

Are regulatory T cells the target of venom immunotherapy?

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Purpose of review

Allergen-specific immunotherapy is the only treatment that leads to lifelong tolerance to previously disease-causing allergens by restoring normal immunity against allergens. T-regulatory (T_{Reg}) cells are involved in preventing sensitization to allergens and represent a major target for venom- or other allergen-specific immunotherapy.

Recent findings

Induction of peripheral tolerance in T cells, which is characterized mainly by suppressed proliferative and cytokine responses against the T-cell epitopes of major allergens, is an essential step in specific immunotherapy. It is initiated by the autocrine action of interleukin-10 and/or transforming growth factor- β , which are produced by antigen-specific T_{Reg} cells. Tolerized T cells can be reactivated to produce distinct T-helper-1 or T-helper-2 cytokine patterns, thus directing allergen-specific immunotherapy toward successful or unsuccessful outcomes. T_{Reg} cells directly or indirectly influence effector cells of allergic inflammation, such as mast cells, basophils and eosinophils. In addition, there is accumulating evidence that they may suppress IgE production and induce IgG₄ and IgA production against allergens. In addition, histamine released from mast cells and basophils may efficiently contribute to immunoregulation during specific immunotherapy, and affect T_{Reg} cells and the production of their cytokines via histamine receptor 2.

Summary

By applying recent knowledge in T_{Reg}-cell-dependent mechanisms of peripheral tolerance, more rational and safer approaches to the prevention and cure of venom hypersensitivity may be developed in the future.

Keywords

histamine, IgE, IgG₄, immunotherapy, interleukin-10, T-regulatory cells, venom

Abbreviations

IL	interleukin
PIT	peptide immunotherapy
TGF	transforming growth factor
Th	T-helper (cell)
T_{Reg}	T-regulatory (cell)
VIT	venom-specific immunotherapy

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Introduction

Although it is well documented that venom-specific immunotherapy (VIT) is the only approach by which tolerance to insect stings can be achieved, its mechanism of action was unclear for some time. VIT has been demonstrated to influence the aberrant immune response in allergic individuals in a specific manner and eventually redirect the immune system toward normal immunity. A rise in allergen-blocking IgG antibodies, particularly of the IgG₄ class, which supposedly block allergen- and IgE-facilitated antigen presentation [1]; the generation of IgE-modulating CD8⁺ T cells [2]; and a reduction in the numbers of mast cells and eosinophils, including release of mediators [3,4], were shown to be associated with successful allergen-specific immunotherapy. Later, specific immunotherapy was found to be associated with a decrease in production of interleukin (IL)-4 and IL-5 production by CD4⁺ T-helper (Th)2 cells [5,6], and in some experimental conditions with a shift toward increased interferon- γ production [7–9]. Distinct Th1 and Th2 subpopulations of T cells counter-regulate each other and play specific roles in certain diseases [10]. Whether the responsible mechanism was a repolarization of specific T-cell activity from a dominant Th2-type response to a Th1-type response had been a matter of controversy.

Further light was shed on the issue following the description of a further subtype of T cells that have immunosuppressive function and cytokine profiles distinct from those of either Th1 and Th2 cells, termed regulatory/suppressor T cells (T_{Reg}) [11,12]. Evidence for their existence in humans has been reported [13,14^{••}]. Like Th1 cells, T_{Reg} cells can inhibit the development of allergic Th2 responses and play a major role in allergen-specific immunotherapy [13,15] (Fig. 1). Recent studies [13,14^{••},15] have also demonstrated that peripheral T-cell tolerance is crucial for a healthy immune response and success of treatment for allergic disorders. This review examines the role played by T_{Reg} cells in the

