

STATE OF THE ART REVIEW

T regulatory cells and their counterparts: masters of immune regulation

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Summary

The interaction of environmental and genetic factors with the immune system can lead to the development of allergic diseases. The essential step in this progress is the generation of allergen-specific CD4⁺ T-helper (Th) type 2 cells that mediate several effector functions. The influence of Th2 cytokines leads to the production of allergen-specific IgE antibodies by B cells, development and recruitment of eosinophils, mucus production and bronchial hyperreactivity, as well as tissue homing of other Th2 cells and eosinophils. Meanwhile, Th1 cells may contribute to chronicity and the effector phases. T cells termed T regulatory (Treg) cells, which have immunosuppressive functions and cytokine profiles distinct from that of either Th1 or Th2 cells, have been intensely investigated during the last 13 years. Treg cell response is characterized by an abolished allergen-specific T cell proliferation and the suppressed secretion of Th1 and Th2-type cytokines. Treg cells are able to inhibit the development of allergen-specific Th2 and Th1 cell responses and therefore play an important role in a healthy immune response to allergens. In addition, Treg cells potently suppress IgE production and directly or indirectly suppress the activity of effector cells of allergic inflammation, such as eosinophils, basophils and mast cells. Currently, Treg cells represent an exciting area of research, where understanding the mechanisms of peripheral tolerance to allergens may soon lead to more rational and safer approaches for the prevention and cure of allergic diseases.

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Introduction

The immune system has to work in a synchronized manner to maintain immune homeostasis. Stimuli from both internal and external sources must be recognized and processed to make appropriate decisions for 'self' or 'non-self' responses. This highly interactive network operates at a high level of complexity, where effector cells and their products activate, induce or suppress other cells of the immune system. CD4⁺ T-helper (Th) cells were shown to exert distinct cellular functions and cytokine secretion capacities, initially being classified as Th1 and Th2 cells by Mosmann et al. [1]. The roles of these distinct cell populations differ and a close and balanced interaction exists among them. Th1 cells are characterized by their secretion of IL-2, IFN- γ and TNF- α and - β . These cells are responsible for delayed-type hypersensitivity reactions as well as autoimmune diseases and rejection of allografts [2]. On the other hand, Th2 cells play a major role in parasitic infections and allergic inflammatory responses of the body [2, 3]. In atopic individuals, presentation of allergens to naïve T cells leads to the development of

effector Th2-type cells and production of Th2-type cytokines (IL-4, IL-5, IL-9 and IL-13). Generation of allergen-specific IgE antibodies responsible for subsequent hypersensitivity reactions is the hallmark of this type of immune response [4–8] (Fig. 1). This is followed by a cellular-driven process with infiltration of eosinophils, neutrophils, basophils, T cells and macrophages, and release of additional inflammatory mediators and cytokines, perpetuating the pro-inflammatory response. Chronic late-phase response due to continuous antigen exposure is thought to be responsible for the persistent, chronic signs and symptoms [9, 10].

Formerly, pathogenesis of both autoimmune and atopic diseases could be explained by a dysregulation in the Th1/Th2 balance. However, this concept of immune regulation has been revised with the description of a new subset of CD4⁺ T cells called 'regulatory T cells (Treg)' by Sakaguchi et al. [11]. Treg cells maintain immune homeostasis and various effector functions. Subsets of Treg cells with distinct phenotypes and mechanisms of action include the naturally occurring, thymus-selected CD4⁺CD25⁺FOXP3⁺ Treg cells [12–14] and the inducible type 1 IL-10-secreting

