD25^+$ T cells in peanut sensitized mice by means of anti-CD25 monoclonal antibody results in impaired oral tolerance development and leads to an heightened allergic[77]. The central role of T_{Reg} cells in oral tolerance development to food allergen has been confirmed in human studies where children who had outgrown milk allergy exhibited higher frequencies of milk protein-specific $CD4^+$ $CD25^+$ T_{Reg} cells and where the emergence of allergen-specific T_{Reg} cells is highly correlated with a favourable disease outcome[78,79].

Among the T_{Reg} cell populations, $Foxp3^+$ iT_{Reg} cells play an essential role in maintaining tolerance at environmental interfaces, including small and large intestinal and the lung respiratory mucosa[32]. Allergen-specific iT_{Reg} cells are involved in controlling inflammation severity and the IL-4 Th2 cell immune response[35]. Accordingly, the development of allergic reactions may result from decreased induction and/or impaired function of allergen-specific iT_{Reg} cells in genetically allergy-prone subjects. This proposition is supported by a study that took advantage of mice lacking *CNS1*, an intronic *Foxp3* enhancer which contains binding sites for multiple transcription factors such as NFAT and Smad3 and is required for the differentiation of iT_{Reg} cells *in vivo*[80,81]. Despite decreased iT_{Reg} cells expansion, *CNS1*-deficient mice do not develop a fatal autoimmune lymphoproliferative disease. Nonetheless, with time *CNS1*-deficient mice develop a pro-allergic phenotype associated with Th2 cell-induced pathologies at mucosal surfaces[81]. We have recently demonstrated using mice with a gain of function mutation in the IL-4Rα chain (*Il4raF709* mice) that heightens susceptibility to oral sensitization that food allergy development is associated with impaired generation and function of allergen-specific T_{Reg} cells[82]. In peanut allergic patients undergoing successful oral immunotherapy (OIT) leading to peanut tolerance induction demonstrated increased numbers of circulating allergen-specific iT_{Reg} cells with heighten suppressive capacities and augmented stability, as evidenced by increased demethylation of CpG islands within the *FOXP3* gene[83]. Evidences also point towards a reduced frequency of T_{Reg} cells associated with allergic asthma[84,85]. Compared to healthy controls, frequencies of pulmonary $CD4^+$ $CD25^{high}$ T_{Reg} cells in the bronchoalveolar lavage fluid (BALF) were significantly decreased in untreated asthmatic children[86]. Four weeks of inhaled corticosteroid treatment were sufficient to restore the T_{Reg} cells compartment in the blood and the BALF[86].

A specific requirement for the cytokines IL-10 and TGF-β1 expressed by T_{Reg} cells in the control of allergic responses has emerged. Kearley *et al.* have demonstrated that adoptively transferred allergen-specific T_{Reg} cell suppressive functions during allergic airway inflammation rely on their capacity to induce IL-10 production by $CD4^+$ T cells[87]. However, subsequent studies by Rubstov *et al.* employed a genetic approach to show that T_{Reg} cell-specific deletion of *Il10* promoted allergic airway inflammation; thereby suggesting that T_{Reg} cell-derived IL-10 plays a “privileged”, non-redundant role in the induction of immune tolerance in allergic airway inflammation[88]. IL-10 has immunosuppressive functions and can modulate the activity of key cell subset involved in the allergic reaction such as mast cells[82,89], Th2 T cells[90], eosinophils and DCs[91]. The Similar to IL-10, TGF-β1 specifically expressed by T_{Reg} cells also appears to play a privileged role in the regulation of allergic responses. In mice, T_{Reg} cell-specific deletion of *Tgfb1* heightens susceptibility to food

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allergy (M. NR and T. A. C; unpublished data). The respective role of T_{Reg} cell IL-10 and TGF-β1 in the regulation of different allergic responses remains to be fully mapped.

In addition to the defect in allergen-specific iT_{Reg} cell induction, aberrations of the T_{Reg} cell compartment during allergic disease can also be attributed to a decreased or failure of their suppressive functions. *In vitro* studies with peripheral T_{Reg} ($CD4^+CD25^+$) cells isolated from the blood of atopic and non-atopic patients demonstrated that atopic T_{Reg} cells can be distinguished from non-atopic T_{Reg} cells by decreased capacities to suppress allergen-driven proliferation of effector $CD4^+$ T cells as well as their Th2 cell cytokines secretion[92].

T_{Reg} cells regulation of the innate immune response in allergic diseases

T_{Reg} cells can exert their immunosuppressive functions on a broad variety of different cell types, including innate immune cells. Accumulating evidences demonstrate that T_{Reg} cells control the immediate hypersensitivity response by acting directly on mast cells and blocking their degranulation (Fig 3)[82,89,93]. After allergen-sensitization, triggering of mucosal mast cells via the high affinity receptor for IgE (FcεRI) will induce the release of preformed mediators and elicit an IgE-mediated hypersensitity response[94]. One mechanism by which T_{Reg} cells modulate IgE-mediated mucosal mast cells degranulation and decrease mast cells effector mediators release is through the OX40-OX40L pathway[93]. Direct cell-to-cell contact between OX40 expressed on T_{Reg} cells and OX40L on mast cells will lead to increased intracellular levels of cyclic adenosine monophosphate (cAMP) and result in a blockage of extracellular $Ca2^+$ influx and mast cell mediator release inhibition[93].

IL-4 production by mast cells is critical in food allergic reaction. IgE interaction with the FcεRI expressed on mast cells act as amplifier of the Th2 cell and IgE responses during allergic sensitization[89]. Importantly, dysregulated IgE mast cell activation and their subsequent IL-4 production profoundly inhibits allergen iT_{Reg} cells generation during allergic processes[82]. This IL-4 inhibition of iT_{Reg} cells generation is mediated through increased intracellular levels of GATA-3 which acts as a FOXP3 inhibitor in early T cell differentiation [95]. In contrast, food allergy prone mice that lack the α chain of the high affinity IgE receptor FcεRI (*Il4raF709* $Fcer1a^{-/-}$) were protected from anaphylaxis[82]. This protection was reflected in decreased mast cell expansion and degranulation and inhibition of the conventional $CD4^+$ cell response, consistent with the key function of IgE/FcεRI axis in mediating not only anaphylaxis but also driving the food allergen-associated cell response [82,89]. Moreover, FcεRI deficiency completely corrected the impaired allergen-specific iTreg cell generation[82]. Similar results were obtained by targeting IgE production ($IgE^{-/-}$ mice or anti-IgE treatment) or by using mast cells ablation genetic murine models [89]. The key role of T_{Reg} cells in inhibiting mast cell degranulation and their Th2 cell cytokine secretion is thus critical to the prevention of food allergy (Fig 3).

