

Review article

T-cell tolerance in allergic response

C. B. Schmidt-Weber, K. Blaser

Swiss Institute of Allergy and Asthma Research (SIAF),
Obere Str. 22, CH-7270 Davos, Switzerland

C. B. Schmidt-Weber
Swiss Institute of Allergy and Asthma Research (SIAF)
Obere Str. 22
CH-7270 Davos
Switzerland

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Introduction

Allergic diseases are immunological disorders (1), which originate from the activation of T-cell subsets secreting allergic, inflammatory factors including interleukin (IL)-4 and IL-5 and/or IL-13. The regulation of allergen-specific T cells is one of the strategies to control disease. However, available drugs such as steroids are not allergen-specific and only temporarily suppress the activity of immune cells. Thus, they are not curative and accompanied by side effects. In contrast, allergen-specific immunotherapy (SIT) can specifically restore normal immunity against allergens. Successful SIT is characterized by the induction of peripheral T-cell tolerance against the allergen (2, 3). Although successful SIT applies predominantly for treatment of mono- or oligo-specific allergies, the underlying immuno-regulatory mechanism can possibly be projected on allergic disease in general. Lymphocyte tolerance describes a complex process, which balances the reactivity against foreign vs. autologous antigens. Autoreactive lymphocytes are eliminated early in their thymic development, a process, which is covered by the term central tolerance (4). T cells, however, might escape thymic selection or might face harmless antigens later in their development, which are meant to be under the control of peripheral tolerance (5). Lymphocyte tolerance is likely to be a dynamic process (6), which adapts to the development of the organism to effectively distinguish autologous from foreign antigens. Recent investigations on peripheral tolerance revealed several basic mechanisms. These mechanisms comprise cell survival, as cells might undergo apoptosis, which would

delete antigen specificities. Furthermore, antigens located in anatomically 'hidden' areas could be neglected by the immune system. Finally, cells could be suppressed and/or undergo clonal anergy, which keeps already existing lymphocyte specificities under control (7–9). The latter mechanism appears to be particularly interesting for the understanding of the pathogenesis of allergic disease, because allergen-specific T cells can be isolated, even from donors, who do not suffer from allergies. Moreover, it is frequently observed that patients are sensitized against two allergens but only show clinical symptoms against one of them. Interestingly, both the clinically silent and the pathogenic T-cell specificities, express both Th2-like cytokines.

Defective peripheral T-cell tolerance as a cause of allergies

Normal immune responses to allergens are balanced by the activity of T cells, which express suppressive cytokines (Th3, Tr). This balance probably allows a limited activity of allergen-specific cells to eliminate the antigen, but prevents more excessive activity, which would result in inflammation and tissue destruction. Although the exact mechanism of this suppressive balance is still poorly understood, recent studies demonstrated that the suppressive cytokines IL-10 and transforming growth factor (TGF)- β are important mediators in this process. Experimental models demonstrate that functional interference of T-regulatory molecules such as TGF- β induces allergy like symptoms (10) and autoimmune diseases (11, 12). However, major

differences exist in the regulation of peripheral T-cell tolerance between mice and man. In particular, regulation of the IL-10 gene differs in that murine IL-10 gene expression is linked to the IL-4 locus and can be induced by IL-4 alone in the absence of TCR engagement (13). In contrast, human IL-10 can also be expressed in Th1-like cells and cannot be induced by IL-4 in the absence of TCR stimulation (C.B. Schmidt-Weber, unpublished observations).

Considerable knowledge on peripheral tolerance induction in allergy of human could be raised in SIT of bee venom allergy and has previously been reviewed (14–17). The mechanism of SIT has been attributed to the expression of suppressive cytokines by allergen-specific T cells, but also by monocytes and B cells, which however, start to express IL-10 at the later course of SIT (14). For allergens, which enter the organism via mucosal surfaces, TGF- β plays an important role in addition to IL-10. In case of SIT against house dust mite, IL-10 and TGF- β are induced and result in decreased allergen-specific proliferation and cytokine secretion (18, 19). Moreover, the cytokines IL-10 and TGF- β also affect B-cell activity towards immunoglobulin (Ig)G4 and IgA production. Both are suppressive for IgE. Accordingly, the allergen-specific isotype profile shifts during SIT towards normal IgG4/IgA levels and the ratio of specific IgE to IgG4 or IgA changes about 100–1000-fold. Interestingly, individuals who had been multiply stung and are hyperimmunized against bee venom (e.g., bee keepers) also show, as observed in BV-SIT, a high IL-10 expression in T cells within 7 days and decreased cytokine and proliferative responses (20). Successful SIT in bee venom patients results in tolerization of bee stings without the risk of anaphylaxes and clinical symptoms of house dust mite or cat allergies can also be improved (18, 21).