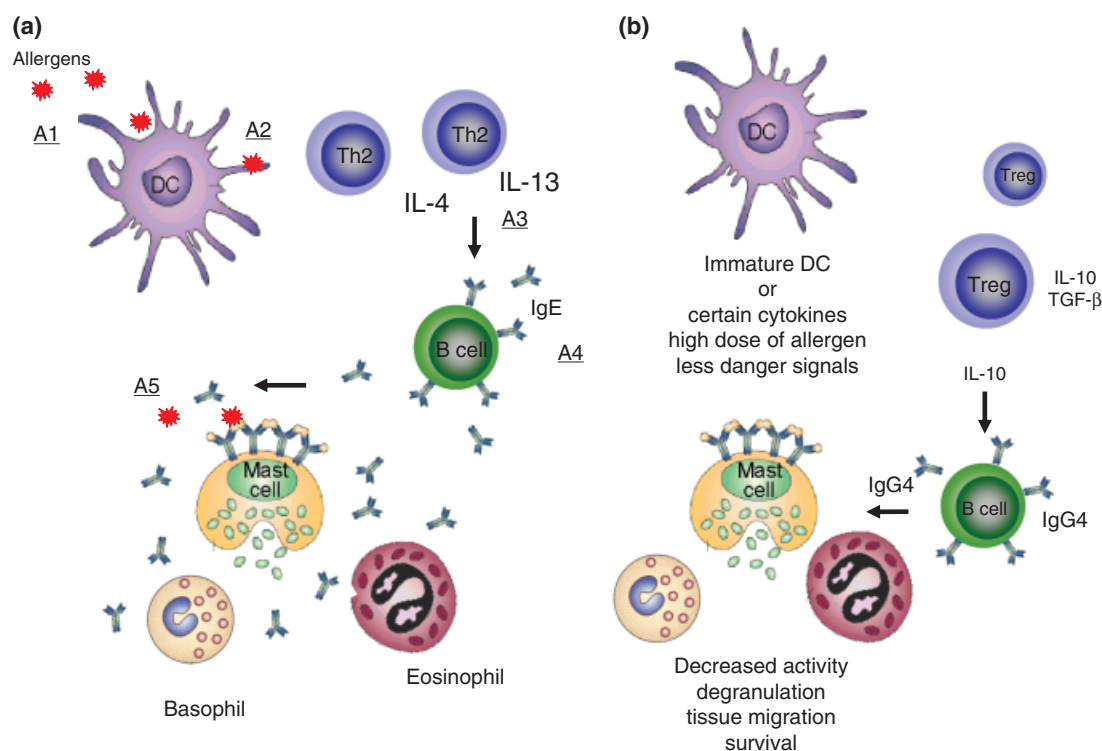


Fig. 1. (a) Generation and function of T-helper type 2 (Th2) cells. *Sensitization*: Antigen was captured by antigen-presenting cells (A1) and presented to (A2) naïve T cells and induced the generation of memory/effector Th2 cells. Th2-type cytokines, IL-4 and IL-13, synthesized (A3) by these cells induce a class switch to IgE in B cells to produce allergen-specific IgE (A4). Exposure to the same allergen causes activation of effector cells, mainly mast cells, resulting from the release of mediators via a membrane-bound allergen-specific IgE-allergen interaction, which cause clinical signs of type I hypersensitivity (A5). (b) T regulatory (Treg) cells in the control of an allergic reaction. Immature dendritic cells (DCs) control peripheral tolerance by inducing the differentiation of Treg cells. Antigen presentation by partially mature airway DCs that express IL-10 induces the formation of Treg cells, which produce IL-10, and TGF- β can potentially suppress IgE production while simultaneously increasing the production of the non-inflammatory antibody isotypes IgG4. Treg cells directly or indirectly suppress effector cells of allergic inflammation, such as mast cells, basophils and eosinophils.

Treg cells (Tr1 cells) [15, 16]. In addition, subsets of CD8⁺ T cells, $\gamma\delta$ T cells, dendritic cells (DCs), IL-10-producing B cells, natural killer (NK) cells and resident tissue cells, which might promote the generation of Treg cells, could contribute to suppressive and regulatory events [17, 18]. Numerous studies have been conducted to understand the molecular and cellular mechanisms involved in Treg development as well as their functions in various human diseases.

Recently, a new subset of Th cells has been described, called Th17 cells, which has major functions with the induction of tissue inflammation and host protection against extracellular pathogens. Th17 cells mediate immune responses against extracellular bacteria and fungi [19, 20] and can induce many organ-specific autoimmune diseases. It has been shown that in the presence of dual cytokine interaction of TGF- β and IL-6, Th cells differentiate into Th17 cells [21]. A reciprocal relationship between Treg cells and Th17 cell has been suggested; manipulation of this pathway may result either in the induction of Treg cells that will suppress autoimmunity or in the induction of Th17 cells that initiate tissue inflammation [22]. This review is focused on how Treg cells

function and how they interact with other components of the immune system based on recent developments and current understanding of immune regulation in allergic disease.

Naturally occurring regulatory T cells

Naturally occurring thymus-derived CD4⁺CD25⁺ Treg cells play a central role in the control of immune responses in autoimmune diseases, allergic disorders, infections, transplantation and cancer [23–25].

The importance of these cells has been shown by neonatal thymectomy performed at day 3 after birth, which resulted in autoimmune diseases in mice, with a concomitant decrease in CD25⁺ cells in the periphery [26]. CD25⁺ cells begin to appear in the periphery immediately after day 3, rapidly increasing within 2 weeks to nearly adult levels. The transfer of CD25⁺ splenic T cells prevented the development of autoimmune disease [26], supporting the idea that self-tolerance is maintained by Treg cells. Several subsequent experiments have delineated the importance of Treg cells in disease models. Both transfer [27] and depletion [28] experiments have

highlighted the role of Treg cells in experimental autoimmune encephalomyelitis (EAE) model. It has also been shown that antigen specificity is also essential for CD4⁺CD25⁺ Treg development and effector functions. This has been best exemplified in an EAE model that has been developed in mice transgenic for anti-myelin basic protein T cell antigen receptor (TCR) [29]. Transfer of CD4⁺ T cells to immune-deficient mice in the absence of the CD25⁺ subset leads to the development of colitis, indicating that Treg cells are capable of controlling a bacteria-driven inflammatory response in normal mice [30].

Multiple studies marked the role of Treg cells in human diseases. In type 1 diabetes, defective suppressor function in CD4⁺CD25⁺ T cells has been emphasized in several studies [31–33]. In patients with myasthenia gravis, the numbers of Treg cells were found to be less [34]. Also, patients with systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis exhibit deficiencies in Treg cell numbers and function [35–37]. In addition, pregnancy represents a semi-allograft situation to the maternal host. It has been shown that the early decidua contains abundant numbers of CD4⁺CD25⁺ T cells [38]. Treg cell numbers increase during early pregnancy, peaking during the second trimester and then declining post-partum [39]. Supporting this concept, adoptive transfer of pregnancy-induced CD4⁺CD25⁺ Treg cells prevents abortions in mice [40].