Innate lymphoid cells type 2 (ILC2), a population of mucosal innate cells, are simultaneously characterized by a lack of antigen specificity (absence of T and B cell receptor) and lymphoid traits, as demonstrated by a shared development origin and phenotypic traits with T cells[96]. ILC2 produce large amounts of Th2 cell cytokines and are linked to allergic disorders such as asthma, chronic rhinosinusitis and atopic dermatitis[97-100]. In mice, ILC2 can be identified based on the expression of CD25 (IL-2Rα),

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IL-33R (ST2) and CD127 (IL-7Rα)[101] . ILC2 are located in the blood and various organs such as the spleen, the gastrointestinal tract, the liver, the lungs and the lymph nodes[102,103]. The transcription factor GATA-3 is required for ILC2 differentiation, their stability and Th2 cell cytokines production[104,105]. Halim *et al.* demonstrated that ILC2 are required for the development of protease allergen papain-induced airway inflammation as ILC2 deficient mice (RORα$^{-/-}$) were incapable of mounting an effective Th2 cell immune response and had reduced type 2 lung inflammation[102]. The critical role of ILC2 in triggering Th2 cell adaptive immune responses involves their production of IL-13, which promotes migration of DC to the draining lymph nodes and enhance the conversion of naïve CD4$^+$ T cells into Th2 cells[102]

The role of ILC2 in food allergy has been less documented. It appears that IL-13 production by ILC2 enhances allergic mucosal inflammation and promotes IgE-mediated experimental food allergy[106]. Food allergy development is associated with defective allergen-specific T_{Reg} cells induction, consequently resulting in disease promotion[82]. We have recently demonstrated that increased IL-33 production at the intestinal mucosa during food allergy promotes ILC2 expansion, which further enhances the IgE-mediated food allergic response through their IL-4 production[107]. ILC2-derived IL-4 inhibits T_{Reg} cell response and promotes mast cells activation. Reciprocally, T_{Reg} cells block ILC2 expansion and suppress their IL-4 production[107](Fig 3). Together, these findings point to the disruption of T_{Reg} cell control of mast cells and ILC2 as a key mechanism in the pathogenesis of food allergy. At steady state, T_{Reg} cells control both mast cells and ILC2 by restricting their capacity to promote food allergy. Perturbation of this regulatory interaction will subsequently result in a dysregulated pro-allergic innate immune response skewing the immunological balance towards food allergy.

By processing and presenting antigens to naïve T cells, dendritic cells (DCs) are key initiators and master regulators of the allergen-specific immune response. T_{Reg} cells also directly act on DCs by down-modulating their surface expression of CD80/CD86 expression and subsequently blocking the generation of an allergen-specific Th2 cell immune response. T_{Reg} cell suppression of DCs appears to be mediated via CTLA-4, LAG-3 and Leukocyte Function-Associated antigen-1 (LFA-1)[63,64,108]. DCs, mostly plasmacytoid DCs (pDCs) have the capacities to prime and naïve T cells and induced their differentiation into IL-10 secreting T_{Reg} cells upon ICOS-ICOS ligand interactions[109,110].

T_{Reg} cells regulation of the adaptive immune response in allergic diseases

Allergic disorders are characterized by increased dysregulated and aberrant immune responses mediated by the Th2 cell cytokines IL-5, IL-4 and IL-13. T_{Reg} cells have also the capacities to regulate allergen-induced adaptive T and B cells responses through diverse mechanisms, either soluble or membrane-bound suppressive molecules (Fig 4). T_{Reg} cells express constitutively CTLA-4, a negative co-stimulatory molecule which is essential to their suppressive functions. Mice deficient for CTLA-4 exhibit a lethal multi-organ lymphoproliferative disease[111]. T_{Reg} cell-specific deletion of CTLA-4, by means of crossing *Foxp3-Cre* with *Ctla4*$^{fl/fl}$ mice, leads to an autoimmune disease characterized by an increased Th2 cell immune response as evidenced by elevated IL-4 production by CD4$^+$

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Foxp3$^-$ T_{conv} cells and increased serum IgE levels[91]. Ovalbumin (OVA)-specific nT_{Reg} cells are efficient in controlling *in vitro* Th2 cell immune responses and IL-4 production by inhibiting the polarization of naive CD4$^+$ T cells into Th2 cells via a GITR-dependent suppressive mechanisms[112]. Circulating CD4$^+$ CD25$^+$ T_{Reg} cells isolated form the blood of atopic human subjects were also less efficient *in vitro* than heathy controls CD4$^+$ CD25$^+$ T_{Reg} in controlling the Th2 cell cytokines production by effector CD4$^+$ T cells[92]. Furthermore, frequencies of allergen-specific T_{Reg} cells secreting IL-10 with suppressive functions were predominant in peripheral blood mononuclear cells (PBMCs) from healthy subject whereas frequencies of Th2 CD4$^+$ IL-4 secreting T cells frequencies were overrepresented in allergic subjects[90]. Effector CD4$^+$ IL4$^+$ Th2 cells and suppressive T_{Reg} IL-10$^+$ cells are present in both healthy and allergic patients, their ratio frequencies determine either tolerance induction or allergic response development[90].

Through their production of IgE, B cells are essential in the development of allergic immune responses. IgE responses are highly dependent of the immune response Th polarization; the Th2 cell cytokines IL-4 and IL-13 and CD40-CD40L cognate interactions are two signals required to class switching and IgE production by B cells[113]. *In vitro*, peripheral allergen-specific T_{Reg} cells from healthy subjects repress B cells IgE production by inducing IgG4 class switching[114]. T_{Reg} cells can also exercise their suppressive functions through the release of immunosuppressive cytokines such as IL-10, B cell suppression by T_{Reg} cells appears to be cell-to-cell contact mediated and probably occur via CTLA-4 and and TGF-β1[114].

Pathogenic T_{Reg} cell Th-reprogramming in allergic diseases

An important problem in chronic allergic diseases relates to the mechanisms that enable persistence of inflammation in the face of T_{Reg} cell responses[115]. In the course of regulating Th cell immune responses, T_{Reg} cells appropriate partial or “aborted” forms of the transcriptional programs of the target Th cells by expressing their master transcription factors, such as T-bet for Th1 cells and IRF-4 for Th2 cells, and co-opting their function [116,117]. Whereas under physiological conditions such partial Th cell programming remains restrained, such restraint is lost under the influence of chronic inflammation leading to pathogenic reprograming of T_{Reg} cells into Th cells[118,119]. In the context of allergic diseases, emerging evidence indicates that a sharply skewed inflammatory environment can overcome the allergen-specific T_{Reg} cell regulatory response and redirect those cells towards a pathogenic and pro-inflammatory phenotype (Fig 5 and 6). Recent studies from our laboratory have provided two examples of how allergen-specific T_{Reg} cells may acquire T effector (T_{Eff}) cell programs and in the process contribute to disease pathogenesis. In the first set of studies, a tyrosine (Y) to phenylalanine (F) mutation at position 709 of the murine IL-4 receptor alpha chain (IL-4Rα) inactivated the receptor’s immunotyrosine inhibitory motif and resulted in augmented activation by IL-4 and IL-13 of the downstream transcription factor STAT6[120,121]. This mutation, which models human polymorphisms that promote STAT6 activation via the IL-4Rα, imparted on mice heightened susceptibility to allergic diseases, including food allergy and allergic airway inflammation, and reproduced a Th2 cell-high disease “endotype” common in some subjects with those disorders. Importantly, allergen-specific T_{Reg} cells became reprogrammed to express a Th2 cell-like

