

Review article

Genes of tolerance

Activation-induced cell death, anergy and/or immune response modulation by T-regulatory cells (T_{Reg}) are essential mechanisms of peripheral T-cell tolerance. There is growing evidence that anergy, tolerance and active suppression are not entirely distinct, but rather, represent linked mechanisms possibly involving the same cells and multiple suppressor mechanisms. Skewing of allergen-specific effector T cells to T_{Reg} cells appears as a crucial event in the control of healthy immune response to allergens and successful allergen-specific immunotherapy. The T_{Reg} cell response is characterized by abolished allergen-induced specific T-cell proliferation and suppressed T helper 1 (Th1)- and Th2-type cytokine secretion. In addition, mediators of allergic inflammation that trigger cAMP-associated G-protein coupled receptors, such as histamine receptor 2 may contribute to peripheral tolerance mechanisms. The increased levels of interleukin-10 (IL-10) and transforming growth factor- β (TGF- β) that are produced by T_{Reg} cells potentially suppress immunoglobulin E (IgE) production, while simultaneously increasing production of noninflammatory isotypes IgG4 and IgA, respectively. In addition, T_{Reg} cells directly or indirectly suppress effector cells of allergic inflammation such as mast cells, basophils and eosinophils. In conclusion, peripheral tolerance to allergens is controlled by multiple active suppression mechanisms. It is associated with regulation of antibody isotypes and effector cells to the direction of a healthy immune response and opens a window for novel therapies of allergic diseases.

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The T-cell tolerance is characterized by functionally inactivation of the cell to antigen encounter, which remains alive for an extended period of time in an unresponsive state. In recognition of the importance of the phenomenon of immunological tolerance, the Nobel Prize in Physiology and Medicine was awarded in 1960 to Medawar for discovering that skin allografts in mice and chicken can be accepted if they had been preinoculated during embryonic development with allogeneic lymphoid cells (1), and to Burnet for first proposing that exposure to antigens before the development of immune response, specifically abrogates the capacity to respond to that antigen in later life (2, 3). During the last decade, this area of immunology research had become so popular and promiscuous. The overall evaluation of the studies on T cell unresponsiveness suggest that anergy, tolerance and active suppression are not entirely distinct, but rather, represent linked mechanisms possibly involving the same molecular events. The term anergy was first

coined by Von Pirquet in 1908 to describe the loss of delayed-type hypersensitivity to tuberculin in individuals infected with measles virus (4). The term was clinically accepted since then to describe negative tuberculin skin test results in conditions, where it is expected to be positive. In 1980, the term 'anergy' was used to describe the specific inactivation of B cells in mice by high doses of antigen (5). It was subsequently used for T cells to describe a phenomena in which antigen presentation to T-cell clones in the absence of professional antigen-presenting cells (APC) induced a hyporesponsive state affecting subsequent interleukin (IL)-2 production and proliferation upon restimulation (6). A variety of reversible functional limitations characterize the anergic state, including cell division, cell differentiation, and cytokine production (7, 8). It is important to note here that early studies, which are referred as a basis for the definition of anergy/tolerance, functional unresponsiveness was analysed by nonsophisticated assays such as antigen-induced [^{3} H]-thymidine incorporation, IL-2 and total immunoglobulin G (IgG) production. In addition, antigens used in mouse models until the last few years contained high amounts of impurities, such as lipopolysaccharide (LPS) and other innate immune response stimulating substances, which may influence the outcome of the

Abbreviations: SIT, specific immunotherapy; PIT, peptide immunotherapy; APC, antigen-presenting cells; Th, T helper; T_{Reg} , T regulatory; LPS, lipopolysaccharide; HR, histamine receptor; HDM, house dust mite; TGF, transforming growth factor- β ; DC, dendritic cell; OVA, ovalbumin.

experiments. Although some of the biochemical steps overlap with anergy, activation-induced cell death induced by trigger of the death receptors and caspase activation represents a distinct physiological response (9, 10).

It is still not understood why exposure to allergens causes atopic disorders in some individuals, but not others, however, it is clear that strong interaction of environmental and genetic factors is involved. Four cardinal events during allergic inflammation can be classified as activation of memory/effector T cells and other effector cells such as mast cells, eosinophils and basophils, their organ-selective homing, prolonged survival and reactivation inside the allergic organs and effector functions (11). The T cells are activated by aeroallergens, food antigens, autoantigens and bacterial superantigens in allergic inflammation (12, 13). They are under the influence of skin, lung- or nose-related chemokine network and they show organ-selective homing (14–16). A prolonged survival of the inflammatory cells and strong

interaction with resident cells of the allergic organ and consequent reactivation is observed in the subepithelial tissues (17, 18). The T cells play important effector roles in atopic dermatitis and asthma with induction of hyper IgE, eosinophil survival and mucus hyperproduction (12, 18, 19) (Fig. 1). In addition, activated T cells induce bronchial epithelial cell and keratinocyte apoptosis as major tissue injury events (20–23). Peripheral T-cell tolerance to allergens can overcome all of the above pathological events in allergic inflammation, because they all require T-cell activation.

The initial event responsible for the development of allergic diseases is the generation of allergen-specific CD4⁺ T-helper cells (Th). The current view is that under the influence of interleukin 4 (IL-4), naive T cells activated by APC differentiate into Th 2 cells (24–26). Once generated, effector Th2 cells produce IL-4, IL-5 and IL-13 and mediate several regulatory and effector functions. These cytokines induce the production of