There is growing evidence that natural CD4⁺CD25⁺ Treg cells play key roles in the control of tumour immunity. The presence of antigen-specific Treg cells in tumour-infiltrating lymphocytes obtained from fresh tumour samples supports their role in tumour tolerance [41]. Furthermore, reduction of CD4⁺CD25⁺ Treg cells in mice by administration of cell-depleting anti-CD25 mAb before inoculation of tumour cells can elicit effective immunity, leading to eradication of tumours [42, 43]. Treatment modalities altering CD4⁺CD25⁺ Treg cell functions in tumour research can be used as new therapeutic ways of cancer immunotherapy. Several monoclonal antibodies have been used in this context including anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) [44] and anti-glucocorticoid-induced TNF receptor (GITR) antibodies [45].

It is well established that, in particular, the lineage-specific transcription factor forkhead box (FOX) P3, as well as cytokines (including IL-2, IL-10 and TGF- β), characteristic surface markers such as CTLA-4 and members of the TNF superfamily are critically involved in the thymic development, peripheral maintenance and suppressive activity of CD4⁺CD25⁺ Treg cells [23, 46]. FOXP3 acts as a master switch gene for Treg cell development and function. Deletion in the forkhead domain of FOXP3 in scurfy mice results in failure to develop thymus-derived, naturally occurring Treg cells and presents with lymphoproliferative disease, hyper-IgE levels and eosinophilia in the absence of Th2 skewing. The mice die

after 3 weeks [46–48]. The human counterpart of this deficiency arises and termed as immune dysregulation polyendocrinopathy enteropathy-X-linked syndrome and X-linked autoimmunity allergic dysregulation syndrome [48, 49]. Foxp3 amplifies and stabilizes expression of several genes encoding cell surface or secreted molecules – like Fgl2, CD73, CD39, TRAIL or CTLA4 – normally elaborated by conventional T cells upon TCR stimulation and capable of negative feedback regulation of T-cell activation. Foxp3 has the capacity to inhibit TCR-activation-dependent immune response of effector cytokines including IL-4, IFN- γ , TNF- α , IL-17 and IL-21 [50].

Induced regulatory T cells

In addition to development in the thymus, Foxp3⁺ Treg cells can also be differentiated or induced from CD4⁺CD25[–]Foxp3[–] T cells in the periphery with some stimuli such as TGF- β supplementation [51]. The gut is an entry site for numerous antigens, where tolerance is a requisite for healthy immune responses. It has been shown that peripheral conversion of CD4⁺ T cells to Treg cells occurs primarily in gut-associated lymphoid tissue (GALT) [52]. It was also shown that DCs purified from the lamina propria promote a high level of Treg cell conversion relative to lymphoid organ-derived DCs. This enhanced conversion is dependent on TGF- β and retinoic acid, which is a vitamin A metabolite highly expressed in GALT [52, 53].

Although the pathways regulating FOXP3 expression are yet unknown, a mechanism controlling Treg cell polarization, which is overruled by the Th2 differentiation pathway, has been recently described [54]. It was demonstrated that IL-4 present at the time of T cell priming inhibited FOXP3 expression. The findings were confirmed in Th2 cells and in transgenic mice overexpressing GATA-3 in T cells. A novel mechanism for the inhibition of tolerance development controlled by a Th2-type immune response was described by inhibition mediated by direct binding of GATA-3 to the FOXP3 promoter [54]. These data demonstrate a molecular mechanism for the counter-regulation of Th2 and Treg cells [55].

Other regulatory cells

There are groups of cells other than naturally occurring and induced Treg cells, which play inhibitory roles on immune system. Tumour-infiltrating $\gamma\delta$ Treg cells have similar cytokine profiles that resemble Tr1 cell clones, playing a significant role in the inhibition of immune responses against tumours [56]. Several studies point out that $\gamma\delta$ T cells are important mediators of mucosal tolerance and regulate autoimmunity [57]. It has been shown that aerosolized insulin induces regulatory CD8 $\gamma\delta$ T cells that prevent murine insulin-dependent diabetes [58]. Also, injection of a monoclonal anti- $\gamma\delta$ T cell

monoclonal antibody blocked the induction of oral tolerance to ovalbumin (OVA) in mice [59]. Furthermore, role of CD8⁺ Treg cells has been shown in an OVA oral tolerance murine model by demonstration of inhibition of priming of CD8⁺ T cells, CD4⁺ T cells and antibody responses with oral antigens, while CD8⁺ suppressor T cells have been activated [60]. This suppression depends on expression of the major histocompatibility complex (MHC) class Ib molecule Qa-1 on activated target cells, which resembles human leucocyte antigen-E in humans [61]. A subset of T cells has been described that interact vigorously with human endothelial cells, which are known to activate T cell responses to alloantigens. These cells are alloantigen-specific CD8⁺CD28⁻ T suppressor cells and are FOXP3 positive *in vitro* [62]. Furthermore, presence of double negative (CD4⁻CD8⁻ $\alpha\beta$ ⁺) T cells, which promote tolerance induction and regulate autoimmunity, has been described [63]. On the other hand, a subset of B cells with regulatory functions has been defined with a unique CD1dhiCD5⁺ phenotype, which controls T cell-dependent inflammatory responses [64].