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phenotype, including IL-4 production, all the while retaining their Foxp3 expression[82]. Whereas the Th2 cell master transcription factor GATA-3 normally plays a positive role in the accumulation of T_{Reg} cells at sites of inflammation and prevents their polarization into Th17 cells, its abnormally expression in T_{Reg} cells may contribute to their Th2 cell-like reprogramming under conditions of intense cell polarization[67,122,123]. The significance of this reprogramming was underlined by the observation that T_{Reg} cell-specific deletion of the *Il4/Il13* genes restrained the induction of food allergy and allergic airway inflammation in these mice[82]. Consistent with these results, human food allergic subjects manifest increase expression of Th2 cell cytokines in their circulating allergen-specific T_{Reg} cells, indicative of their acquisition of a "Th2-cell like" T_{Eff} phenotype [82]. Oral immunotherapy was associated with reversal of TH2 cell–like reprogramming of allergen-specific T_{reg} cells, which is coincident with their improved suppression function (data not shown).

A second example of allergen-specific T_{Reg} cell reprogramming came from studies on a human IL-4Rα allele that bears a glutamine (Q) to an arginine (R) substitution at position 576 (IL-4Rα-Q576R)[124]. This allele is associated with asthma severity, while introduction of the Q576R substitution into the murine IL-4Rα results in exaggerated allergic airway inflammation when the mice are sensitized then challenged in their airways with allergens[125-127] (Fig 6). Signalling via IL-4Rα-Q576R allele does not impact the activation by the IL-4R of its dedicated transcription factor STAT6, and Th2 cell responses promoted via the IL-4R are preserved. Nevertheless, in both humans and mice, the Q576R substitution acts to create a novel branch of signalling via IL-4Rα that activates microtubule-associated protein kinases (MAPK), leading to the induction by IL-4 of IL-6 production[128] (Fig 6). The newly produced IL-6 destabilizes newly formed allergen-specific T_{Reg} cells towards the Th17 cell lineage, thus giving rise to mixed Th2-Th17 cell responses in the context of allergic inflammation[128]. Inhibition of the capacity of allergen-specific T_{Reg} cells to differentiate into Th17 cells, whether by neutralization of IL-6 or by T_{Reg} cell-specific deletion of genes encoding IL-6 receptor alpha chain (*Il6ra*) or the Th17 cell master transcription factor RORγt, reversed the exaggerated allergic inflammation induced by IL-4Rα-Q576Rmice[128] (Fig 6).

Microbiome – T_{Reg} cell interactions in allergic diseases

Altered environmental exposures early in life may play a critical role in setting in motion the atopic diseases of childhood[129]. The "hygiene hypothesis" stipulates that increased allergic rates observed over the years result from reduced microbial exposures arising from lifestyle changes, such as family size reduction, use of antibiotics and improved hygiene[130]. The influence of the intestinal microbiome in tolerance induction and allergies development is becoming more appreciated. The intestinal colonization of neonates starts at birth from the mother's vaginal flora, as the microbiota composition of vaginally delivered infants is similar to the maternal vagina[131]. Infants born by cesarian section have a different microbiota composition, mostly derived from maternal skin, and are at increased risks of developing asthma and allergy[132]. The first months of life are a critical period for the intestinal flora to settle and stabilize as children exhibiting intestinal dysbiosis in this "time-window" are at increased risks of developing asthma[133]. Exposure to a farm environment and the associated subsequent large diversity of environmental microbial signals reduce the

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risk of developing allergies[133,134]. The importance of the microbial flora for allergic diseases development is further emphasized by the observation that Germ Free (GF) mice cannot be tolerized to oral antigens and develop a Th2 cell-biased immune response[135]. In humans, a polymorphism in the promoter of CD14, a high affinity receptor for bacterial lipopolysaccharide (LPS) and co-receptor of TLR-4, has been associated with the development of atopic disease[136]. Food allergic responses are also aggravated in TLR4$^{-/-}$ mice or WT mice treated with antibiotics and repopulation of commensal flora in antibiotics treated mice result in reduced allergen-specific IgE and Th2 cell cytokines responses[137]. These observations highlighted an important function of the intestinal microbial flora and microbial exposure in maintaining and shaping the immune response and inducing protection against the development of atopy[138,139].

By using *Il4raF709* food allergy-prone mice, we have demonstrated that food allergy is associated with the emergence of an altered intestinal microbial flora[10]. The microbiome of allergic *Il4raF709* mice exhibits decreased relative abundances of members from the Firmicutes phylum and increased abundance of bacteria belonging to the Proteobacteria phylum. Adoptive transfer of allergen-specific T_{Reg} cells prevented the development of food allergy in allergic-prone mice as well as the emergence of the food-allergy associated microbial dysbiosis[10]. Importantly, disease susceptibility can be transferred from allergic *Il4raF709* mice to GF mice via transplantation of commensal flora from allergic donors. Allergy susceptibility transfer was associated with increase allergen-specific IgE production and expansion of IL-4 secreting Th2 CD4$^+$ T cells in GF mice reconstituted with the allergic microbial flora[10].

The commensal flora can target different immune cell subsets belonging to either the innate or/and adaptive allergic effector responses (Fig 7). In the steady-state, the microbial flora promotes intestinal IgA production by a T_{Reg} cell intrinsic MyD88-dependent mechanism that enables the generation of iT_{Reg} cells in the gut and their differentiation into T follicular helper cells (T_{FH})[140]. Depletion of the commensal flora by antibiotic treatment[141] or the use of GF mice[11] is associated with the development of Th2 cell-type allergic responses and higher serum IgE levels. Th2 cell-type allergic immune responses were held in check by MyD88-dependent microbial sensing by B cells, which suppressed the IgE responses[141]. The commensal microbiota also influence the outcome of the allergic response modulating the ILC (Fig 7). Mono-colonization of GF mice with anaerobic bacteria belonging to the Clostridia class blocks and protects from oral allergen sensitization by inducing IL22$^+$ ROR-γt$^+$ ILC at the intestinal mucosa[11]. ILC2 are expanding in the course of allergic disorders and have a pathogenic function in further promoting disease through their Th2 cell type cytokines secretion[102,107]. However, how the intestinal or the upper airways microbial flora could affect, regulate or promote the ILC2 immune response needs still to be further investigated.