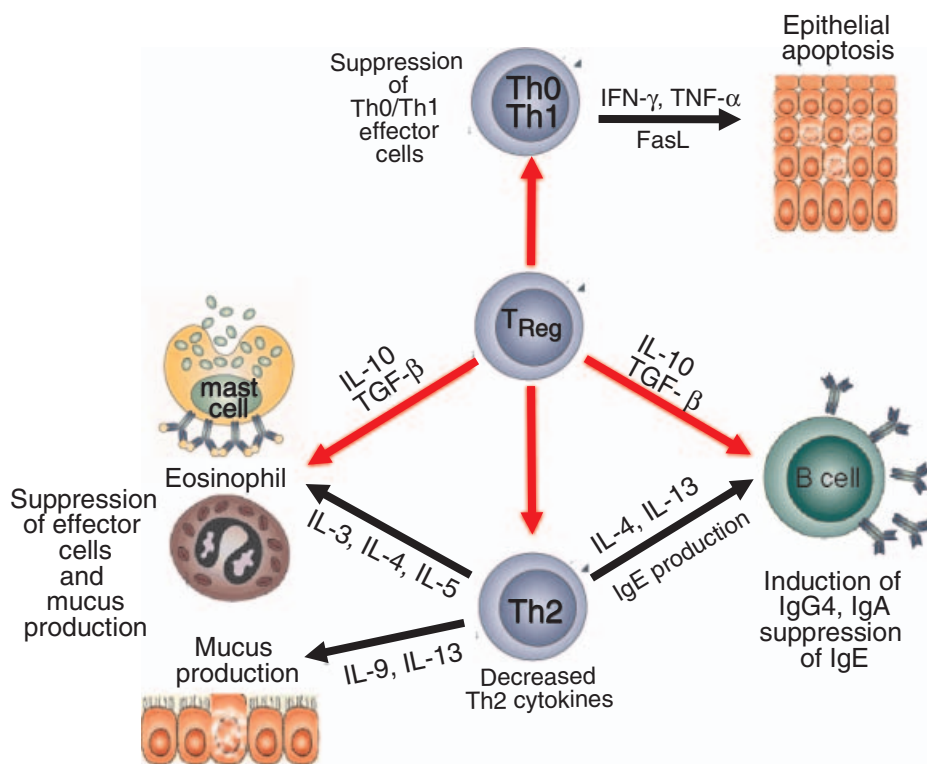


Figure 1. Peripheral tolerance mechanisms in allergen-specific immunotherapy and healthy immune response. Immune deviation towards T-regulatory (T_{Reg}) cell response is an essential step in allergen-specific immunotherapy and natural allergen exposure of nonallergic individuals. The T_{Reg} cells utilize multiple suppressor factors, which influence the final outcome of allergen-specific immunotherapy. In addition, IL-10 and transforming growth factor (TGF)- β induce immunoglobulin (Ig)G4 and IgA respectively from B cells as noninflammatory immunoglobulin isotypes and suppress IgE production. These two cytokines directly or indirectly suppress effector cells of allergic inflammation such as mast cells, basophils and eosinophils. In addition, T helper (Th)2 cells, which are dominated by T_{Reg} cells cannot anymore induce IgE by interleukin (IL)-4 and IL-13 and cannot provide cytokines such as IL-3, IL-4, IL-5 and IL-9, which are required for the differentiation, survival and activity of mast cells, basophils and eosinophils and mucus producing cells. Furthermore, the suppressed Th0/Th1 compartment of allergic inflammation cannot mediate essential tissue injury mechanisms via interferon (IFN)- γ , tumour necrosis factor (TNF)- α and Fas-ligand (L) such as apoptosis of skin keratinocytes and bronchial epithelial cells (red line: suppression, black line: stimulation).

allergen-specific IgE by B cells, development and recruitment of eosinophils, production of mucus and contraction of smooth muscles (24, 25, 27). Furthermore, the degranulation of basophils and mast cells by IgE-mediated cross-linking of receptors is the key event in type I hypersensitivity, which may lead to chronic allergic inflammation. Importantly, although Th2 cells are responsible for the development of allergic diseases, Th1 cells may contribute to chronicity and effector phase in allergic diseases (20–23, 28, 29). Distinct Th1 and Th2 two subpopulations of T cells counter-regulate each other and play a role in distinct diseases (24, 25). In addition, recent studies have demonstrated that peripheral T-cell tolerance is crucial for a healthy immune response and successful treatment of allergic disorders (30–32). A further subtype of T cells, with immunosuppressive function and cytokine profiles distinct from either Th1 and Th2 cells, termed regulatory/suppressor T cells (T_{Reg}) has been described (33–35) and evidence for their existence in humans has been demonstrated (31, 36, 37). In addition to Th1 cells, T_{Reg} cells are able to inhibit the development of allergic Th2 responses (38) and play a major role in allergen-specific immunotherapy (SIT) (31, 32). This review will examine allergen-specific peripheral tolerance mechanisms in humans and discuss novel ways of T-cell suppression.

Peripheral T-cell tolerance in allergen-specific immunotherapy

The symptoms of IgE-mediated allergic reactions – such as rhinitis, conjunctivitis and asthma – can be ameliorated by temporary suppression of mediators and immune cells (by antihistamines, antileukotrienes, β_2 -adrenergic receptor antagonists and corticosteroids) (39–42). However, a more long-term solution is allergen-specific immunotherapy that specifically restores a normal immunity against allergens. Allergen-SIT is most efficiently used in allergy to insect venoms and allergic rhinitis (43–47). Despite its usage in clinical practice for nearly a century, the underlying immunological mechanisms of allergen-SIT are slowly being elucidated (30, 32, 48–52). A rise in allergen-blocking IgG antibodies particularly of the IgG4 class, which supposedly block allergen and IgE facilitated antigen presentation (53–55), the generation of IgE-modulating $CD8^+$ T cells (56) and a reduction in the number of mast cells and eosinophils, including the release of mediators (57–59) were shown to be associated with successful allergen-SIT. Furthermore, allergen-SIT was found to be associated with a decrease in IL-4 and IL-5 production by $CD4^+$ T cells (47, 60, 61). Also a shift from Th2 cytokine pattern towards increased interferon (IFN)- γ production in allergen-SIT of allergy to bee venom, wasp venom, grass pollen and house dust mite (HDM) was observed (60, 62). It appears, however, that the induction of a tolerant state in peripheral T cells

represents an essential step in allergen-SIT (Fig. 1) (30–32, 48). Peripheral T-cell tolerance is characterized mainly by suppressed proliferative and cytokine responses against the major allergens and its T-cell recognition sites (48). The T-cell tolerance is initiated by autocrine action of IL-10, which is increasingly produced by the antigen-specific T cells (30, 31). Tolerized T cells can be reactivated to produce either of distinct Th1 or Th2 cytokine patterns depending on the cytokine present in the tissue microenvironment, and thus directing allergen-SIT towards successful or unsuccessful treatment (48).

