

EDITORIAL

Interleukin-10, T regulatory cells and specific allergy treatment

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Allergen-specific immunotherapy induces peripheral tolerance owing to generation of T regulatory cell response

Allergen-specific immunotherapy (SIT) is efficiently used in allergy to insect venoms and allergic rhinitis [1, 2]. Currently, it represents the only curative approach for type I allergy. A rise in allergen-blocking IgG antibodies, particularly of the IgG4 class, and a reduction in mast cell and eosinophil numbers including the release of mediators were shown to be associated with successful SIT [3–5]. Furthermore, SIT was found to be associated with a decrease in IL-4 and IL-5 production by CD4⁺ T cells [6, 7]. However, it is now clear that the induction of peripheral tolerance in antigen-specific T cells and increased production of IL-10 and/or TGF- β is essential for successful SIT [8–10]. Similar to SIT with whole allergens, injection of minimal length T cell peptides of bee venom phospholipase A₂ (PLA) or peptides of the cat allergen Fel d 1, showed specific tolerance in peripheral T cells [11–13].

T cells predominantly secreting IL-10 are termed T-regulatory 1 cells (Tr1) [14]; those producing TGF- β T cells are termed Th3 cells [15]. Apparently, both IL-10 and TGF- β cooperate in peripheral T cell tolerance to mucosal allergens. House dust mite (HDM) allergens represent typical aero-allergens, which preferentially interact with the mucosal immune system. The changes in T cell response to Der p1, the major HDM allergen, were investigated during the first 70 days of SIT with whole allergen extract [16]. Within this time the suppressive cytokines IL-10 and TGF- β significantly increased, whereas allergen-induced T cell proliferation and IL-5, IL-13 and IFN- γ secretion substantially decreased. It was found that SIT generates regulatory T cells of the CD4⁺ CD25⁺ type, which secrete IL-10 and TGF- β and specifically suppress allergen-induced responses [16]. In contrast, the CD4⁺ CD25⁺ T cells taken before therapy did not display such suppressive properties. Thus, allergen-specific T regulatory cells (T_{reg}) represent a fraction of the peripheral CD4⁺ CD25⁺ T cell population and secrete IL-10 and/or TGF- β .

Early IL-10 response, a predictor of successful SIT

Up to now, no clear markers predicting the beneficial outcome of SIT were demonstrated. However, since the induction of a tolerant state and development of T_{reg} is a key event in SIT, it is obvious that IL-10 and TGF- β could potentially serve as markers for successful therapy. Indeed, production and increase of IL-10, most prominently observed during venom allergy treatment and also in SIT of pollen and mite allergy, could be used to predict the outcome of successful SIT. Whether it represents a long-time predictor,

however, is not yet known. In this issue, Savolainen et al. [17] report that the levels of IL-10 mRNA in allergen-stimulated T cells of successfully treated patients with allergic rhinitis undergoing SIT, is higher than in those of poor or moderate outcome. Patients allergic to birch pollen or timothy grass pollen or to both were included in the study and either conventional or a clustered rush protocols were used. All treated patients showed increased mRNA for IL-10 in allergen-stimulated PBMC. Most likely this may reflect the generation of Tr1 cells. Interestingly, those patients who demonstrated the highest increase in IL-10 mRNA after a short time (1 month) finally showed the best clinical score after allergen exposure in the next pollen season. This group of successfully treated patients showed decreased IL-10 mRNA production after 1 year, at the time they were protected. In contrast, patients with delayed increase in IL-10 showed a poor clinical outcome after 1 year. Thus, these data suggest that successful SIT depends on fast development and accessibility of IL-10 secreting T cells. Early increase in IL-10, which in venom therapy is within 5–7 days [9], may predict the successful outcome of SIT. Interestingly, TGF- β production after 1 year was only observed in successfully treated patients. It was not altered in patients with poor outcome. Thus, particularly in aeroallergens entering the mucosal route, generation of T_{reg} cells secreting TGF- β may be required for stable tolerance to non-pathogenic environmental antigens. These findings support the key role of IL-10- and TGF- β -secreting T_{reg} cells in natural tolerance to allergens and successful SIT.