NK cells represent a distinct lymphocyte population with a variety of functions including cytolytic activity, regulation of haemopoiesis, suppressor functions and Ig production [65]. NK cells may have influence in maintaining the delicate balance between the regulatory and effector arms of the immune response [66, 67]. Human NK cells have been subdivided into subsets as NK1 and NK2, which have been determined by purification of IFN- γ -secretion capacities. The IFN- γ -secreting NK subset showed a typical cytokine pattern with the predominant expression of IFN- γ , but almost no IL-4, IL-5 and IL-13, whereas the IFN- γ -non-secreting NK subset was composed of IL-4-, IL-5- and IL-13-producing NK cells [68]. These findings suggested that NK1 and NK2 subsets play counter-regulatory and provocative roles in immune responses, similar to Th1 and Th2 cells. The regulatory roles of NK cells were investigated in humans, by characterization of NK cell subsets according to their IL-10-secretion property. Freshly purified IL-10-secreting NK cells expressed up to a 40-fold increase in IL-10, but not FOXP3 and TGF- β . Stimulation with phytohaemagglutinin and IL-2 in addition to vitamin D3/dexamethasone and anti-CD2/CD16 monoclonal antibodies induced IL-10 expression in NK cells. IL-10⁺ NK cells significantly suppressed both allergen/antigen-induced T cell proliferation and secretion of IL-13 and IFN- γ , particularly due to secreted IL-10 as demonstrated by blocking of the IL-10 receptor, suggesting that a distinct small fraction of NK cells display regulatory functions in humans [66]. It has been recently reported that cytokines IL-22, IL-26 and leukaemia inhibitory factor secreted from subsets of human NK cells located in mucosa-associated lymphoid tissues, such as tonsils and Peyer's patches, can stimulate epithelial cells to secrete IL-10, proliferate and express a

variety of mitogenic and anti-apoptotic molecules. These cells provide an innate source of IL-22 for mucosal immunity [69].

On the other hand, recently, a subgroup of T cells that expresses an invariant T cell receptor and has potent immunoregulatory functions, known as CD1d-restricted NK T cells, has been identified [70]. There has been some controversial progress in understanding the complex role of NK-T cells in asthma. Initial presentation of the activation of invariant TCR natural killer T (iNKT) cells has been shown to be sufficient to induce airway hyperreactivity (AHR) in murine asthma models even in the absence of eosinophils, B cells and MHC class II-restricted CD4⁺ T cells [71], which supports the conclusion that iNKT cell-driven AHR can occur in the complete absence of adaptive immunity. However, there are conflicting reports on the numbers of iNKT cells in the lungs of patients with asthma. The suggestion that a Th2-like subset of iNKT cells is selectively recruited or expanded in the lungs of patients with asthma needs to be confirmed by using different methods [70].

Antigen presentation and T regulatory cell development

DCs are complex cell populations that differ in their anatomic location, antigen recognition, processing machinery and migratory capacity. Cutaneous DCs (Langerhans cells as well as dermal DCs) function as sentinels that survey invading agents and transmit the information into immune system by taking up exogenous antigens, migrating to draining lymph nodes, and presenting the processed antigens to T cells resulting in T cell differentiation and activation [72]. Different subpopulations may sequentially present skin-acquired antigens, possibly serving as a regulatory mechanism of cell-mediated immunity and adding further complexity to established concepts [73]. Cutaneous DCs are involved in pathologies including infections, inflammatory disorders or skin cancers. These cells play a role in regulating the balance between immunity and peripheral tolerance. It is widely accepted that cutaneous DC in an immature state may have tolerogenic properties, resulting in the induction or expansion of Treg cells [74].

In relation to the prevention and development of asthma, airway DCs control the pulmonary immune response and determine tolerance and immunity to newly encountered antigens [75, 76]. Tolerogenic DCs, modulated by IL-10, are able to convert peripheral T cells into iTreg cells [77]. These Treg cells are anergic and characterized by a G₁ cell cycle arrest, dependent on elevated levels of the cyclin-dependent kinase inhibitor p27 (Kip1). Novel data revealed a distinct pattern of mitogen-activated protein (MAP) kinase activation in iTreg, different from clonal T cell anergy, with enhanced activation of the p38-MAPKAP-K2/3 pathway. p38 is involved in cell cycle

control and its activity is a prerequisite for the induction and maintenance of the anergic state in iTreg. Inhibition of p38 leads to down-regulation of p27 (Kip1), cell cycle progress and loss of Treg cell function [77].

Immature DCs are distributed throughout the lungs and capture allergens, subsequently migrating to the T cell area of mediastinal lymph nodes within 12 h [78]. Depletion and adoptive transfer of pulmonary plasmacytoid DCs have demonstrated an important role for these cells in protection from allergen sensitization and asthma development in mice [79]. Moreover, existing therapies for allergic diseases, such as treatment with glucocorticoids and β_2 -agonists, might function to promote the numbers and function of IL-10-secreting Tr1-like cells [67, 80].

TNF superfamily member, receptor activator for nuclear factor κ -B ligand (RANKL) is thought to be a DC survival factor, which augments the ability of DCs to stimulate naïve T cell proliferation and increases the survival of RANK⁺ T cells generated with IL-4 and TGF- β [81, 82]. Another TNF receptor family member GITR-related protein gained attention as a potential marker of Treg cells, because of its purported role in reversing the immunosuppressive effects of Treg cells and extending the survival of T-effector cells [14, 83]. In addition, the gene related to anergy in lymphocytes (GRAIL) is involved in the induction of anergy in CD4⁺ T cells and is differentially expressed in naturally occurring and peripherally induced CD25⁺ Treg cells where expression of GRAIL is linked to regulatory activity [84].

Recently, new methods for generating and expanding Treg cells *in vitro* have emerged and supported the use of CD4⁺CD25⁺ T cells as a novel strategy for the treatment of patients suffering from autoimmune diseases [85–88]. IL-2R signalling seems essential for Treg cell development and function. Genetic defects disrupting the IL-2R signalling pathway result in severe T cell-mediated autoimmunity rather than immunodeficiency. Signalling downstream of the IL-2R can act through the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR pathway, the Janus kinase (Jak)/signal transducers and activators of transcription and the MAP kinase pathways [89].