Mechanisms of T-cell tolerance

Two signal hypothesis

Despite considerable advances in the understanding of SIT, it still remains unclear how high-dose allergen administration can induce IL-10 production in lymphocytes. Several mechanisms were reported in the literature, which provide models for regulation of peripheral tolerance (Fig. 1). However, T-cell unresponsiveness (anergy) can also be induced *in vitro*, when pure antigen-specific T cells are incubated with peptides in the absence of antigen presenting cells (APCs) (25). Under these conditions, T cells are possibly presenting the antigens themselves, but fail to costimulate each other and therefore, receive only one T-cell activation signal. For successful T-cell stimulation, two signals are necessary, whereas one signal alone results in T-cell anergy, which can be resolved by the addition of IL-2 (26) and IL-15 (27). Therefore, T-cell anergy can be

transient and anergized T cells are potentially available for later immune responses. Although the function of anergic cells as 'inactive memory' is speculative, it is interesting to note that peptide-induced T-cell anergy does not shut down the entire effector program of T cells (23): increased IL-10 production (28), IL-5 and IL-13 as well as upregulation of the IL-2R α were reported [CD25 (23)]. The array of the remaining effector function can vary and might be dependent on the anergizing stimuli (5). Recently, CD25⁺ T cells were described to possess suppressive activity, if they were freshly isolated from the peripheral blood. This population appears to possess suppressor functions (29–32), which are currently difficult to distinguish from very recently activated T cells expressing CD25 for a short time. The expression of CD62 ligand and CTLA-4 might be useful for the identification of the suppressive fraction of CD25⁺ cells (31). Thus, peptide treatment might induce an IL-10-secreting and CD25-expressing suppressor population, which shows features of anergic cells. That anergic T cells can contribute to suppression has been previously suggested by *in vitro* studies, where the addition of anergic cells blocked the activation of antigenic and allogenic responses (33) as well as by studies *in vivo* (34–35). In turn, CD25⁺ T cells can induce anergy in CD25[−] T cells (36). These data reflect a growing concept that anergic cells and suppressor cells share common features and/or are identical.

Although it is now generally accepted that suppressor cells exist, the origin of these cells is still unclear. It is possible that they derive as alternative differentiation pathway ('Th3' cells) starting from a naive cell, which is then driven to IL-10 production in the presence of certain signals such as IL-10, interferon (IFN)- α (37, 38). However, there is also evidence that IL-10 is induced in any T-cell response, but occurs as late as after eight cell cycles (39) as a programmed mechanism to shut down a T-cell response (40). On the background of these findings the question arises how much IL-10 needs to be expressed by a cell to be a suppressor cell or whether there is a dynamic range of suppression.

Suppression

Altered antigen presentation

T cells require a set of signals for full activation, which are provided by APC. The most powerful population of APS are dendritic cells (DC), which reside in lymphnodes, but also in peripheral tissues. Presenting capacity of APCs can be reduced by suppressive cytokines, resulting in reduced MHC-II expression (41). Interestingly, IL-10 can also affect (42) or prevent (43) differentiation into mature DC to drive T cells into an anergic state (for review see reference 41). This mechanism can create the phenomena of bystander suppression, if two antigens are presented at the same time on the same 'tolerogenic' APC