Immunotherapy using peptides (PIT) is another attractive approach for investigation of peripheral T-cell tolerance in humans. Short allergen peptides, either native sequences or altered peptide ligands, with amino acid substitutions do not contain epitopes for IgE cross-linking to induce anaphylaxis. There is considerable rationale for targeting T cells with synthetic peptides based on such T-cell epitopes. To date, clinical trials of PIT have been performed in two allergies (63–67). Relatively long peptides of 27 and 35 amino acids of the major cat allergen Fel d 1 containing the T-cell epitopes or mixture of peptides spanning the whole protein sequence were used to treat allergy to cats and resulted in the induction of tolerance in IL-4-producing cells (64, 67). In the other trial, PIT of bee venom allergy was performed with a mixture of short peptides that directly represent the T-cell epitopes (17, 12, 11 amino acids) of the bee venom major allergen, phospholipase A_2 (63). The study showed modulation of the immune response against the whole allergen, inducing specific T-cell tolerance and a decrease in the specific IgE : IgG4 ratio (63). Single amino acid alteration in T-cell epitopes can modify specific T-cell activation and cytokine production (68). Rodent studies suggest that, under highly controlled experimental conditions, allergic diseases can be inhibited by altered peptide ligand administration. Whether this is due to Th2–Th1 immune deviation or the induction of T_{Reg} cells remains to be elucidated (68, 69). Although PIT is theoretically attractive as a means to avoid IgE-mediated early phase reactions, it is important to note that serum IgE in allergic individuals may sometimes bind to relatively short linear epitopes of protein allergens (70). A potential barrier to PIT of allergy is the apparent complexity of the allergen-specific T-cell response in terms of epitope usage and dominant epitopes in humans (71–73).

Peripheral T-cell tolerance to allergens is associated with regulation of antibody isotypes and suppression of effector cells

The serum levels of specific IgE and IgG4 antibodies delineate allergic and normal immunity to allergen. Although peripheral tolerance was demonstrated in specific T cells, the capacity of B cells to produce specific

IgE and IgG4 antibodies was not abolished during SIT (48). In fact, specific serum levels of both isotypes increased during the early phase of treatment. However, the increase in antigen-specific IgG4 was more pronounced and the ratio of specific IgE to IgG4 decreased by 10- to 100-fold. Also the *in vitro* production of PLA-specific IgE and IgG4 antibodies by peripheral blood mononuclear cells (PBMC) changed in parallel to the serum levels of specific isotypes. A similar change in specific isotype ratio was observed in SIT of various allergies. Moreover, IL-10 which is induced and increasingly secreted by SIT, appears to counter-regulate antigen-specific IgE and IgG4 antibody synthesis (31). The IL-10 is a potent suppressor of both total- and allergen-specific IgE, while it simultaneously increases IgG4 production (31, 74). Thus, IL-10 not only generates tolerance in T cells; it also regulates specific isotype formation and skews the specific response from an IgE to an IgG4 dominated phenotype (Fig. 1). The healthy immune response to Der p1 demonstrated increased specific IgA and IgG4, small amounts of IgG1 and almost undetectable IgE antibodies in serum (32). The HDM-SIT did not significantly change specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1 and IgG4 was observed (32). The increase of specific IgA and IgG4 in serum coincides with increased transforming growth factor- β (TGF- β) and IL-10 respectively. This may account for the role of IgA and TGF- β as well as IgG4 and IL-10 in peripheral mucosal immune responses to allergens in healthy individuals (31, 75).

Despite the fact that definite decrease in IgE antibody levels and IgE-mediated skin sensitivity normally requires several years of SIT, most patients are protected against bee stings already at an early stage of BV-SIT. The reason for this is that effector cells of allergic inflammation, such as mast cells, basophils and eosinophils require T-cell cytokines for priming, survival and activity (76, 77), which are not efficiently provided by suppressed Th2 and T_{Reg} cells (Fig. 1). The SIT efficiently modulates the thresholds for mast cell and basophil activation and decreases IgE-mediated histamine release (78, 79). In addition, IL-10 was shown to reduce proinflammatory cytokine release from mast cells (80). Furthermore, IL-10 down-regulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 cells (81). Moreover, IL-10 inhibits endogenous granulocyte monocyte colony-stimulating factor (GM-CSF) production and CD40 expression by activated eosinophils and enhances eosinophil cell death (82).

Peripheral T-cell tolerance by inhibition of T cell co-stimulation

Inhibition of T cell co-stimulatory molecules at the cell surface or their intracellular signal transduction has been

repeatedly reported to play an important role in T-cell tolerance (83–85). The T cells could be anergized in experimental models that bypass co-stimulatory signals (6, 7, 86). The interaction between B7 and CD28 may determine whether a T-cell response develops. Blocking antibodies to B7-2 inhibit the development of specific IgE, pulmonary eosinophilia and airway hyperresponsiveness in mice (87). A molecule on activated T cells, cytotoxic T-lymphocyte antigen 4 (CTLA4), seems to act on endogenous inhibitor of T-cell activation and CTLA4-Ig, a soluble fusion-protein construct, is also effective in blocking airway hyperresponsiveness in mice (88). Anti-CD28, anti-B7-2 and CTLA4-Ig also block the proliferative response of T cells to allergen (89). Furthermore, cytokine production by memory/effector T cells, particularly those of Th2 subset, is highly dependent on co-stimulation via the ICOS/B7RP1 (ICOS-ligand) pathway. Blockage (90) or genetic disruption (91) of this co-stimulatory pathway markedly reduced allergen-induced asthma in mice. Thus, blockage of B7-2/CD28 and ICOS/B7RP1 interaction may be a promising approach for the treatment of allergic disease in humans.

One mechanism of direct T-cell suppression by IL-10 is due to the inhibition of CD28 co-stimulation. The IL-10 inhibits the proliferative T-cell response in PBMC to various antigens, and the superantigen staphylococcal enterotoxin B (92). However, IL-10 does not affect the proliferative responses of T cells that were stimulated by anti-CD3. In contrast, IL-10 significantly inhibits the anti-CD28-stimulated proliferation. The analysis of T-cell receptor (TCR) numbers on T cells demonstrated the essential requirement for co-stimulation in T-cell activation and its relation to the number of triggered TCRs (92). The IL-10 inhibited the T-cell proliferation within a certain range of triggered TCRs that T cells require co-stimulation. The T cells, which were stimulated by different concentrations of anti-CD3, and a constant amount of anti-CD28 showed that low number of triggered TCRs required CD28 co-stimulation. Thus, IL-10 suppressed only those T cells that had low number of TCRs triggered and which required CD28 for proliferation (92).