Role of regulatory T cells in healthy immune response to allergens

Treg cells strongly control T cell repertoire of healthy individuals that harbours self-reactive and non-infectious non-self-reactive lymphocytes with a potential to cause autoimmune disease and allergy [90, 91]. Allergen-specific T cells can be differentiated within this repertoire according to cytokine profile. IFN- γ -, IL-4- and IL-10-producing allergen-specific CD4⁺ T cells display characteristics of Th1, Th2 and Treg cells, respectively [55]. In sensitized, hyperexposed, but non-allergic individuals, specific Tr1 cells consistently represent the dominant

subset against common environmental allergens in contrast to the high frequency of allergen-specific IL-4-secreting T cells in allergic individuals. In healthy donors, the suppressive role of CD4⁺CD25⁺ Treg cells on proliferation and IL-5 production on allergen-stimulated CD4⁺CD25⁺ T cells has been shown. However, this inhibition was significantly reduced in atopic donors, whereas even more diminished in CD4⁺CD25⁺ T cells isolated from patients with hayfever during the pollen season [92]. Similarly, asthmatic individuals have quantitative and functional impairments in CD4⁺CD25⁺ cells, in which an improvement has been observed after inhaled corticosteroid treatment trials [93].

Development of a healthy or an allergic immune response seems to be determined by the ratio between Treg cells and particularly Th2 and also Th1 cells [15, 55]. Healthy and allergic individuals exhibit all three allergen-specific subsets in different proportions, indicating that a change in dominant subset might lead to allergy development or recovery [15, 55]. In non-allergic individuals, understanding the mechanisms of the rapid switch and expansion of IL-10-producing Tr1 cells and the use of multiple suppressive factors are essential. Repeated exposure of non-allergic healthy beekeepers to bee venom antigens represents a valuable model to ascertain mechanisms of T cell tolerance induction. After multiple bee stings, venom antigen-specific Th1 and Th2 cells show a switch towards IL-10-secreting Tr1 cells, which results in diminished T cell-related cutaneous late-phase responses in parallel with suppressed allergen-specific T cell proliferation and Th1 and Th2 cytokine secretion [94]. This regulation is marked as long as venom exposure persists and returns to initial levels within 2–3 months after bee stings. Also up-regulation of histamine receptor 2 on specific Th2 cells suppresses allergen-stimulated T cells and increases IL-10 production [94]. However, although circulating CD4⁺CD25⁺ Treg cells in non-allergic healthy individuals has been shown to suppress proliferation of allergen-specific effector cells upon exposure to allergens, Treg cells from sensitized individuals are not able to do so to the same extent [95]. Neutralization of IL-10 enhances the production of TNF- α , suggesting counter-acting properties of IL-10 and TNF- α , where IL-10 promotes tolerance and suppression by Treg cells and TNF- α promotes inflammatory responses [95].

Role of regulatory T cells in allergen-specific immunotherapy

Allergen-specific immunotherapy (SIT) is accepted as a cornerstone in the management of allergic diseases [96, 97]. The mechanisms underlying this method of treatment have been slowly elucidated [17]. The effectiveness of allergen SIT has been especially proved in insect venom allergies and allergic rhinitis [96, 97]. In allergen SIT, the

essential step is the induction of a tolerant state in peripheral T cells [98–100]. Generation of allergen-specific Treg cells and production of their suppressive cytokines IL-10 and TGF- β are a requisite for the induction of peripheral T cell tolerance that is characterized by suppressed proliferative and cytokine responses against major allergens and their T cell recognition sites [15, 100] (Fig. 2). In concordance with clinical efficacy, local induction of CD4⁺CD25⁺ Treg cells has been recently shown in allergic patients with rhinitis in the nasal mucosa after SIT [101]. Generation of antibodies, especially of IgG4 type that are blocking IgE-facilitated allergen presentation, may play an important role in this treatment modality [102]. Also, a reduction in the number of effector cells including mast cells and eosinophils results in decreased amounts of

mediator releases from these cells [103, 104], which is associated with improvements in clinical and laboratory outcomes of the disease.

Rapid development to Tr1-type Treg cell development has been shown in bee venom (BV)-SIT. The specific proliferative and cytokine responses against the main allergen, phospholipase A2 (PLA) and T cell epitope-containing PLA peptides were significantly suppressed even after 7 days of treatment [15]. During BV-SIT, the production of IL-10 was increased and after 28 days of treatment a non-responsive state was established in allergen-specific T cells due to direct suppressive effects of IL-10. In addition, progressive expansion of circulating CD4⁺CD25⁺ Treg cells has been demonstrated in the course of venom immunotherapy [105].