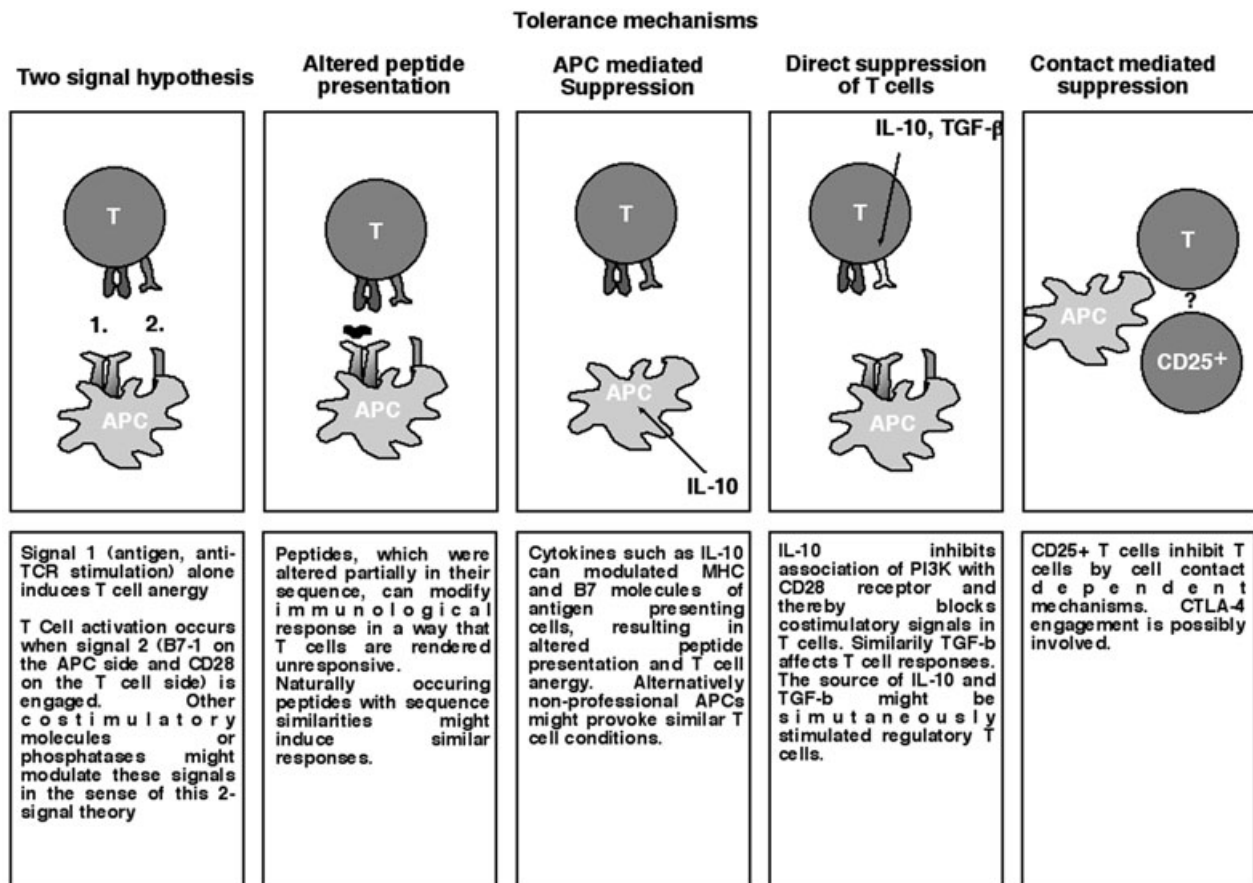


Figure 1. Schematic summary of T-cell tolerance inducing condition. Shown is the T cell (T) with the TCR and the CD28 surface antigen, facing the MHC-II and the CD86 molecule on the antigen presenting cell (APC).

and in the context of the same MHC-II (45, 46). Altered antigen presentation can also occur, when nonprofessional APCs such as endothelial cells trigger T cells (46). Under these circumstances APCs might utilize different subsets of costimulatory molecules (e.g., PD:L1 interaction), which might differentially regulate tolerance (47). Interestingly, suppressor T cells can reduce the DCs costimulatory capacity by reducing CD28 ligands B7 (CD80) and B7.2 (CD86 (48)).

Direct suppression of T cells

A direct effect of suppressive cytokines on T cells was controversial for a long time, but recent experiments clearly demonstrate that both IL-10 and TGF- β directly suppress T cells in the absence of APCs (15, 49–51). Both cytokines are well known as immune suppressive cytokines and genetic deletion of these genes result in severe inflammatory reactions (52–54). For the understanding of cytokine-induced suppression it is important to consider that IL-10 (55) and possibly also TGF- β (C. Akdis, personal communication) suppress costimulatory signals and therefore induce a similar or identical state of anergy as described for the two signal paradigm. Therefore, sup-

pression maintains the antigen-specific pool, but modulates effector functions of lymphocytes. In addition, IL-10 (56, 57) and TGF- β (58–60) can contribute to the survival of lymphocytes. Although IL-10 and TGF- β share features of lymphocyte suppression, some differences exist; whereas IL-10 only suppresses proliferation prior TCR engagement (56), it is possible that TGF- β also affects already activated T cells, because molecules within the TGF- β signaling cascade are upregulated following T cell stimulation (S. Kunzmann, unpublished results). Current investigations do also consider resistance of T cells against TGF- β . The TGF- β RIII endoglin/CD105 has been demonstrated to be present on T cells (61) and is known to negatively regulate the TGF- β signal transduction (62). The endoglin-mediated resistance might enable T cells to become active in environments, which are rich in TGF- β as it is the case in atopic skin lesions. Another negative regulator, SMAD7, has been shown to be induced by IFN- γ (63), which could be important along current Th1/Th2 paradigms of allergic diseases.