Stimulation of CD28 by B7 surface molecules leads to tyrosine phosphorylation of CD28. Ligation of IL-10 receptor (IL-10R) at the time of CD28 stimulation inhibits tyrosine phosphorylation of CD28 as detected after 10 min (92, 93). The inhibitory effect of IL-10 on CD28 appeared to be specific for the CD28 pathway, because IL-10 did not affect ZAP-70 tyrosine phosphorylation stimulated by CD3 cross-linking. As a consecutive event for signal transduction, the association of CD28 with the phosphatidylinositol 3-kinase (PI3-K) p85 molecule was inhibited by IL-10. This inhibition can be specifically blocked by preventing IL-10 binding to its receptor with an anti-IL-10R mAb. The PI3-K is a heterodimer that comprises an 85 kDa regulatory and a 110 kDa catalytic subunit possessing both protein serine

kinase and lipid kinase activity (94). Binding of PI3-K to CD28 occurs by direct interaction between SH2 domain motifs of p85 PI3-K and a (p)YXXM motif in the cytoplasmic part (95).

Taken together, after a century of its first usage, the term 'anergy' sits on an immunological basis. Containment and cure of tuberculosis requires an effective cell-mediated immune response and its absence during active tuberculosis infection has been demonstrated to be related to IL-10-mediated peripheral tolerance (96–98). Accordingly, T-cell response in IL-10-mediated peripheral tolerance is the same as definition of anergy, because co-stimulatory signals are suppressed and T cells receive a weak signal only via the TCR without co-stimulation (6). To date, there was no report of clinical studies, which relates inhibition of co-stimulation to the specific treatment of allergy and asthma. Nevertheless, it remains as a fruitful approach for the treatment of both autoimmunity and allergy.

T-regulatory cells

The T cells that were able to suppress immune responses were described first in the early 1970s (99). Suppressor T cells were thought to be a specialized subpopulation, the effects of which were mediated in an antigen-specific fashion (56, 100). Unfortunately, the failure to clearly identify the mechanisms underlying immune suppression led to a collapse in the entire field in 1980s (101). The concept of T cell-mediated immune suppression is being strongly explored starting from the mid-1990s. Many types of suppressor T cells have been described in a number of systems, and their biology has been the subject of intensive investigation (Table 1). Although many aspects of the mechanisms by which suppressor cells exert their effects remain to be elucidated, it is well-established that T_{Reg} cells suppress immune responses via cell-to-cell interactions and/or the production of suppressor cytokines (30, 31, 35, 102).

Tr1 cells

Type 1 T-regulatory (Tr1) cells are defined by their ability to produce high levels of IL-10 and TGF- β (35, 102). The Tr1 cells specific for a variety of antigens arise *in vivo*, but may also differentiate from naive $CD4^+$ T cells. The Tr1 cells have a low proliferative capacity, which can be overcome by IL-15 (103). The Tr1 cells suppress naive and memory Th1 or -2 responses via production of IL-10 and TGF- β (102). The use of Tr1 cells to identify novel targets for the development of new therapeutic agents, and as a cellular therapy to modulate peripheral tolerance in allergy and autoimmunity, can be foreseen (30, 63, 104).

The generation *in vitro* of a T_{Reg} cell subset by stimulating naive $CD4$ T cells in the presence of IL-10, IFN- α or a combination of IL-4 and IL-10, has

Table 1. Regulatory/suppressor cells and their subsets

Regulatory/suppressor cells		Suppressor mechanism*	References
T cells	Tr1	IL-10, TGF- β	30–35, 102–106
	Th3	TGF- β	33, 107
	$CD4^+CD25^+ T_{Reg}$	IL-10, TGF- β , CTLA-4, PD-1, GITR	108–113
	$CD8^+CD25^+$	Same as $CD4^+ CD25^+$	114–117
	$CD28^- T_{Reg}$		
	$CD4^-CD8^- T_{Reg}$	Induction of apoptosis	118–119
	$TCR\gamma\delta T_{Reg}$	IL-10, TGF- β	120–123
B cell subset	B_{Reg}	IL-10	124
DC	DC_{Reg}	IL-10	125–127
NK cell subset†	NK_{Reg}	IL-10	131, 132
Macrophages		IL-10, TGF- β	131
Resident tissue cells†		IL-10, TGF- β	128–133

* Studies that investigate suppressor mechanisms are not conclusive. It is possible that multiple other suppressive mechanisms exist.

† NK cells and resident tissue cells are included in the Table because of expression of suppressive cytokines by these cells.

DC, dendritic cell; NK, natural killer; Tr1, type 1 T-regulatory (Tr1) cells; IL, interleukin; TGF, transforming growth factor; PD-1, programmed death-1.

previously been reported (35, 102). To overcome the problems in cytokine profiles of T_{Reg} cells, it has been demonstrated that a combination of vitamin D3 and dexamethasone, induced human and mouse naive $CD4^+$ T cells to differentiate *in vitro* into T_{Reg} cells (105). In contrast to the previously described *in vitro*-derived $CD4^+$ T cells, these cells produced only IL-10, but no IL-5 and IFN- γ , and furthermore, retained strong proliferative capacity and prevented central nervous system inflammation in an IL-10-dependent manner. There is now clear evidence that IL-10- and/or TGF- β -producing Tr1 cells are *in vivo* generated in humans during the early course of allergen-SIT, suggesting that high and increasing doses of allergens induce Tr1 cells in humans (31, 32, 106).

Th3 cells

Regulatory/suppressor T-cell clones have been induced by oral feeding of low doses of antigen in a TCR-transgenic experimental encephalitis model (33, 107). The $CD4^+$ T-cell clones isolated from mesenteric lymph nodes in orally tolerated animals produced high levels of TGF- β , and variable amounts of IL-4 and IL-10 upon activation with appropriate antigen or anti-CD3 antibody (33). These cells functioned *in vivo* to suppress encephalitis induction with myelin basic protein and were designated as Th3 cells. The TGF- β and IL-10 seemed critical, as treatment with neutralizing antibodies abrogated the disease-protective effects of these cells. These T_{Reg} cells also exerted bystander immune suppression *in vitro*.