The microbiota promotes either a tolerant or a pro-Th2 cell type allergic immune response by interacting directly with immune cells and their TLRs or indirectly through the release of microbial products. Polysaccharide A (PSA), produced by the commensal bacterium *Bacteroides fragilis,* acts by a TLR2-dependent mechanisms to induce the conversion of CD4$^+$ T cells into functional iT_{Reg} cells with enhanced suppressive activities and increased

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IL-10 production[142,143]. In mice, Clostridial species are among the most abundant Gram-positive bacteria present at the intestinal mucosa. Colonization of GF mice with a mix of Clostridium isolated from either murine or human faeces resulted in a strong induction of iT_{Reg} cells in the colonic lamina propia of the reconstituted mice[144,145]. Through their production of IL-10, Clostridium-induced T_{Reg} cells also controlled the systemic IgE production by reducing *in vitro* the IL-4 production by splenic $CD4^+$ T cells[144].

Short chain fatty acids (SCFAs) produced by bacterial fermentation of dietary fibers act on T cells via a G protein coupled receptor (GPR43) and protect mice from intestinal inflammation by expanding the pool of the colonic T_{Reg} cells[146]. SCFAs also promote the generation of intestinal iT_{Reg} cells from naïve $CD4^+$ T cells by T-cell intrinsic epigenetic mechanisms[147,148]. Butyrate, a SCFA known as an histone deacetylase (HDAC) inhibitor, increases Foxp3 protein acetylation conferring thereby increased stability and enhanced suppressive function to *de novo* generated intestinal iT_{Reg} cells[148]. Accordingly, a high-fiber diet results in modulation of the intestinal flora composition characterized by increased Bacteroidetes and decreased Firmicutes abundances, resulting in increased circulating levels of SCFAs and allergic airways inflammation protection[149]. More recently, it has been reported that the microbiome and oral antigen promote the induction of iT_{Reg} cells expressing RORγt at the intestinal mucosal surfaces[150]. Specific ablation of RORγt in T_{Reg} cells resulted in increased frequencies of GATA-3^+ $Foxp3^+$ T_{Reg} cells and increased production of IL-4 and IL-13 by $CD4^+$ $Foxp3^-$ T_{conv} cells, leading to the conclusion that the microbiome control the Th2 cell immune response through the expansion of T_{Reg} RORγt^+ cells and the regulation of DC activation[150]. Whether “pro-allergic” or “pro-tolerant” bacterial species are linked to and directly affect the generation of those $GATA3^+$ or RORγt^+ T_{Reg} cells needs to be further investigated. Since atopic disease are associated with a defect in the generation of allergen-specific iT_{Reg} cells, it will be of interest to pursue investigation of these pathways to identify potential therapeutic targets to promote allergen-specific tolerance.

Conclusion

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

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Abbreviations

T_{Reg}	Regulatory T cells
BALF	Bronchoalveolar Lavage Fluid

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ILC	innate lymphoid cells
Th	T helper
IgE	Immunoglobulin type E
Areg	Amphiregulin
Ahr	Aryl hydrocarbon receptor
SCFA	Short Chain Fatty Acids
HDAC	Histone Deacetylase
T_{Eff}	T effector cells
T_{Conv}	Conventional T cells
DCs	dendritic cells
APCs	antigen presenting cells
CTLA-4	Cytotoxic T Lymphocyte Antigen 4
LAG3	Lymphocyte-Activation Gene 3
TCR	T cell receptor
Tr1	Type 1 T regulatory cells

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What do we know?

- T_{Reg} cells have a key role in promoting and maintaining tolerance to allergens by regulating both innate and adaptive allergen-triggered immune response.
- Allergic diseases are associated with a failure to develop tolerance towards a specific allergen leading to the emergence of a pathogenic Th2 immune response.
- A pro-allergic inflammatory environment may skew allergen-specific T_{Reg} cells towards a pathogenic phenotype that perpetuates and aggravates disease.
- Allergic responses are influenced by the commensal flora, acting in part via T_{Reg} cells.

What is still unknown?

- Mechanisms by which T_{Reg} cells fail to maintain tolerance in allergic diseases are not well understood and require further investigation.

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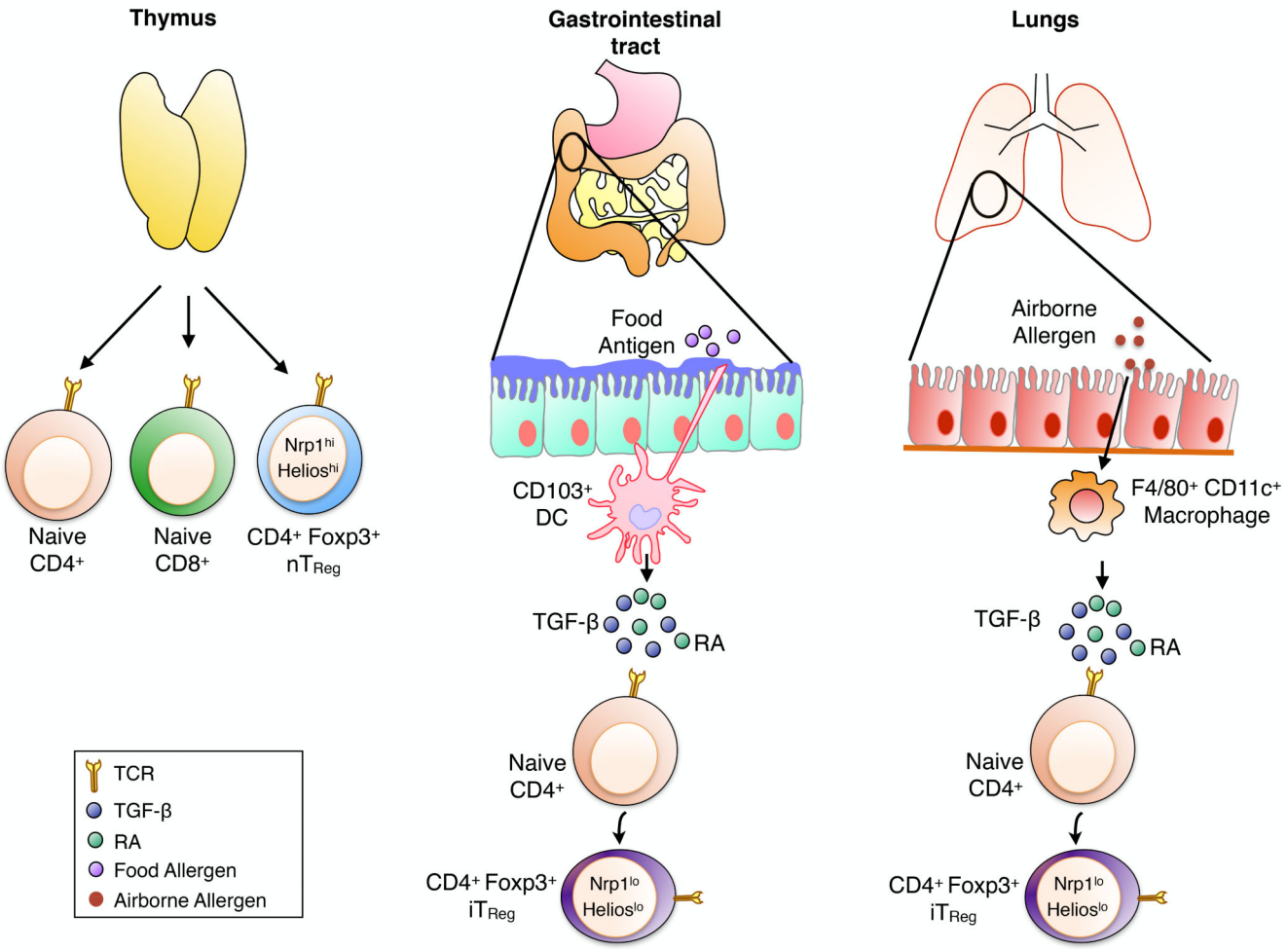