Glucocorticoids and T_{reg} cell response

Glucocorticoids (GC) are the most widely used immunosuppressive drugs in allergic diseases. Several recent studies have shown that IL-10 and GC have strong interrelationship within the immune regulation. GC down-regulate most cytokines and pro-inflammatory mediators released by different types of immune cells. In some studies of different immune disorders, an increase in IL-10 was observed after GC treatment [18, 19]. Furthermore, it was shown that inhaled GC increases IL-10 in serum and BAL fluid of asthmatic patients [20]. In this issue, Mozo et al. [21] demonstrate that after dexamethasone treatment, IL-10 increases at both mRNA and protein levels in monocytes and not in lymphocytes. This indicates a different way of action in suppression involving IL-10 than in normal immunity to allergenic proteins. Whereas GC act during their pharmacologically active lifetime, T_{reg} cells exist for a longer period of time after allergen contact and activation.

Although IL-10, which is constitutively up-regulated by GC, may exert its suppressive effect on all cells expressing the specific IL-10 receptor, the cytokine appears to be induced by monocytes only. A reason for this may be that GC mainly exert their suppressive activity by specifically acting on the intracytoplasmic GC receptor, and not all immune cells and tissues express the GC receptor in same and sufficient numbers. GC freely penetrate into the cell and bind to their receptor to form the GC receptor complex. This complex becomes activated and gains enhanced nuclear binding affinity to regulatory GC response elements. GC up-regulate inhibitor $\kappa B\alpha$ levels, which traps NF κ B in inactive cytoplasmic complexes and functions to block nuclear translocation and DNA binding of NF κ B [22]. In addition, monocytes and other immune cells may show different kinetics in response to GC. For example, B cells are both directly or indirectly affected by GC leading to suppression of newly generated allergen specific IgE [23].

There are two major, non-exclusive hypotheses concerning the generation of regulatory T cells. T_{reg} cells emerge from the thymus as a distinct subset of mature T cells with defined functions [24]. The generation *in vitro* of a regulatory T cell subset by stimulating naïve CD4⁺ T cells in the presence of IL-10, or a combination of IL-4 and IL-10, has previously been reported [14]. The generation of T_{reg} cells in the periphery was demonstrated in studies analyzing the early course of SIT [9, 16]. The article by Mozo et al. [21] suggests that GC-induced IL-10-producing monocytes may further play a role in generation of T_{reg} cells. Studies which demonstrate whether the generation of dendritic cells is affected by GC in the same way should now be performed. In addition, the use of IFN- α as a possible cofactor led to a population of T cells producing high levels of IL-10, but in addition IFN- γ [25]. Moreover, GC can contribute to the differentiation of human and mouse naïve CD4 T cells into T_{reg} cells [26].

Regulation of antibody isotypes and effector cells of allergy by T_{reg} cell cytokines

The serum levels of specific IgE and IgG4 antibodies delineate allergic and normal immunity to allergen. Whereas peripheral tolerance was demonstrated in specific T cells, the capacity of B cells to produce specific IgE and IgG4 antibodies was not abolished [8]. 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 10–100 fold. Also the *in vitro* production of PLA-specific IgE and IgG4 antibodies by 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 that is induced and increasingly secreted by SIT, appears to counter-regulate antigen-specific IgE and IgG4 antibody synthesis [9]. IL-10 is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production [9, 27]. 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. The healthy immune response to Der p1 demonstrated increased specific IgA and IgG4, small amounts of IgG1 and almost undetectable IgE antibodies in serum [16]. HDM-SIT did not change significantly specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1 and IgG4 was observed [16]. The increase of specific IgA and IgG4 in serum coincides with increased 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 [9, 28].

Despite the fact that definite decrease in IgE antibody levels and IgE-mediated skin sensitivity normally requires several years of treatment, 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 [29, 30]. In addition, IL-10 was shown to reduce pro-inflammatory cytokine release from mast cells [31]. Furthermore, IL-10 down-regulates eosinophil function and activity and suppresses IL-5 production by human resting Th0 and Th2 cells [32]. Moreover, IL-10 inhibits endogenous GM-CSF production and CD40 expression by activated eosinophils and enhances eosinophil cell death [33]. In addition, mediators of allergic inflammation, such as histamine, act on dendritic cells to promote the differentiation of naïve T cells to IL-10-producing cells, and histamine directly suppresses Th2 cells through histamine 2 receptors [34, 35]. Histamine 2 receptors also play a role in the enhancement of susceptibility of Th2 cells to TGF- β -mediated suppression [36].