CD4⁺CD25⁺ T_{Reg} cells

There is clear evidence from various animal models and human studies for an active mechanism of immune suppression, whereby a distinct subset of T cells inhibits the activation of conventional T cells in the periphery (108–111). This T_{Reg} cell population has been determined as CD4⁺CD25⁺ T cells. The CD4⁺CD25⁺ T cells constitute 5–10% of peripheral CD4⁺ T cells and express the IL-2R α chain (CD25) (108). They can prevent the development of autoimmunity indicating that the normal immune system contains a population of professional T_{Reg} cells. Elimination of CD4⁺CD25⁺ T cells leads to spontaneous development of various autoimmune diseases, such as gastritis or thyroiditis in genetically susceptible hosts. In mice these cells have been shown to express CD45RB^{low} (34). The CD38[−]CD25⁺ CD4⁺CD45RB^{low} subpopulation contains T cells, which respond to recall antigens and produced high levels of cytokines in response to polyclonal stimulation. In contrast, the CD38⁺ cells within this subpopulation fails to proliferate or to produce detectable levels of cytokines, and furthermore inhibits anti-CD3-induced proliferation induced by CD38[−] population (112).

There are two major hypotheses concerning the generation of CD4⁺CD25⁺ T_{Reg} cells. One of these, suggests that T_{Reg} cells emerge from the thymus as a distinct subset of mature T cells with defined functions (108, 110). On the contrary, several studies have shown that T_{Reg} cells may differentiate from naive T cells in the periphery upon encountering antigens present at high concentrations (35, 105, 113). It can be proposed that thymic differentiation accounts for T_{Reg} cells that are specific for self-peptides and are devoted to the control of autoimmune responses, whereas peripheral differentiation may be required for environmental antigen-specific T cells for which an undesired immune response results in pathology.

Other T-regulatory cells

It has been proposed that, in addition to CD4⁺ T cells, CD8⁺ T_{Reg} cells may have a role in oral tolerance (114, 115). Recent efforts to generate suppressor cell lines *in vitro* resulted in a population of CD8⁺CD28[−] T cells restricted by allogeneic HLA class I antigens, which were able to prevent up-regulation of B7 molecules induced by Th cells on APCs (116). This resulted in the suppression of CD4⁺ T cells in an HLA-nonrestricted fashion (116). Interestingly, the magnitude of a CD8⁺ T cell-mediated immune response to an acute viral infection is also subject to control by CD4⁺CD25⁺ T_{Reg} cells. If natural T_{Reg} were depleted with specific anti-CD25 antibody before infection with virus, the resultant CD8⁺ T-cell response was significantly enhanced suggesting that controlling suppressor effects at the time of vaccination could result in more effective immunity (117).

Double negative (CD4[−]CD8[−]) TCR $\alpha\beta$ ⁺ T_{Reg} cells that mediate tolerance in several experimental autoimmune diseases have been described (118). These double-negative T cells are specific for major histocompatibility complex (MHC) class I molecules and the suppressive effect of these cells on the proliferation and cytotoxic activity of CD8⁺ T cells with the same antigen specificity was not mediated by cytokines, but instead was attributed to Fas-mediated apoptosis of alloreactive T cells (119).

The $\gamma\delta$ T cells with regulatory functions have also been described. A population of $\gamma\delta$ T_{Reg} cells with a cytokine profile reminiscent of Tr1 cell clones has been isolated from tumor-infiltrating lymphocytes (120). These T_{Reg} cells could play a role in the inhibition of immune responses to tumors (121). It has also been shown that aerosol delivery of protein antigens resulted in the differentiation of $\gamma\delta$ T cells with regulatory functions (120). It has been observed that induction of tolerance by various doses of ovalbumin (OVA) was abrogated in mice lacking TCR δ (122). In contrast, TCR $\gamma\delta$ -deficient mice have the same degree of IgE-specific unresponsiveness after aerosol priming and immunization with OVA (123).

B_{Reg} cells

A regulatory role for IL-10-secreting B cells has been recently proposed (124). These B cells prevented the development of arthritis and their suppressive effect was particularly IL-10-dependent, because the B cells isolated from IL-10-deficient mice failed to protect from arthritis.

Dendritic_{Reg} cells

It is generally thought that immature dendritic cells (DC) do not appropriately activate T cells, which may lead to tolerance (125). In normal immunity, DC should not have any restriction in antigen presentation and they should appropriately receive maturation signals given by the surroundings of the antigen, T cells and other tissue cells, such as co-stimulatory ligands, cytokines, innate immune response stimulating (i.e. Toll-like receptor triggering) substances. However, there are some indications that DC can induce peripheral T-cell tolerance and a regulatory DC subset may exist. Pulmonary DC from mice exposed to respiratory antigen transiently produce IL-10 (126). These phenotypically mature pulmonary DCs, which were B7^{hi} stimulated the development of CD4⁺ Tr1-like cells that also produced high amounts of IL-10. Adoptive transfer of pulmonary DCs from IL-10^{+/+}, but not IL-10^{−/−}, mice exposed to respiratory antigen-induced antigen-specific unresponsiveness in recipient mice. In accordance with these findings, IL-10 inhibited the development of fully mature DC, which induced a state of alloantigen-specific anergy in CD4⁺ T cells (127). These studies show that IL-10 production by DCs is critical for

the induction of tolerance, and that phenotypically mature DC_{Reg} may exist under certain circumstances.

Other cells with possible regulatory function

It has been clearly demonstrated that natural killer cells, epithelial cells, macrophages, glial cells etc. express suppressor cytokines such as IL-10 and TGF- β . Although their role has not been coined as professional regulatory cells, these cells may efficiently contribute to the generation and maintenance of a regulatory/suppressor type immune response (128–133). The expression of suppressor cytokines in resident tissue cells may additionally contribute to this process.