Fig 1. Natural and inuced Foxp3$^+$ T$_{Reg}$ cells subsets

The T$_{Reg}$ cell pool is composed by two different sub-populations, nT$_{Reg}$ and iT$_{Reg}$ cells, both expressing the transcription factor Foxp3 crucial for their development and regulatory functions. Foxp3$^+$ Nrp-1^{high} Helios^{high} nT$_{Reg}$ cells arise in the thymus and mediate tolerance to self- antigens. Foxp3$^+$ Nrp-1^{low} Helios^{low} iT$_{Reg}$ cells, which mediate tolerance to foreign antigens, are induced extra-thymically from naïve CD4$^+$ Foxp3$^-$ T$_{conv}$ cells in the presence of TCR stimulation, TGF-β and RA by either CD103$^+$ DCs at the intestinal mucosa or F4/80$^+$ CD11c$^+$ macrophages at the airways epithelial surfaces.

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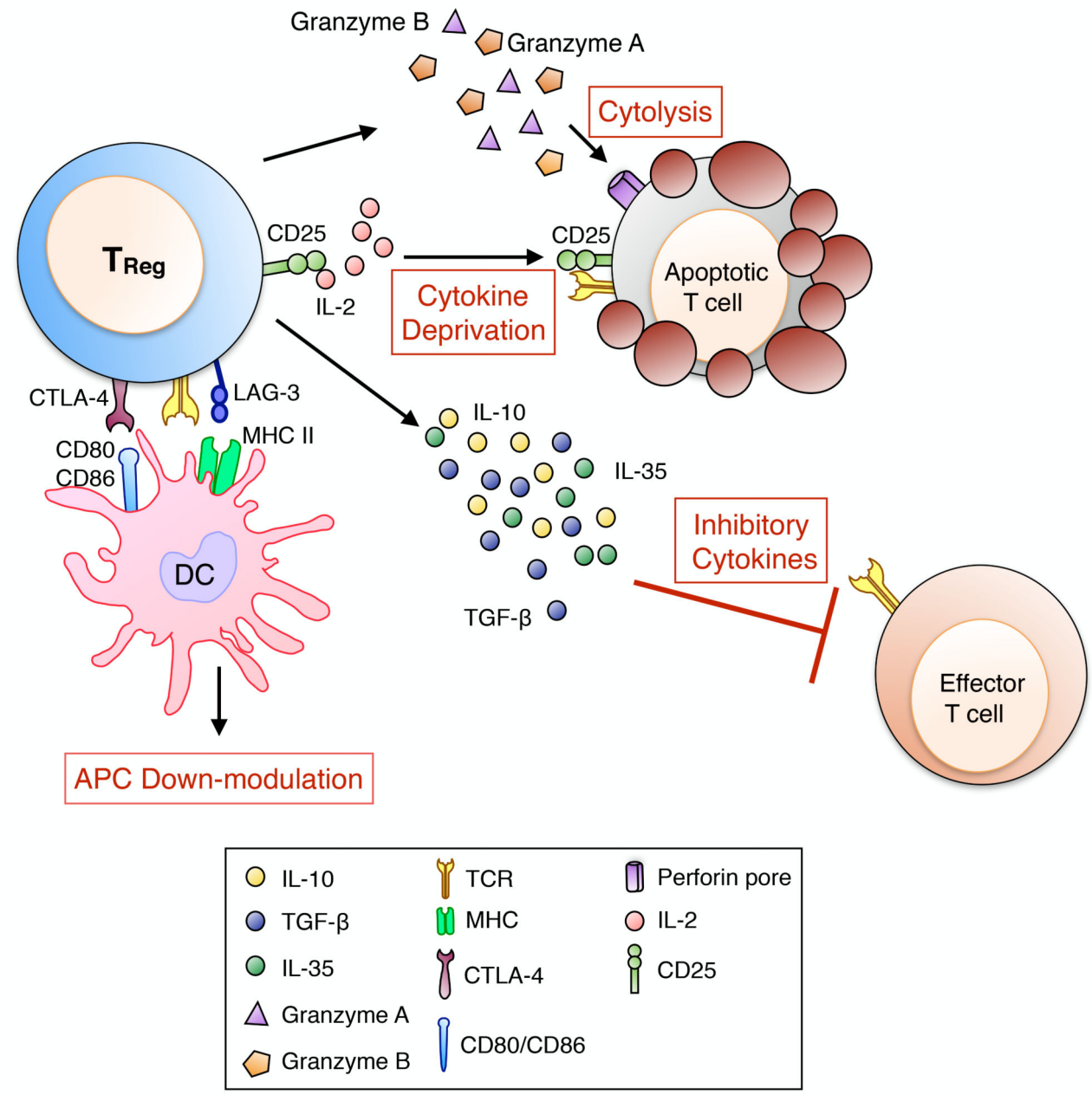

Fig 2. Mechanisms of Foxp3$^+$ T_{Reg} cell-mediated suppression

Foxp3$^+$ T_{Reg} cells mediate tolerance to allergens by diverse suppressive mechanisms. These include T cell cytolysis by a granzyme dependent mechanism, IL-2 deprivation, production of inhibitory cytokines including IL-10, IL-35 and TGF-β capable of blocking the proliferation of T_{eff} cells and down-modulation of antigen presenting cells (APCs) via LAG-3-MHC II and CTLA-4-CD80/CD86 interactions.