IL-10 elicits tolerance in T cells by selective inhibition of the CD28 costimulatory pathway and thereby controls suppression and development of antigen-specific immunity [37]. IL-10 only inhibits T cells stimulated by low numbers of triggered T cell receptors and which therefore depend on CD28 costimulation. T cells receiving a strong signal by the T cell receptor alone and thus do not require CD28 costimulation, are not affected by IL-10. IL-10 inhibits CD28 tyrosine phosphorylation, the initial step of the CD28 signalling pathway and consequently the phosphatidylinositol 3-kinase p85 binding to CD28 [37]. Previous studies demonstrated that IL-10 is also a potent inhibitor of activated monocytes and macrophages [38]. Since monocytes and macrophages do not express CD28, the inhibitory impact of IL-10 is likely to occur through other mechanisms in non-T cells. IL-10 exerts its biological functions through the activation of Jak1 and Tyk2, the members of the receptor-associated Janus tyrosine kinases family and Stat1 and Stat3 and in certain cells Stat5 [39]. In monocytes, IL-10 was shown to induce expression of the cytokine-signalling-3 (SOCS3) gene suppressor, which may inhibit IFN- γ -induced tyrosine phosphorylation of STAT-1.

Conclusion

There is now clear evidence that T_{reg} cells are required for specific allergy suppression (Fig. 1). Similar to the normal allergen response in healthy individuals, SIT generates IL-10- and TGF- β -producing regulatory T cells in allergic patients.

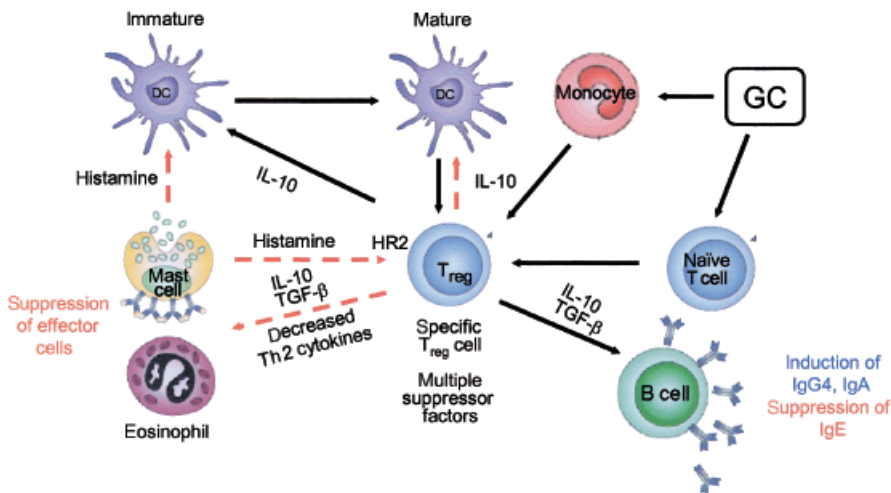


Fig. 1. Immune deviation towards T regulatory cells (T_{reg}) cell response is an essential step in SIT and natural allergen exposure of non-allergic individuals. Black arrows indicate activation and differentiation of cells; dotted lines indicate suppressive activity on cells. The fast activation of a T_{reg} cell response may be an essential factor for successful SIT. T_{reg} cells utilize multiple suppressor factors, which influence the final outcome of SIT. In addition, IL-10 and TGF-β induce IgG4 and IgA, respectively, from B cells as non-inflammatory Ig isotypes and suppress IgE production. These two cytokines directly or indirectly suppress effector cells of allergic inflammation such as mast cells, basophils and eosinophils. The controlled release of histamine from effector cells of allergy induces IL-10 in dendritic cells (DC) and suppresses both Th1 and Th2 responses through the histamine receptor 2 (HR2). GC may additionally play a role in peripheral tolerance by IL-10 production in antigen-presenting cells. Furthermore, IL-10 affects the maturation of DC to IL-10-producing DC, which may further contribute in T_{reg} cell generation.

The skewing of allergen-specific effector T cells towards a regulatory phenotype is a crucial event in the control of immune response to high allergen doses. Although various suppressor factors, including contact-dependent or -independent mechanisms, may be involved in the allergen-specific T_{reg} cell function, IL-10 and TGF-β play an essential role against allergens. The immune deviation towards T_{reg} cells during SIT modulates the allergen-specific antibody response and directly or indirectly suppresses mast cells, basophils and eosinophils. Increased IL-10 and TGF-β levels potentially suppress the IgE production and simultaneously increase IgG4 and IgA. In this issue, two articles point to the mechanisms, which may play a role in peripheral tolerance to allergens: the importance of the speed of a T_{reg} cell response generation in successful SIT [17] and the role of GC in the induction of IL-10 by monocytes [21].

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