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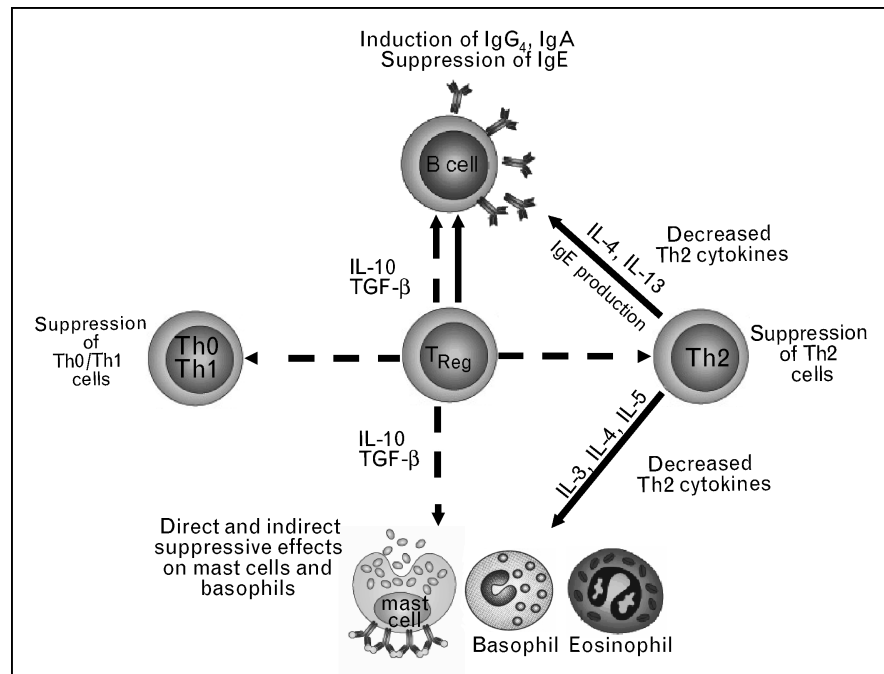
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Figure 1. Role of regulatory T cells in specific immunotherapy

Induced repolarization of the immune system toward a regulatory T (T_{Reg}) cell response leads to peripheral tolerance in allergen-specific immunotherapy and in the healthy immune response. T_{Reg} cells utilize multiple suppressor factors to regulate undesired activity of effector cells. Interleukin (IL)-10 and transforming growth factor (TGF)- β suppress IgE production and induce noninflammatory immunoglobulin isotypes IgG₄ (more relevant in venom specific immunotherapy) and IgA, respectively. Furthermore, these two cytokines directly suppress allergic inflammation induced by effector cells such as mast cells, basophils and eosinophils. In addition, T_{Reg} cells inhibit T-helper (Th)2 cells, preventing them from producing cytokines such as IL-3, IL-4, IL-5 and IL-13. These cytokines are required for the differentiation, survival and activity of mast cells, basophils, eosinophils (dashed line = suppression, solid line = stimulation).



induction of allergen-specific peripheral tolerance during VIT. An enhanced understanding of these processes, which determine whether VIT is successful, may stimulate further developments in specific immunotherapy.

T-regulatory cells and allergic responses to insect venom

The initial event responsible for the development of allergic disease is the generation of allergen-specific $CD4^+$ Th cells. Under the influence of IL-4, naïve T cells activated by antigen-presenting cells differentiate into Th2 cells [10,16^{••}]. 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 allergen-specific IgE by B cells, development and recruitment of eosinophils, production of mucus, and contraction of smooth muscle [10]. The degranulation of basophils and mast cells resulting from IgE-mediated crosslinking 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 the effector phase in allergic diseases [17–19].

The cardinal difference between anaphylactic forms of allergy (e.g. venom allergy) and atopic diseases (e.g. allergic rhinitis, asthma and atopic dermatitis) is the absence of many chronic allergic inflammatory events that lead to tissue injury and remodelling [20]. Peripheral T-cell tolerance to allergens, which is characterized by

functional inactivation of the cell–antigen encounter, can overcome both acute and chronic events in allergic reactions. It is well established that T_{Reg} cells suppress immune responses via cell–cell interactions and/or production of suppressor cytokines [12,13,21].