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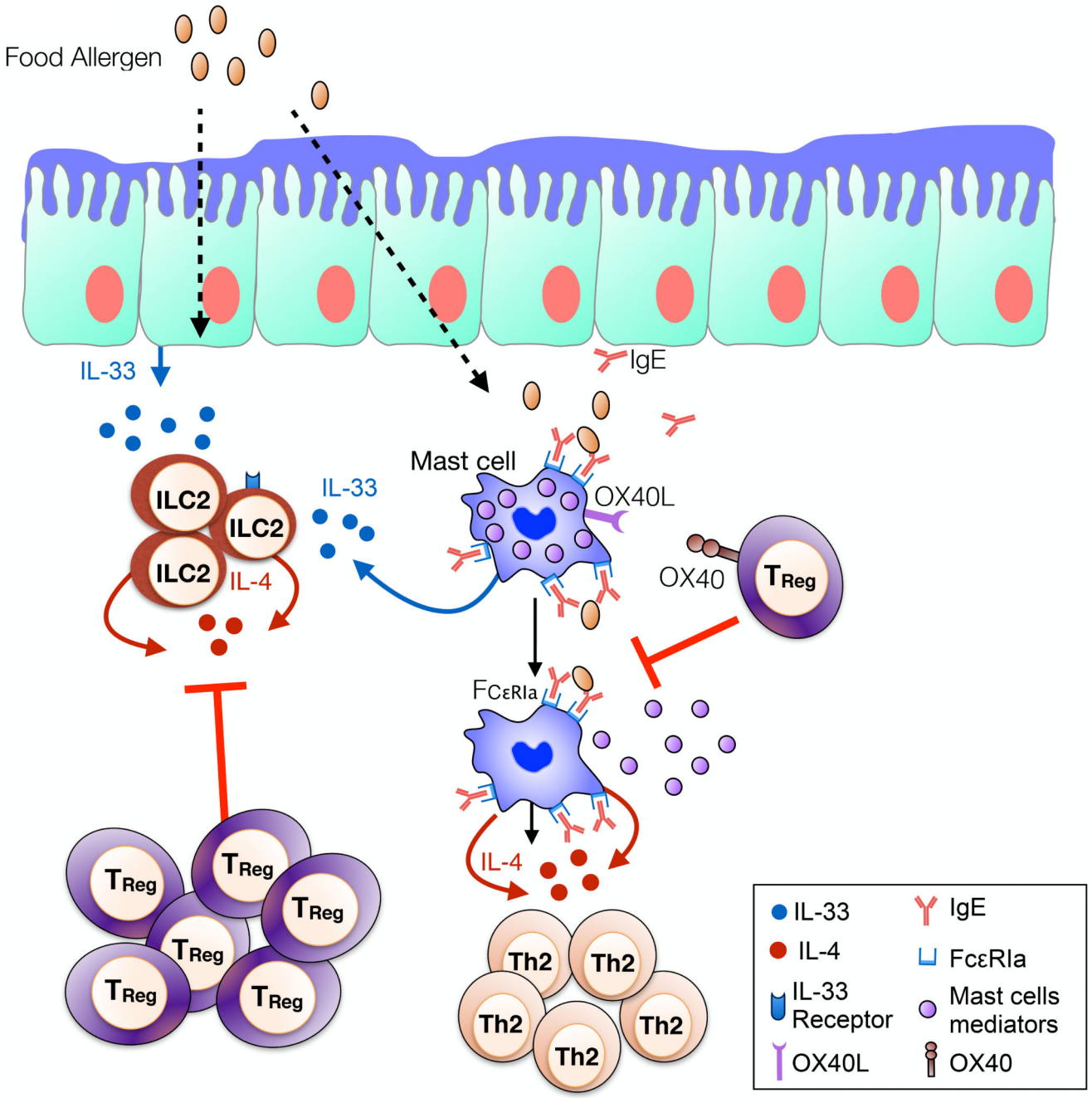

Fig 3. Regulation and suppression of allergic innate immune responses by T_{Reg} cells

T_{Reg} cells control innate immune cell subsets involved in promoting allergy. T_{Reg} cells block mast cell activation and the release of pre-formed anaphylactic mediators through OX40-OX40L mediated interactions. T_{Reg} cells also impede the IL-33-driven ILC2 expansion in the intestinal mucosa and their subsequent IL-4 production. Adapted version from the graphical abstract of ref. 107.

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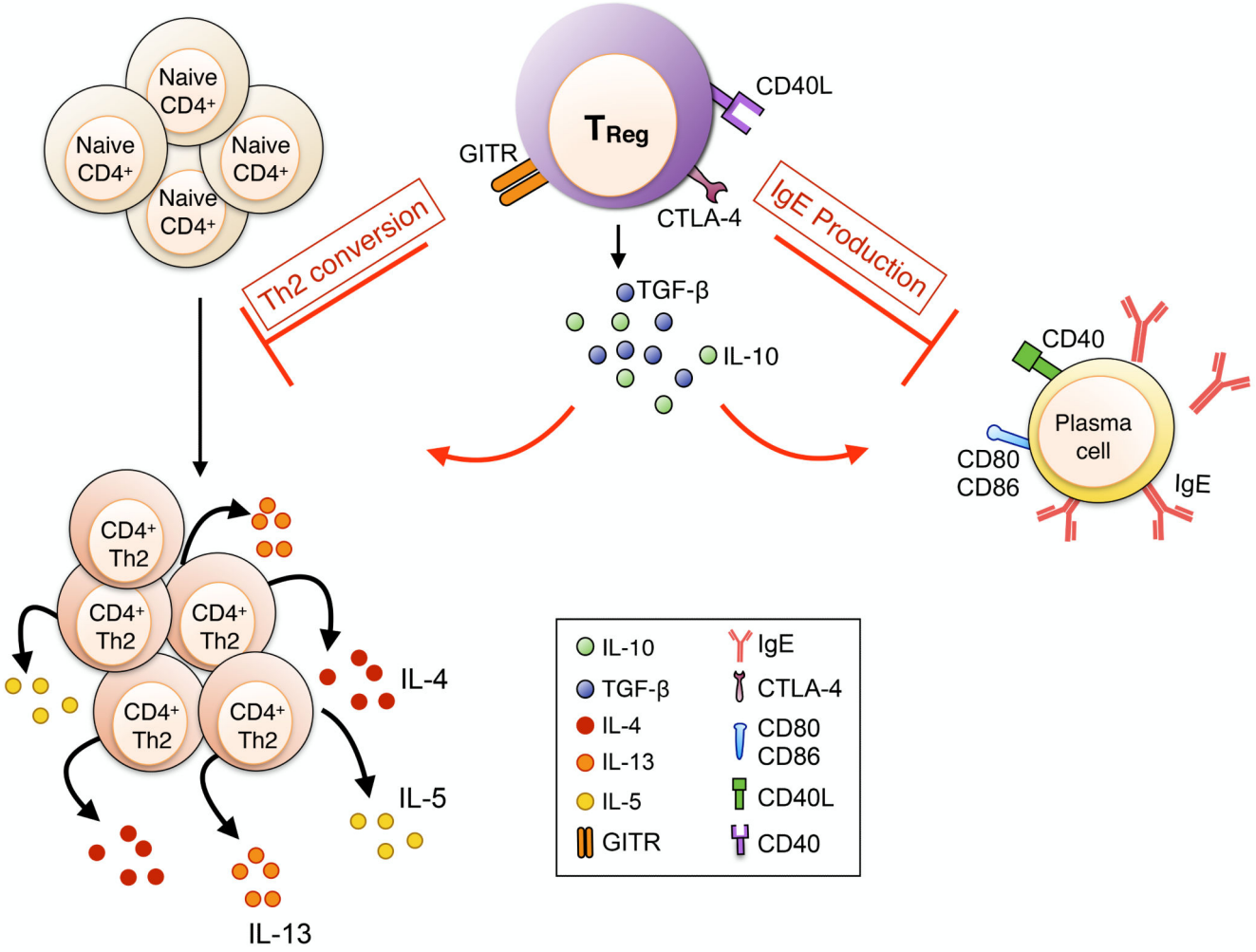