Suppression mechanisms of T-regulatory cells

A great deal of uncertainty remains about mechanisms of action of T_{Reg} cells. Initial studies have shown that T_{Reg} cells act as suppressor T cells, which down-regulate effector cells and inflammation models in chronic infection, organ transplantation and autoimmunity (33, 35, 134). Most studies have failed to find a soluble factor as a suppressive mechanism of CD4⁺CD25⁺ T_{Reg} cells. Antigen-induced proliferation of CD4⁺ T cells was dramatically reduced following coculture with activated T_{Reg} clones, which had been separated from the responding T cells by a Transwell insert (135). However, in Transwell membrane cultures that separate suppressor cells and target cells, the distance between two populations is approximately 2 mm and this may influence the concentration of suppressor cytokines. Accordingly, it cannot be possible to rule out an effect of a cytokine that acts in short distances or a membrane bound cytokine. Indeed, membrane bound TGF- β might be one of the mechanisms of suppression of CD4⁺CD25⁺ T_{Reg} cells (136). In contrast to CD4⁺CD25⁺ T_{Reg} cells, suppressive effects of Tr1 cells was reversed by addition of neutralizing mAb, directed against TGF- β and IL-10, implicating the role of suppressive cytokines in the mechanism of immune suppression both *in vitro* and *in vivo* in different settings and different autoimmune as well as allergy models (32, 35, 92, 136, 137). This suppression was a hallmark of Tr1 clones, as OVA-specific Th1 or Th2 clones, derived from the same mice, had no suppressive effects, but rather enhanced OVA-induced proliferation of naive CD4⁺ T cells (38).

One group of CD4⁺CD25⁺ T_{Reg} cells originate from the thymus as a distinctive subset (108, 110, 138). Thymectomy at a very early stage of animal development induces various autoimmune diseases in genetically susceptible animals (139, 140). Furthermore, induction of autoimmune diseases in an immunodeficient animal model was prevented by adoptively transferred CD4⁺ T cells or CD4⁺CD8⁻ thymocytes isolated from normal syngeneic animals. In a rat model, CD4⁺ T_{Reg} cells were found to

be of the CD45RC^{low} phenotype and to produce IL-2 and IL-4, but not IFN- γ , upon *in vitro* stimulation (139). The IL-4 and TGF- β are critical in preventing autoimmunity, as neutralization of either of these two cytokines abrogates the protective response. In another study, CD4⁺CD25⁺ T_{Reg} cells from thymus were shown to exert their suppressive function via the inhibition of IL-2R α chain in target T cells, induced by the combined activity of CTLA-4 and membrane TGF-B1 (141).

Studies of this activated CD4⁺ T cell subpopulation have shown that they do not proliferate upon normal TCR-mediated stimulation and suppress proliferation of other T cells. The TCR stimulation was required for these cells to exert suppression of other T cells; such suppression, however, was not confined to T cells specific for the same antigen. The CD4⁺CD25⁺ T cells are the only lymphocyte subpopulation in both mice and humans that express CTLA4 constitutively. The expression apparently correlates with the suppressor function of CTLA4. The addition of anti-CTLA4 antibody or its fragment of antigen binding (Fab) reverses suppression in cocultures of CD4⁺CD25⁺ and CD4⁺CD25⁻ T cells (142). Similarly, the treatment of mice, which are recipients of CD4⁺CD45RB^{low} T cells with these agents abrogated the suppression of inflammatory bowel disease (143). These studies indicate that signals that result from the engagement of CTLA4 by its ligands, CD80 or CD86, are required for the induction of suppressor activity. Under some circumstances, the engagement of CTLA4 on the CD4⁺CD25⁺ T cells by antibody or by CD80/CD86 might lead to inhibition of the TCR-derived signals that are required for the induction of suppressor activity.

Programmed death-1 (PD-1) is an immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptor expressed upon T-cell activation. The PD-1-deleted mice develop autoimmune diseases, suggesting an inhibitory role for PD-1 in immune responses (144). Members of the B7 family, PD-L1 and PD-L2, are ligands for PD-1. The PD-1 : PD-L engagement on murine CD4 and CD8 T cells results in inhibition of proliferation and cytokine production. The T cells stimulated with anti-CD3/PD-L1 display dramatically decreased proliferation and IL-2 production (145). The PD-1 : PD-L interactions inhibit IL-2 production even in the presence of costimulation and, thus, after prolonged activation, the PD-1 : PD-L inhibitory pathway dominates. Exogenous IL-2 is able to overcome PD-L1-mediated inhibition at all times, indicating that cells maintain IL-2 responsiveness.

Glucocorticoid-induced tumour necrosis factor receptor family related gene (TNFRSF18, GITR) is expressed by CD4⁺CD25⁺ alloantigen-specific and naturally occurring circulating T_{Reg} cells (146, 147). Stimulation of CD25⁺CD4⁺ regulatory T cells through GITR breaks immunological self-tolerance (147). The GITR is up-regulated in CD4⁺CD25⁻ T cells after TCR stimulation and it also functions as a survival signal for activated cells (148). In addition, CD103 (α E β 7 integrin) and

CD122 (β chain of IL-2R) are highly expressed on $CD4^+CD25^+$ T_{Reg} cells, which correlates with their suppressive activity (149, 150).

An X-linked forkhead/winged helix transcription factor, FoxP3 (Scurfin) is essential for the suppressive function of $CD4^+CD25^+$ T_{Reg} cells (151, 152). It is highly expressed in $CD4^+CD25^+$, but not $CD4^+CD25^-$ T cells (151, 152). It acts as a silencer of cytokine gene promoters and programmes the development and function of $CD4^+CD25^+$ T_{Reg} cells (151–154). Mutations in the FoxP3 gene in human leads to a severe immune dysregulation with polyendocrinopathy, enteropathy and hyper-IgE known as IPEX syndrome (155).

The failure of T_{Reg} cells to proliferate after TCR stimulation *in vitro* has suggested they are naturally anergic. However, T_{Reg} cells expressing a transgenic TCR were shown to proliferate and accumulate locally in response to transgenically expressed tissue antigen, whereas their $CD25^-$ counterparts are depleted at such sites (156). $CD4^+CD25^+$ T_{Reg} cells population is composed of two T_{Reg} subsets that have distinct phenotypes. Some T_{Reg} remain quiescent and have a long lifespan, in the order of months, whereas the other T_{Reg} cells (mainly the autoantigen-specific ones) divide extensively and express multiple activation markers (157).