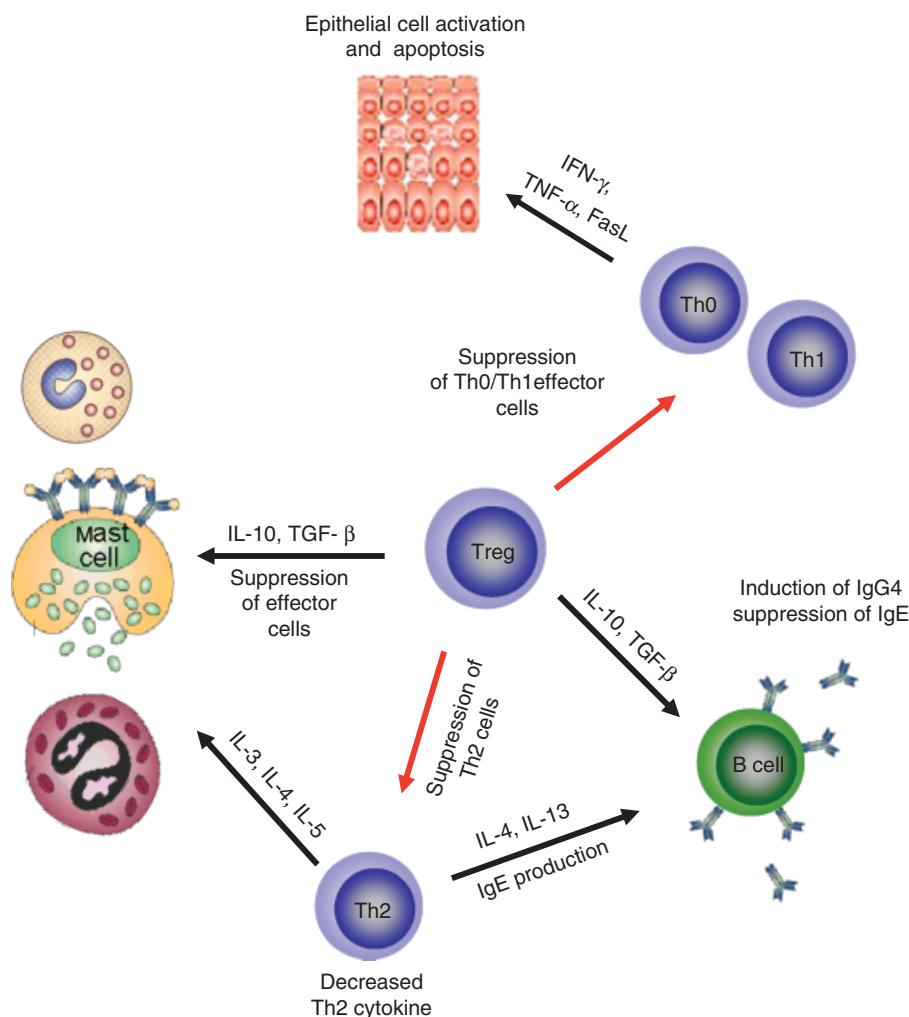


Fig. 2. T regulatory (Treg) cells in allergen-specific immunotherapy. Treg cells utilize multiple suppressor factors to regulate the undesired activity of effector T-helper type 2 (Th2) cells. IL-10 and TGF- β suppress IgE production and induce non-inflammatory Ig isotype IgG4, respectively. Th2 cells are suppressed by Treg cells and can therefore no longer provide cytokines such as IL-3, IL-4, IL-5, IL-9 and IL-13. These cytokines are required for the differentiation, survival and activity of mast cells, basophils, eosinophils and mucus-producing cells, and the tissue homing of Th2 cells. The Th1 cytokine, IFN- γ , in combination with TNF- α and/or FasL, induces activation, chemokine production and apoptosis of skin keratinocytes and bronchial epithelial cells as essential tissue injury events in atopic dermatitis and asthma. Treg cells may be involved in tissue remodelling via TGF- β in asthma. Treg cells suppress the stimulation of Th0/Th1 cells, leading to the abrogation of tissue injury mechanisms.

The therapeutic use of proteins to which the patient is sensitized results in frequent allergic adverse events, most commonly related to cross-linking of allergen-specific IgE on mast cells and basophils. A number of strategies aimed at reducing the allergenicity of the treatment preparation, while maintaining immunogenicity, have been described. The use of short, soluble synthetic peptides for the treatment of allergic disease offers the potential to avoid IgE-mediated allergic reactions, while targeting T cell epitopes and inducing modulation of allergen-specific responses. Synthetic peptides have been evaluated in a number of experimental animal models and, more recently, in human studies. Peptide-induced, antigen-specific non-responsiveness, also known as tolerance, has been demonstrated in numerous murine models of both autoimmunity and allergy [106, 107]. Initial experiments demonstrated that it was possible to induce systemic tolerance to peptides administered in incomplete Freund's adjuvant during the neonatal period. Peptide immunotherapy studies in subjects with allergic disease have been performed with peptides from the major allergen of cat dander Fel d 1 [108, 109] and bee venom PLA [98]. T cell epitope (peptide)-SIT of bee venom-allergic individuals and cat-allergic individuals induced a Tr1-type allergen-specific immune response with decreased IL-4, IL-5, IL-13 and IFN- α and increased IL-10 production [110–112]. All these studies have shown promising results with variable clinical outcomes. Improvement in lung and nasal symptoms [113–115] and reductions in skin reactivity have been reported [109, 112, 113, 116]. However, several adverse events were reported in common with other studies.