Fig 4. T_{Reg} cell mediated suppression of the adaptive allergic immune response

T_{Reg} cells regulate allergen-specific Th2 immune responses and B cell IgE production. GITR stimulation of T_{Reg} cells increase their suppressive functions, leading to their blockade of naïve $CD4^+$ T_{conv} cells conversion into allergen-specific Th2 T cells. T_{Reg} cells are also able to control B cells and block their IgE production by a direct CTLA-4 and cell contact-dependent mechanism and through the production of immunosuppressive cytokines such as IL-10.

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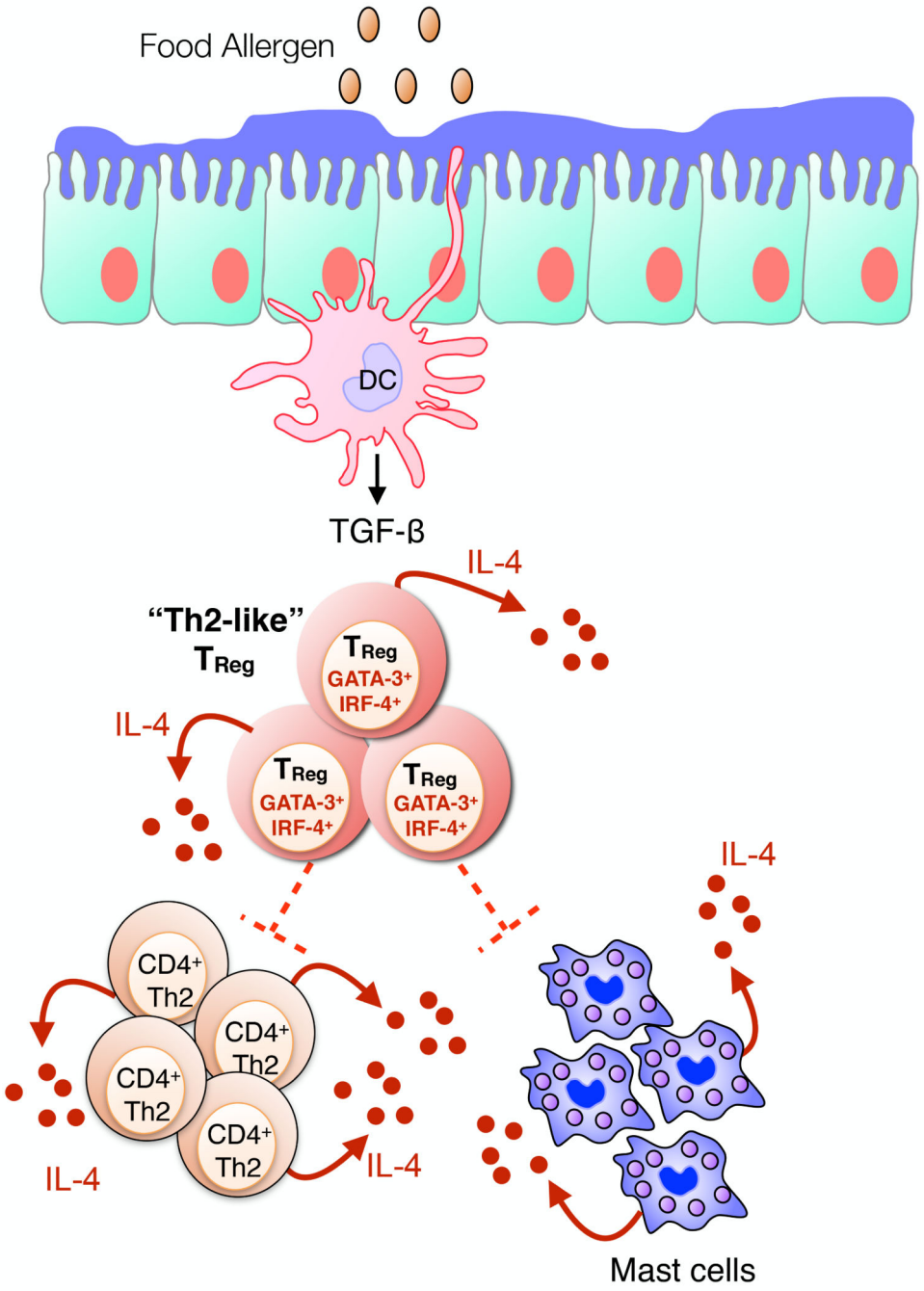

Fig 5. Pathogenic "Th2 cell-like" T_{Reg} cell reprogramming in food allergy

Food allergy is characterized by a decreased induction of allergen-specific iT_{Reg} cells at the intestinal mucosa. Induced allergen-specific T_{Reg} cells in food allergic subjects are prone to acquire a pathogenic skewed "Th2-like" phenotype resulting in increased GATA-3 expression and IL-4 secretion. "Th2 cell-like" iT_{Reg} cells are dysfunctional and lacking in suppressor function. They are not able to control the T_{eff} Th2 cell immune response and mast cells expansion, perpetuating in the process the allergic phenotype.