Clinical relevance of T-regulatory cells

As the concept of professional suppressor cells is recovering interest among the immunological community, it is now time to consider how the manipulation of regulatory/suppressor T cells might be used clinically. As tumour antigens are an important group of autoantigens, the depletion of T_{Reg} cells should result in an enhanced immune response to tumour vaccines. Several studies have shown that the antibody-mediated depletion of $CD25^+$ T cells facilitates the induction of tumour immunity (158, 159).

Currently, the relationship between the different T_{Reg} cell populations is unclear with respect to their development, and activation. However, numerous animal experiments have clearly shown that T_{Reg} cells can suppress both Th1 and Th2 responses *in vivo* and thereby actively suppress the development of autoimmune and allergic responses. Recent studies reported that the application of *in vitro* engineered allergen-specific T_{Reg} cells lines protected mice from developing allergen-induced Th2 responses (160).

In humans, there is circumstantial evidence to suggest that T_{Reg} cells play a major role in the inhibition of allergic disorders. It has been reported that IL-10 levels in the bronchoalveolar-lavage fluid of asthmatic patients are lower than in healthy controls, and that T cells from children suffering from asthma also produce less IL-10 mRNA than T cells from control children (161, 162). These findings indicate that increased IL-10 production is

associated with decreased allergic reactions. As T_{Reg} cells are a major source of IL-10, it has been speculated that T_{Reg} cells secreting IL-10 are involved in the suppression of allergic Th2 responses in humans. Several human allergen-SIT studies supported this hypothesis (31, 63, 163). In contrast, some studies demonstrated that increased IL-10 levels are not associated with less allergic disease (164). The IL-10 may also promote airway hyperresponsiveness (165) and even eosinophilia (166) in allergy models. In contrast to its known T-cell suppressive activity, some reports imply a role for TGF- β in the pathogenesis of asthma, particularly in remodeling of injured lung tissue in humans (167). A recent report indicated that the increased allergic inflammation observed after blocking of CTLA-4 is clearly associated with decreased TGF- β levels in the bronchoalveolar-lavage fluid of these animals (168). Furthermore, inhibition of experimental tracheal eosinophilia was also due to the induction of $CD4^+$ T cells secreting TGF- β (169).

An alloantigen-independent, systemic expansion of the maternal $CD4^+CD25^+$ T cells with dominant regulatory T cell activity has been demonstrated (170). In addition to their function in suppressing autoimmune responses, maternal T_{Reg} cells suppressed an aggressive allogeneic response directed against the fetus. Their absence led to a failure of gestation because of immunological rejection of the fetus.

To analyse human *in vivo* existence of T_{Reg} cells, lymphocyte populations in human lymph nodes with a special emphasis on the $CD4^+CD25^+$ T_{Reg} cells have been investigated (171). $CD4^+CD25^+$ T cells constitutively co-expressed high levels of CD152. Similar to T_{Reg} cells from peripheral blood, T_{Reg} cells from lymph node were *in vitro* anergic and efficiently inhibited other $CD4^+$ and $CD8^+$ lymphocyte proliferation (171). The T_{Reg} cells may play destructive roles in cancer and chronic infectious diseases (172–177). Further studies are needed to demonstrate in the clinic, whether *in vivo* generation or adoptive transfer of T_{Reg} cells and/or their related suppressive cytokines may change the course of allergy and asthma. Small molecular weight compounds that may generate T_{Reg} cells or increase their suppressive properties is an important target not only for the use in allergy and asthma, but also for transplantation and autoimmunity.

cAmp stimulating G-protein coupled receptors in peripheral tolerance

The superfamily of seven transmembrane G-protein coupled receptors is the largest and most diverse group of membrane spanning proteins (178). Within all identified human genes, approximately 1000 encode G-protein coupled receptors. Many established G-protein coupled receptor systems have been successfully exploited by the pharmaceutical industry to become the target for

approximately 40% of the currently available drugs (178). As a small molecular weight monoamine that binds to four different G-protein coupled receptors, histamine was recently demonstrated to regulate several essential events in the immune response (179, 180). The expression of these receptors on different cells and cell subsets is regulated and apparently, diverse effects of histamine on immune regulation are because of differential expression of these receptors and their distinct intracellular signals. Histamine receptor (HR) 2 is coupled to adenylate cyclase and studies in different species and several human cells demonstrated that inhibition of characteristic features of the cells by primarily cAMP formation dominates in HR2-dependent effects of histamine (181). Recent studies indicated that HR3 and HR4 might antagonize with HR2-mediated suppression of the cells (182–184).

Histamine actively participates in functions and activity of DC precursors as well as their immature and mature forms. Immature and mature DCs express all four HR (185–188). In the differentiation process of type 1 DC from monocytes, HR1 and HR3 act as positive stimulants that increase antigen-presentation capacity and proinflammatory cytokine production and Th1 priming activity. In contrast, HR2 acts as a suppressive molecule for antigen-presentation capacity, enhances IL-10 production and induces of IL-10-producing T cells (189–191).

In monocytes stimulated with Toll-like receptor-triggering bacterial products histamine inhibits the production of proinflammatory IL-1-like activity, TNF α and IL-12, but enhances IL-10 secretion, through HR2 stimulation (191–193). Histamine induces intracellular Ca²⁺ flux, actin polymerization, and chemotaxis in immature DCs due to stimulation of HR1 and HR3 subtypes. Maturation of DCs results in loss of these responses. In maturing DCs, however, histamine dose-dependently enhances intracellular cAMP levels and stimulates IL-10 secretion, while inhibiting production of IL-12 via HR2 (190).

It has been demonstrated that differential patterns of HR expression on Th1 and Th2 cells determine reciprocal T-cell responses following histamine stimulation (40). The Th1 cells show predominant, but not exclusive expression of HR1, while Th2 cells shows increased expression of HR2. Histamine enhances Th1-type responses by triggering the HR1, whereas both Th1- and Th2-type responses are negatively regulated by HR2, because of the activation of different biochemical intracellular signals (40). In mice, deletion of HR1 results in suppression of IFN- γ and dominant secretion of Th2 cytokines (IL-4 and IL-13). The HR2-deleted mice show up-regulation of both Th1 and Th2 cytokines. In addition, histamine stimulation induced IL-10 secretion through HR2 (194). Increased IL-10 production in both DC and T cells may account for an important regulatory mechanism in the control of inflammatory functions through histamine. In accordance with this phenomenon, it has recently been demonstrated that histamine supports the suppressive effect of TGF- β on T cells via HR2 (195). The Th2 cells are more affected

by histamine-enhanced TGF- β suppression, which is particularly important for the regulation of allergen-specific T cells in allergic immune responses.