Type 1 T_{Reg} cells are defined by their ability to produce high levels of IL-10 and transforming growth factor (TGF)- β [12,22] and suppress naïve and memory Th1 or Th2 responses. There is now clear evidence that IL-10- and/or TGF- β -producing type 1 T_{Reg} cells are generated *in vivo* in humans during the early course of allergen-specific immunotherapy, suggesting that high and increasing doses of allergens induce type 1 T_{Reg} cells in humans [13,15,23]. Regulatory/suppressor Th3 cells, which produce high levels of TGF- β and variable amounts of IL-4 and IL-10 upon activation with appropriate antigen or anti-CD3 antibody, have been implicated in mucosal tolerance [24].

$CD4^+CD25^+$ T_{Reg} cells constitute 5–10% of peripheral $CD4^+$ T cells and express the IL-2 receptor α chain (CD25) [25]. They can prevent the development of autoimmunity and transplantation rejection, indicating that the normal immune system contains a population of professional T_{Reg} cells that are actively involved in immune suppression [21,26].

There are other T_{Reg} cells, including $CD8^+$ T_{Reg} cells, which may play a role in oral tolerance [27]; double-negative ($CD4^-CD8^-$) T-cell receptor $\alpha\beta^+$ T_{Reg} cells,

which mediate tolerance in several experimental autoimmune diseases [28]; and $\gamma\delta^+$ T_{Reg} cells, which are involved in inhibiting immune responses to tumours [29]. In addition, a regulatory role for IL-10-secreting B cells and dendritic cells was recently suggested [30]. Some other cells may also have regulatory functions. It has been demonstrated that natural killer cells, epithelial cells, macrophages and glial cells express suppressor cytokines such as IL-10 and TGF- β . Although their roles have not been determined to be those of professional regulatory cells, these cells may efficiently contribute to the generation and maintenance of a regulatory/suppressor-type immune response [31].

Role of T-regulatory cells in venom-specific immunotherapy and natural tolerance

Changes in the immune response to bee venom in conventional VIT and specifically in phospholipase A₂ peptide immunotherapy (PIT) has been intensively investigated [6,32,33]. Successfully treated patients develop specific T-cell unresponsiveness against the entire phospholipase A₂ allergen as well as the T-cell epitope-containing peptides. The decreased proliferative responses did not result from deletion because they were restored by adding IL-2 and IL-15. The same anergic state of specific T cells has been observed in protected hyperimmune individuals such as beekeepers [13]. Because stimulation of anergized cells in the presence of IL-4 induces recovery of a Th2 cytokine pattern typical of an allergy, microenvironmental conditions regulate T-cell phenotypes in VIT. Thus, successful specific immunotherapy may be more difficult to achieve in an established polyspecific allergy and atopy. The unresponsive state of specific cells results from increased IL-10 secretion [13]. The cellular origins of IL-10 were demonstrated to be the antigen-specific T-cell population, and activated CD4⁺CD25⁺ T cells and

type 1 T_{Reg} lymphocytes, as well as monocytes and B cells [13].

It has been shown that tolerance to aeroallergens is associated with activation of TGF- β -secreting T_{Reg} cells [15]. This has not been demonstrated in VIT, however. Discrepancies in the mechanisms of control of immune response to venoms and to aeroallergens might lead to alternative routes of natural allergen exposure, with the involvement of the mucosal immune system in the latter case.