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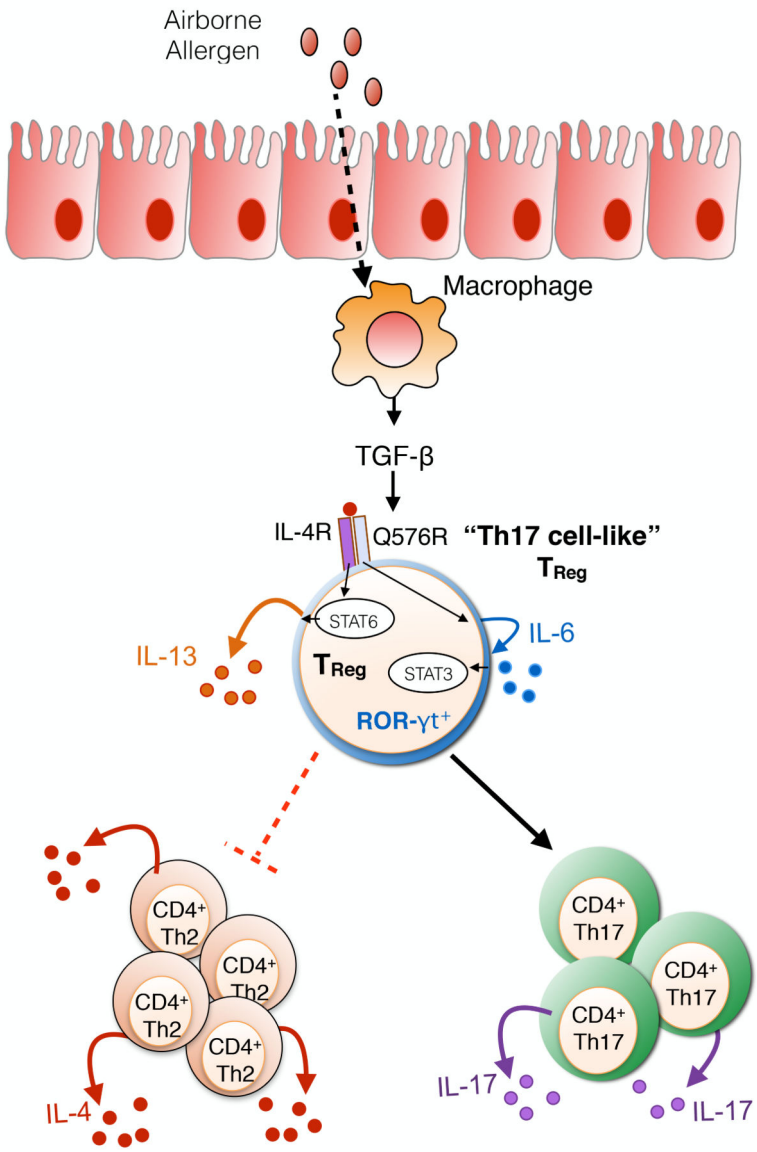

Fig 6. Pathogenic "Th17 cell-like" T_{Reg} cell reprogramming by the IL-4Rα-Q576R allele

Human IL-4Rα-Q576R is associated with increased asthma severity. Signalling through the IL4Rα-Q576R on iT_{Reg} cells induces dual activation of STAT6 and STAT3, the latter through an autocrine IL-6 production loop. The IL-6-STAT3 axis promotes pathogenic "Th17 cell-like" T_{Reg} cell reprogramming resulting in ROR-γt expression and IL-17 secretion by the reprogrammed T_{Reg} cells.

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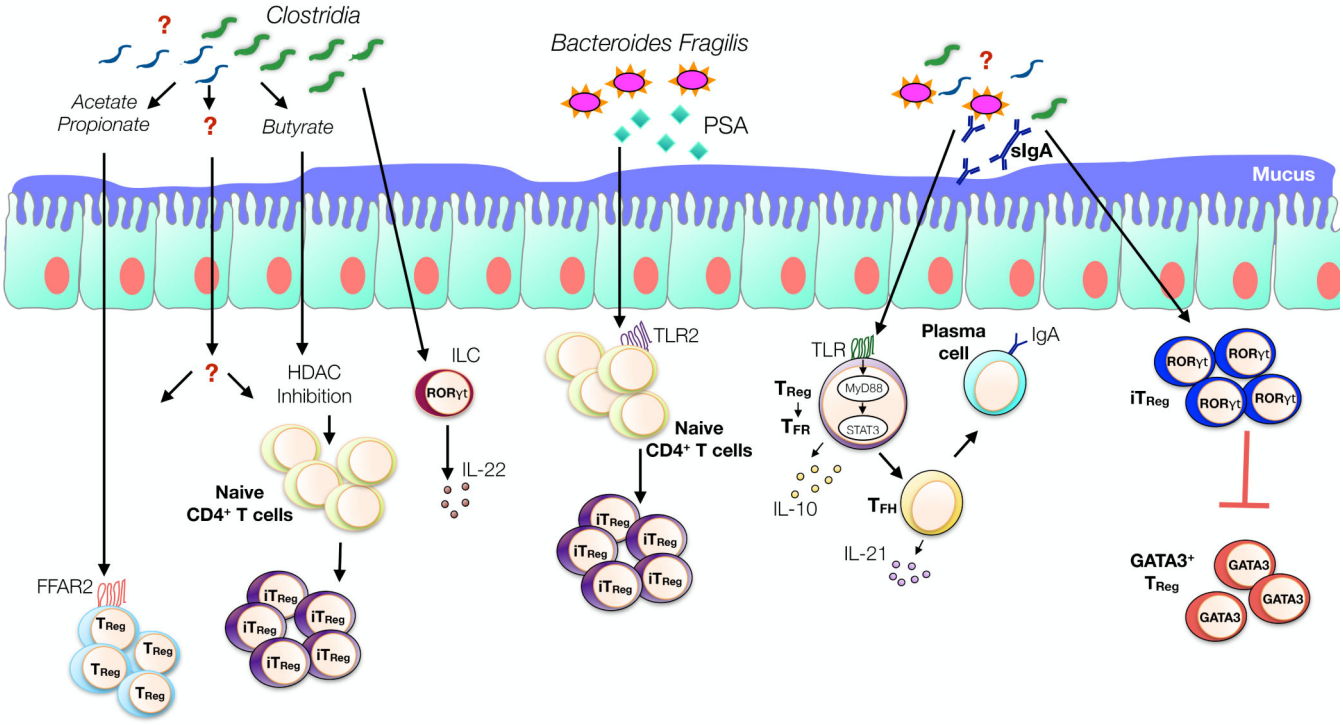

Fig 7. Microbiota-immune cell interactions shape oral tolerance

Metabolites, such as SFCAs produced by bacterial fermentation of dietary fibers promote the proliferation and *de novo* induction of iT_{Reg} cells through FFAR2 (GPR43) receptor and HDAC inhibition. Clostridial bacterial species promote the production of IL-22 by ROR-γt ILC, reinforcing oral tolerance by decreasing gut permeability and oral allergen uptake. *Bacteroides Fragilis* production of PSA promotes *de novo* iT_{Reg} cells generation via TLR2 signalling. MyD88/STAT3-sensing by T_{Reg} cells enforces oral tolerance by inducing and directing the T_{FH} - T_{FReg} and IgA axis. The microbiota also promote the emergence of ROR-γt expressing iT_{Reg} cells. ROR-γt deficiency in T_{Reg} cells promotes a Th2 environment and oral allergen sensitization possibly by inducing iT_{Reg} cell reprograming into "Th2 cell-like" cells expressing GATA-3.