A direct effect of suppressive cytokines is also discussed in the context of T-cell differentiation. Repeated stimulation in the presence of these cytokines

promotes expansion of IL-10 and TGF- β expressing cells (37, 64). Of note, the generation of these cells can be driven by immature DC and independently of IL-10 (65). Moreover IFN- α in combination with IL-10 further promotes the differentiation towards IL-10-secreting cells (38). This finding is of particular interest because: (1) IFN- α induces, such as IL-12, STAT4 activation (66), but is in contrast to IL-12 safe for systemic treatment of allergic diseases (67); (2) IFN- α itself negatively regulates T-cell proliferation possibly by downregulating TCR and CD28 molecules (68). TGF- β secretion was also observed in IL-10 induced T-cell differentiation (37), but might be also dependent on the engagement of CTLA-4 (69) on the surface of T cells, which would be in line with the contribution of CTLA-4 in peripheral tolerance (70, 71).

Intracellular mechanisms of T-cell suppression

The regulation of peripheral tolerance and suppression is linked to costimulation of T cells (72–75). Biochemical analysis of costimulation revealed that the phosphatidyl inositol 3-kinase (PI3K) plays a key role in this process (76, 77). IL-10 suppresses the association of the PI3K with the CD28 costimulatory molecule (20, 55, 78) and thereby directly prevents costimulatory signals. The PI3K phosphorylates phosphatidyl inositol 2-phosphates (PIP2) into phosphatidyl 3-phosphates (PIP3). Similarly immune inhibitory receptors of B cells or NK cells do also address PIP3 lipids by activating PIP-phosphatases (e.g., SHIP (79)), suggesting that the prevention of PIP3 increase is a general principle of suppression of the immune system. The PIP3 lipids recruit molecules to the membrane, which contain pleckstrin or FYVE domains. One of the best known downstream targets of PI3K is the PKB or Akt kinase, which regulates diverse cellular processes such as proliferation and apoptosis (80).

However, PI3K is not only stimulated by costimulatory molecules, it is also a target for growth factor mediated signals, including those by IL-2 and IL-4. Therefore, PIP3 levels can reflect multiple signals from the current environment of T cells, which will influence suppressive mechanisms. The PKB/Akt kinase collectively converts these signals into proliferation- and/or survival-signals. Further research will be necessary to define tissue conditions where suppression operates to control peripheral tolerance.

Suppression of allergic responses

Allergic responses are characterized by the fact that allergen-specific T cells belong to the Th2 cytokine dominated subset and that most allergens are recognized in a mucosal environment. Th1 and Th2 cytokine subsets were described to be differentially sensitive to anergization/suppression *in vitro* (81) and *in vivo* (82). At least in rodents, the Th2 cytokine IL-4 also promotes