Clinical evidence for T regulatory function of histamine receptors

Considerable evidence has emerged to suggest that histamine participates in the immune regulation of the inflammatory response in several diseases (Fig. 2). Histamine interferes with the peripheral tolerance induced during SIT in several pathways. Histamine induces the production of IL-10 by DC (190). In addition, histamine induces IL-10 production by Th2 cells (194). Furthermore, histamine enhances the suppressive activity of TGF- β on T cells (195). All three of these effects are mediated via HR2, which is relatively highly expressed on Th2 cells and suppresses IL-4 and IL-13 production and T-cell proliferation (40). Apparently, these recent findings suggest that HR2 may represent an essential receptor that

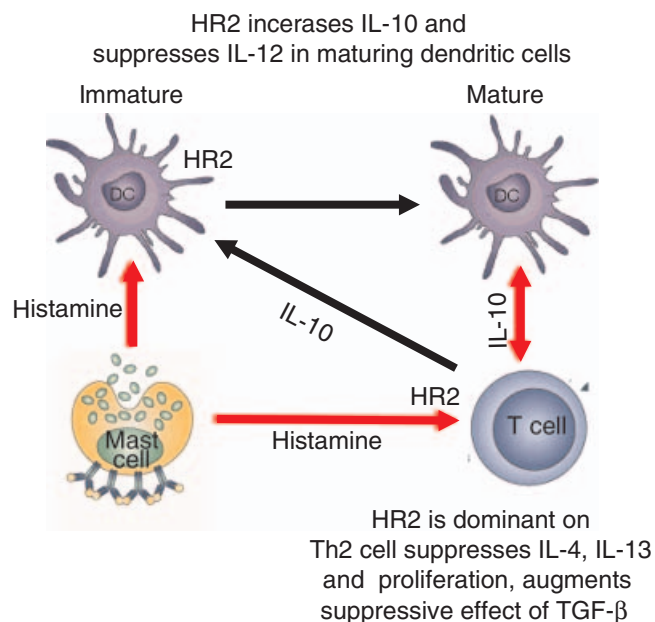


Figure 2. Regulatory/suppressor functions of histamine by histamine receptor 2. In maturing dendritic cells (DCs), histamine enhances intracellular cAMP levels and stimulates interleukin (IL)-10 secretion, but suppresses IL-12 by histamine receptor (HR)2. The IL-10 affects the maturation of DC to IL-10-producing DC and induces of IL-10-producing T cells. The T helper (Th)2 cells show increased expression of HR2 and T-cell proliferation and IL-4 and IL-13 production are negatively regulated by HR2. The IL-10 suppresses the antigen-presentation capacity and proinflammatory cytokine synthesis of DC. In addition, Th2 cells are more efficiently suppressed by transforming growth factor (TGF)- β in the presence of histamine via HR2 (red line: suppression, black line: differentiation).

participates in peripheral tolerance or active suppression of inflammatory/immune responses.

The long-term protection from honeybee stings by terfenadine premedication during rush immunotherapy with honeybee venom in a double-blind, placebo-controlled trial was analysed (196). After an average of 3 years, 41 patients were re-exposed to honeybee stings. Surprisingly, none of 20 patients who had been given HR1-antihistamine premedication, but six of 21 given placebo, had a systemic allergic reaction to the re-exposure by either a field sting or a sting challenge. This highly significant difference suggests that antihistamine premedication during the initial dose-increase phase may have enhanced the long-term efficacy of immunotherapy. Expression of HR1 on T-lymphocytes is strongly reduced during ultrarush immunotherapy, which may lead to a dominant expression and function of tolerance-inducing HR2. This indicates a positive role of histamine in immune regulation during SIT (197).

Selective HR2 antagonists, have attracted interest because of their potential immune response-modifying activity (198). Most data suggest that cimetidine has a stimulatory effect on the immune system, possibly by blocking the receptors on subsets of T-lymphocytes and inhibiting HR2-induced immune suppression. Cimetidine has also been used to restore immune functions in patients with malignant disorders, HIV/AIDS and other viral infections (199–201). Although their systemic usage may cause side-effects such as ulcer development, together these findings are tempting to investigate local usage of selective agonists of HR2 and antagonists of HR3 and HR4 in the treatment of allergic diseases. Apparently, as a result of same signal transduction patterns, β_2 -adrenergic receptors may function similar to HR2 in humans (202, 203). The role of histamine and other redundant G-protein coupled receptors in the regulation of immune/inflammatory pathways in allergic inflammation remain to be intensely focused in future studies.

Conclusion

Peripheral T-cell tolerance is the key immunological mechanism in healthy immune response to self- and noninfectious, nonself-antigens. This phenomenon is clinically well-documented in allergy, autoimmunity, transplantation, tumour and infection. There is a growing evidence supporting the role for T_{Reg} cells and/or immunosuppressive cytokines as a mechanism, by which allergen-SIT and healthy immune response to allergens is mediated (Fig. 1). In addition to the treatment of established allergy, it is essential to consider prophylactic approaches before initial sensitization has taken place. Preventive vaccines that induce T_{Reg} responses can be developed. Allergen-specific T_{Reg} cells may in turn dampen both the Th1 and Th2 cells and cytokines, ensuring a well-balanced immune response. Enhancement of the number and activity of T_{Reg} cells could be an obvious goal for the suppression of allograft rejection, graft-versus-host disease and autoimmunity. The T_{Reg} cells may not be always responsible for healthy immune response, because several studies have shown that they may be responsible for the chronicity of infections and tumour tolerance. The T_{Reg} cell populations have proven possible, but difficult to grow, expand and clone *in vitro*. A crucial area for future studies is the identification of drugs, cytokines or co-stimulatory molecules that induce the growth while preserving the suppressor function of the T_{Reg} cells. In this context, by the application of the recent knowledge in peripheral tolerance mechanisms, more rational and safer approaches are awaiting for the future of prevention and cure of allergic diseases.

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