Mechanisms of direct T cell suppression by interleukin-10 and transforming growth factor- β

IL-10 inhibits the proliferative T cell response in peripheral blood mononuclear cells to various allergens; however, it does not suppress the proliferative responses of T cells that were stimulated by anti-CD3 [117]. IL-10 inhibits T cell proliferation within a certain range of low numbers of triggered T cell receptors that require co-stimulation. IL-10 suppresses T cells by blocking CD2, CD28 and inducible co-stimulatory (ICOS) signals in a rapid signal transduction cascade [118]. In the presence of IL-10, a direct inhibition on CD2 signalling in T cells occurs via utilization of Src-homology-2 domain containing tyrosine phosphatase (SHP-1) by IL-10. Using dominant-negative SHP-1 over-expressing T cells and silencing endogenous SHP-1 by small inhibitory RNA (siRNA), the role of SHP-1 in IL-10-mediated suppression of CD2 co-stimulation on T cells has been demonstrated [119]. Upon binding to its receptor, IL-10 activates two tyrosine kinases, Jak1 and Tyk2. Tyk2 acts as a constitutive reservoir for SHP-1 tyrosine phosphatase in resting T cells. Upon activation, SHP-1 rapidly binds to CD28 and

ICOS and dephosphorylates them within 1.5–3 min. In consequence, the binding of phosphatidylinositol 3-kinase to either co-stimulatory molecule no longer occurs and downstream signalling is inhibited [118]. Supporting these findings, spleen cells from SHP-1-deficient mice showed increased proliferation with CD28 and ICOS stimulation in comparison with wild-type mice, which was not suppressed by IL-10. Generation of dominant-negative SHP-1-overexpressing T cells or silencing of the SHP-1 gene by siRNA both altered SHP-1 functions and abolished the suppressive effect of IL-10 [118]. Interestingly, the suppressive effect of IL-10 was not observed in other IL-10 family cytokines IL-19, IL-20, IL-22 and IL-24 [120]. In addition to the suppressive roles on T cells, IL-10 also exerts inhibitory effect on activated monocytes and macrophages [121]. In monocytes, IL-10 was shown to induce expression of the suppressor of the cytokine-signalling-3 (SOCS3) gene that might play a role in the inhibition of the IFN-induced tyrosine phosphorylation of Stat1 [122].

The TGF superfamily consists of more than 35 members with TGF- β 1 being the proteotypic member. TGF- β 1-deficient mice develop the phenotype, due to activated CD4⁺ T cells, of a rapidly wasting syndrome leading to death by the age of 3 or 4 weeks [123, 124]. Although controversial, TGF- β has been implicated in the conversion of naïve CD4⁺CD25[−] T cells into CD4⁺CD25⁺ T cells by the induction of FOXP3 [51]. In contrast, several recent studies demonstrated that TGF- β plays an essential role in the development of IL-17-secreting T cells, which promote neutrophilic inflammation [21]. TGF- β signalling has been demonstrated to be required for *in vivo* expansion and immunosuppressive capacity of CD4⁺CD25⁺ T cells [125]. However, studies with TGF- β -deficient mice have shown that CD4⁺CD25⁺ T cells can develop up to 2 weeks and autocrine TGF- β production was not essential for these cells to exhibit suppressive activity *in vivo* [126]. In contrast to its known T cell suppressive activity, some reports imply a role for TGF- β in the pathogenesis of asthma, particularly in the remodelling of injured lung tissue in humans, because TGF- β is one of the main fibrotic cytokines [127]. The exact suppressive mechanisms behind TGF- β receptor pathways remain to be elucidated.

T regulatory cells and antibody isotypes

The induction of Treg cells during allergen-SIT displays multiple functions in the immune tolerance network including allergen-specific antibody regulation towards a non-allergic and non-inflammatory direction. Induction and increased secretion of IL-10 by T cells, B cells and monocytes during SIT counter-regulates antigen-specific IgE and IgG4 antibody synthesis [15]. IL-10 increases IgG4 production, whereas simultaneously it is a potent

suppressor of both total and allergen-specific IgE [15]. Specific serum levels of both isotypes initially increase during early phases of immunotherapy, afterwards the increase in allergen-specific IgG4 levels is more pronounced, such that the ratio of specific IgE to IgG4 decreases by time [15]. To understand which subsets of Treg cells cross-talk to B cells and how regulation of antibody production occurs is an essential question. IL-10 has two major effects on B cells, which could result in a shift in balance as observed. IL-10 decreases ϵ transcript expression and therefore down-regulates the production of IgE when added to peripheral blood mononuclear cells during the first 3 days of culture. However, IL-10 induces further up-regulation of IgE production when added to already committed B cells [128]. Furthermore, IL-10 enhances γ 4 transcript expression and IgG4 production induced by IL-4. Thus, IL-10 not only generates tolerance in T cells, but also regulates specific isotype formation and skews the specific response from an IgE- to an IgG4-dominated phenotype. These results have been recently confirmed by direct Treg cell subset–B cell interaction experiments [129].

Meiler and colleagues have recently shown the direct influence of Treg cells on B cells by demonstrating the induction of IgG4 and suppression of IgE in healthy individuals. On the other hand, neither IL-10 secreting Tr1 cells nor CD4⁺CD25⁺ Treg cells have been shown to have an influence on production of IgA. In contrast, it has been shown that production of IgA is strongly dependent on the innate immune system, particularly Toll-like receptor (TLR)7 and TLR9 activation of B cells [130]. The role of mucosal secretory IgA levels in the development of allergic diseases has been questioned. In a mouse model of food allergy, it was observed that antigen-specific secretory IgA antibodies in the gut were decreased suggesting a role for secretory IgA in peripheral tolerance to foods. Peyer's patch CD31 cells were primarily involved by favouring IgA production through the release of IL-10 and TGF- β , and low IL-10 production in Peyer's patches favoured the symptoms of food allergy [131]. In conjunction with the role of TGF- β and IL-10 in IgA regulation in childhood asthma with house dust mite allergy, 6 and 12 months of sublingual immunotherapy down-regulated the specific IgE response, while slightly increasing specific IgA [132].