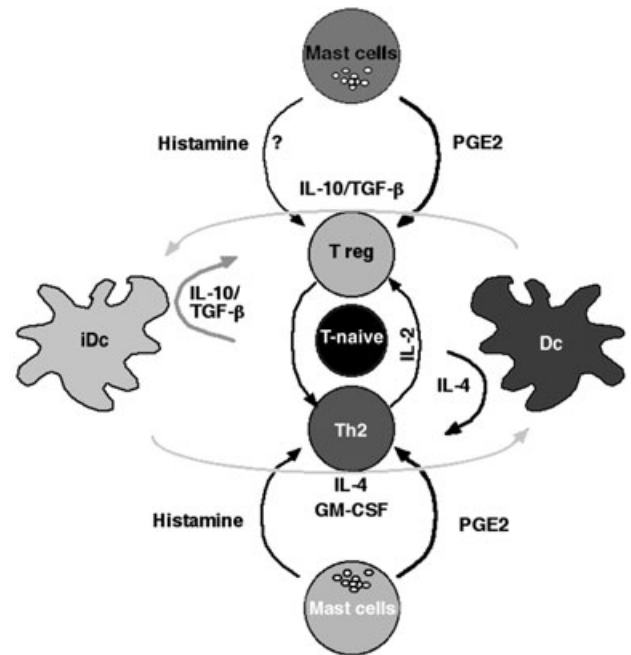


Figure 2. Schematic relationship of regulatory balance of allergen specific T cells. Arrows in light grey indicate differentiation pathways, gray arrows positive and black lines negative regulation pathways. Starting point is the naive T cell, which differentiate into an effector cell and/or regulatory T cell. Whereas regulatory T-cell activity is promoted under conditions where IL-10 and/or TGF- β is abundant, effector T cells are promoted by the presence of IL-4. The differentiation process is linked to the activity of dendritic cells, which are also affected by IL-4 on one hand and IL-10 and/or TGF- β on the other hand. However, undifferentiated DCs do promote T-cell differentiation towards regulatory T cells, which in turn have the potential to limit the activity of differentiated DCs. Th2 type cells affect the activity of Tr or anergic cells by the secretion of IL-2 and Tr inhibiting the activity of Th2 cells by the secretion of IL-10 and/or TGF- β . The environment of allergic inflammation contribute to this balance effector and regulatory cells by secretion of various mediators such as histamine and PGE2, creating conditions which either facilitate or impede induction of peripheral tolerance.

IL-10 production even in the absence of TCR engagement (13) and also promotes differentiation towards TGF- β -secreting cells (83). TGF- β synergizes with IL-4 to induce Th1 cells independently of IL-12 (84). Suppressive cytokines may also drive T-cell differentiation towards cells, predominately expressing IL-10 and/or TGF- β (85). Thus, Th2 type cells promote TGF- β expression intervening a further Th2 differentiation by direct inhibition of Th2 differentiation and/or by promotion of Th1 differentiation. These mechanisms can promote deviations of allergen-induced cytokine secretion and thereby control peripheral tolerance.

In the context of allergen-specific T cells, which are entering inflamed tissues they will be exposed to certain mediators released by allergic effector cells such as mast cells, basophils or eosinophils. Upon IgE crosslinking these inflammatory cells release a variety of proteases, cytokines, leukotrienes, histamine and prostaglandins. In the established disease, these factors can modulate

T-cell responses. Histamine has recently been shown to enhance proliferation of Th1 and to inhibit proliferation of Th2-type cells (86). Furthermore, histamine drives maturing dendritic cells towards an IL-10-producing phenotype, which then negatively regulates T cells (87). Prostaglandins were also reported to influence T-cell anergy. In combination with steroids, prostaglandin E2 (PGE2) promotes anergy when T cells are stimulated with anti-CD3 (88), and PGE2 alone might support anergy induction when T cells are antigen-specifically stimulated (89). Moreover, PGE2 negatively regulates SEB induced upregulation of the skin homing receptor, the cutaneous lymphocyte-associated antigen (CLA), by inhibiting IL-12 secretion (90). Because CLA is necessary for skin homing, PGE2 may reduce the number of skin resident T cells. Transgenic overexpression of prostaglandin D synthase promotes Th2 cytokine release and negatively regulates IFN- γ production (91), which might be related to reduced IL-12 production. Mice lacking the PDG receptor produce similar amounts of specific IgE, but produce less IL-4 and IL-5 and show also reduced eosinophil infiltration and bronchial hyper-reactivity (92). Taken together, it appears that both histamine and PGE2 can deviate cytokine expression and thereby affect peripheral tolerance against allergens.

TGF- β is usually abundant in regenerating tissues particularly where extracellular matrix (EM) is synthesized, because TGF- β is a key regulator for collagen synthesis. The skin-infiltrating T cells are protected against TGF- β -mediated suppression by expressing CD105 (endoglin (61)). Responsiveness to TGF- β can also be reduced by SMAD7, a negative regulator of TGF- β signal transduction, as induced by IFN- γ (63).

Although studies on the negative regulation of suppressive pathways are at a preliminary stage, it is evident that such mechanisms are important for the development of peripheral tolerance in allergic condition. For the development of therapeutic strategies, which aim to restore peripheral tolerance, these environmental factors need to be taken into consideration in particular for established or chronic allergies.

Concluding remarks

The recent development of tolerance research proceeded particularly in the field of DC biology and suppressor T-cell population. Figure 2 schematically describes the relationship of cells encountered in allergic inflammations. Naive T cells encounter the allergen either under the influence of suppressive cytokines, driving them towards regulatory, suppressive T cells (Tr), or T cells secreting more IL-4 (Th2). Corresponding polarizations can also be observed for dendritic cells, which are short lived and can be matured to support certain T-cell polarization pathways. The balance of regulation is affected by tissue factors, released by mast cells, when surface IgE is crosslinked by allergen. The effect of histamine and PGE2 on regulatory T cells or dendritic cells is still unclear and is currently under investigation.

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