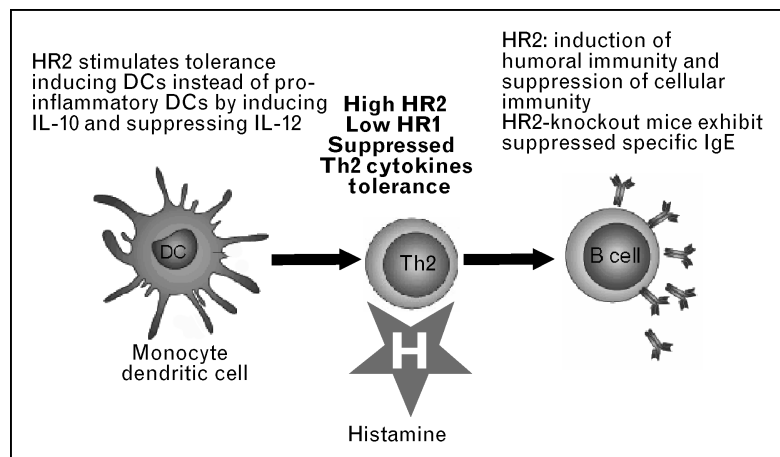
IL-10 also plays an inhibitory role with respect to IgE and effector cells of allergic inflammation (Fig. 1). Although VIT does not abolish the capacity of B cells to produce specific IgE and IgG₄ antibodies, the ratio of specific IgE to IgG₄ antibodies decreases up to 100-fold. IL-10 counter-regulates antigen-specific IgE and IgG₄ antibody synthesis. It is a potent suppressor of both total and allergen-specific IgE, while at the same time IgG₄ formation is increased [13]. Thus, IL-10 regulates specific isotype formation and skews the specific response from an IgE- to an IgG₄-dominated phenotype. Similar findings suggesting induction of IgG₄ by T_{Reg} cells have been observed in other allergies [15,34^{*}].

Most patients are protected against bee stings at an early stage in VIT, which is not paralleled by changes in antibody formation. It has been shown that lower amounts of mediators of anaphylaxis (e.g. histamine and sulphidoleukotrienes) are released during VIT [7]. These effects may be attributed to a direct suppressive effect of IL-10 on effector cells (mast cells, basophils).

PIT is another attractive approach for investigating peripheral T-cell tolerance in humans. In a clinical study using PIT in bee venom allergy with a mixture of short

Figure 2. Role of histamine in the control of T-helper-2 responses

Histamine released during allergen-specific immunotherapy plays a suppressive role on monocytes and monocyte-derived dendritic cells (DCs) via H2 histamine receptor (HR2). T-helper (Th)2 cells predominantly express HR2, which acts as the negative regulator of proliferation, and suppress interleukin (IL)-4 and IL-13 production, suggesting a role for HR2 on T cells in peripheral tolerance. Histamine also modulates antibody production by directly affecting B-cell antibody production as a costimulatory receptor on B cells and enhances humoral immune responses via HR2. Allergen-specific IgE production is differentially regulated in HR1- and HR2-deficient mice. HR1-knockout mice exhibit increased allergen-specific IgE production, whereas HR2-knockout mice exhibit suppressed IgE production.



peptides that directly represent T-cell epitopes (17, 12 and 11 amino acids) of the bee venom major allergen [35], phospholipase A₂ was administered. That study demonstrated modulation of the immune response against the whole allergen, inducing specific T-cell tolerance and a decrease in the specific IgE : IgG₄ ratio. Several ongoing studies [36,37] have suggested a role for T_{Reg} cells and their cytokines in PIT.

Recently, an important role of antihistamine pretreatment in enhancing the clinical efficacy of VIT was suggested [38]. Considerable evidence has emerged to suggest that histamine participates in the immune regulation of the inflammatory response in several diseases. Histamine interferes with peripheral tolerance induced in specific immunotherapy via several pathways. Histamine induces the production of IL-10 by dendritic cells [39], it induces IL-10 production by Th2 cells [40], and it enhances the suppressive action of TGF- β on T cells [41]. All of these effects are mediated via the H2 histamine receptor, which is relatively highly expressed on Th2 cells and suppresses IL-4 and IL-13 production and T-cell proliferation [42] (Fig. 2). Apparently, these recent findings suggest that H2 histamine receptor represents an essential receptor that participates in peripheral tolerance or active suppression of inflammatory/immune responses. These findings provide a possible rationale for recommending antihistamine pretreatment in the induction phase of all VIT patients.

Conclusion

There is mounting evidence for involvement of T_{Reg} cells and/or the immunosuppressive cytokine IL-10 in the mechanisms underlying VIT and the healthy immune response, which lead to suppression of Th2 responses. This ensures a well balanced immune response and a switch from IgE to IgG₄ antibody production (Fig. 1). These mechanisms may be exploited to improve current treatments using recombinant allergens and peptide therapy. Elaboration of more effective desensitization methods, including rapid protocols and antihistamine pretreatment, also hold promise and should be developed further.

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