Effector cells, histamine and regulatory T cells

T cell cytokines are essential for priming, survival and activity of mast cells, basophils and eosinophils during allergic inflammation [133]. Treg cells seem to directly or indirectly influence these effector cells [134]. During SIT, modulation of thresholds for mast cell and basophil activation occurs even during early phases [135, 136]. This results in decreased IgE-mediated histamine release.

This early effect was pronounced especially in patients who received bee venom allergen immunotherapy a few days after starting of the injections [137].

IL-10 plays a role in homeostatic mechanisms of mast cell numbers and function in peripheral tissues by regulating mast cell maintenance and proliferation [138]. It was shown that long-term exposure of murine bone-marrow-derived mast cells to cytokines IL-3, IL-4 and IL-10 induced down-regulation of critical mast cell cKit and Fc ϵ -RI, followed by mast cell apoptosis [139]. IL-10 also down-regulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 cells [140–142].

The various effects of histamine on immune regulation are due to differential expression and regulation of four histamine receptors and their distinct intracellular signals [143, 144]. Histamine acting through its receptor (HR) type 2 positively interferes with the peripheral antigen tolerance induced by Treg cells [94]. Histamine released from mast cells and basophils may efficiently contribute to immunoregulation during SIT [137], and affect Treg cells and the production of their cytokines. Histamine and HR2 may promote the development of peripheral tolerance during SIT in several pathways. Histamine supports the suppressive effect of TGF- β on Th2 cells through HR2 [145]. Histamine also plays a role in induction of IL-10 by DCs and Th2 cells again via HR2 [146, 147].

Role of regulatory T cells in airway hyperreactivity and remodelling

In animal models it was shown that FOXP3⁺ Treg cells protect mice against AHR in an IL-10-dependent manner. A population of Treg cells that developed *in vivo* from naïve CD4⁺CD25[−] T cells during a Th1-polarized response, distinct from CD25⁺ Treg cells, has been described [148]. However, these cells expressed the transcription factor T-bet, and secreted both IL-10 and IFN- γ , but not Th2 cytokines, suggesting a possible relation to the Th1 lineage. It was thought that adaptive Treg cells were heterogeneous and comprised Th1-like Treg cells as well as previously described Th2-like Treg cells, which express FOXP3 and are induced during the development of respiratory tolerance by CD8 α [−] DCs [148]. A recent study reported that functional insufficiency of Treg cells in patients with allergic asthma might be related to the enhanced production of TNF- α and its effect (suppression) on FOXP3 expression. The efficacy of anti-TNF- α therapy in asthma may play a role related to Treg cells; however, it remains to be further elucidated [149].

Moreover, repeated airway injury results in airway remodelling in asthma that is characterized by deposition of various extracellular matrix proteins in the reticular basement membrane and bronchial mucosa, as well as airway smooth muscle thickening, goblet cell hyperplasia

and new blood vessel formation [150, 151]. TGF- β , which serves as a potent Treg cytokine, is a well-known regulator of fibroblasts and myofibroblast function and plays role in the production of collagens, tenascin and proteoglycans, which compose the extracellular matrix [152]. Increases in fibroblasts related to thickening of lamina reticularis were shown to correlate with TGF- β expression levels. The responsiveness of fibroblasts to TGF- β is heterogeneous resulting in different pathological outcomes of inflammation in the upper and lower airways [153]. In addition to smooth muscle cells and fibroblasts, the other cellular sources of TGF- β include eosinophils, macrophages, mast cells, neutrophils, endothelial and epithelial cells [154]. In asthma, even before the initial diagnosis, thickening of lamina reticularis starts. This thickening may create a natural barrier against mucosal allergens, thus reducing the contact with the inner tissues with a possible down-regulation of allergen-induced inflammatory response [155].

Conclusion

Studies on T cell immunity to allergens in healthy individuals have demonstrated a wide range of immune response from non-detectable to involvement of active peripheral tolerance mechanisms mediated by different subsets of Treg cells. Peripheral T cell tolerance represents the key mechanism in healthy immune responses to allergens. A self-check, built-in component of the immune system, which is characterized by Treg cells, suppressive cytokines and non-inflammatory antibody isotypes, organizes these responses. Understanding the mechanisms of action of allergic diseases may enlighten the complex interactions of effector cells with Treg cells. In addition to allergy, these mechanisms may have implications in autoimmunity, graft-vs.-host disease, tumour cell growth, parasite survival/clearance, chronic infections and the development of acquired immune deficiency syndrome. Suppression of an immune response may have beneficial effects in the case of hypersensitivity reactions, but it may also be harmful in other cases such as tumour cell tolerance during cancer development, immune tolerance to chronic infectious agents and tissue remodelling. The overexpression of Treg cells and suppressive cytokines may be responsible for chronicity and tumour tolerance and therefore does not always support a healthy response. Changes in the fine balance between allergen-specific Treg and Th2 and/or Th1 cells is crucial in the development and also in the treatment of allergic diseases. In addition to the treatment of established allergy, it is essential to consider prophylactic approaches before the initial sensitization takes place. Preventive vaccines that induce Treg cell responses could be developed, and allergen-specific Treg cells, which will become predominant, may in turn dampen both the Th1 and the

Th2 cells and their cytokines, ensuring a well-balanced immune response. Considering these findings, along with the recent advances in the knowledge of peripheral tolerance mechanisms, developments of safer approaches and treatment of immune-mediated diseases await.

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