



T-cell responses during allergen-specific immunotherapy

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Purpose of review

Allergen-specific immunotherapy is the only specific, dose-dependent and time-dependent and disease-modifying strategy for the treatment of allergy associated with clinical improvement and biological tolerance which may persist years after discontinuation.

Recent findings

Successful immunotherapy in respiratory allergy is associated with the immunodeviation of Th2 response to a more protective allergen-specific Th1 cells and with the induction of interleukin-10 (IL-10)/transforming growth factor (TGF)- β -producing T regulatory cells in blood and inflamed airways. Subcutaneous treatment and sublingual treatments induce similar alterations which are dose-dependent and time-dependent.

Summary

This study provides an update on the immunological T-cell responses during subcutaneous immunotherapy and sublingual immunotherapy, giving a unifying view of the redirecting mechanisms and regulating mechanisms elicited by these treatments.

Keywords

allergy, cytokines, subcutaneous and sublingual immunotherapy, T effector and T regulatory cells

INTRODUCTION

Allergic disorders recognize type 2 T helper (Th2) cell-mediated response to allergens as the main pathogenetic mechanism. In allergic people the priming of allergen-specific Th2 cells by antigen presenting cells (APCs) results in the production of a panel of cytokines, responsible for the induction, maintenance and amplification of allergic inflammation. Interleukin-4 (IL-4) and IL-13 induce B-cell switch to IgE isotype, IL-4 and IL-9 recruit mast cells and IL-5 activates eosinophils whose persistence favours the evolution to chronicity [1].

Susceptibility genes of asthma can be associated with the allergy *di per se* or with the airway bronchial hyperreactivity (AHR) or the response of cells to allergens [2]. The allergens modify the function of airway epithelial cells or of innate immunity cells by triggering their receptors. More than 80 allergens exert protease activity, increase vascular permeability through the production of vascular endothelial growth factor or directly trigger Toll-like receptors (TLRs) in several cell types [3,4]. Sensitization to inhalants is influenced by a crosstalk among barrier epithelium, mucosal dendritic cells and other cells of innate and adaptive immunity. Allergen-driven protease-activated receptors or TLRs signalling in

epithelial cells induce nuclear factor κ B activation and secretion of cytokines essential for Th2 (IL-25, IL-33, thymic stromal lymphopoietin) and Th17 (IL-1 β , osteopontin, TGF- β , etc.) differentiation [3,5].

In the lung, IL-17 is produced by T cells and natural killer (NK) T cells, alveolar macrophages and epithelium and promotes the neutrophil influx and the production of profibrotic cytokines [6,7]. The Th17 response in respiratory allergy has been associated with chronic evolution and airway remodelling. IL-17 increases in the lung, sputum and bronchial alveolar lavage (BAL) fluids or in the sera of asthmatic patients and its levels correlate with the disease severity [8]. A novel subset of T cells producing both IL-17 and IL-4 has been recently described in asthmatic individuals [9^{***}]. Due to the heterogeneity of

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KEY POINTS

- Allergen subcutaneous immunotherapy (SCIT) is the only effective antigen-specific therapy inducing long-lasting tolerance.
- Successful immunotherapy is associated with the immunodeviation of Th2 response to more protective allergen-specific cells with Th1 profile and with the induction of interleukin-10 (IL-10)/TGF- β -producing T regulatory cells in blood tissues and inflamed tissues.
- SCIT and sublingual immunotherapy (SLIT) induce similar alterations which are dose-dependent and time-dependent.
- The Th1-like cells producing IL-10 cells, which are induced in tissue microenvironment upon chronic antigen stimulation and play a role in successful therapy, can reconcile the dualism between immunodeviating and regulating mechanisms and provide new insights into novel therapeutical strategies.

asthmatic phenotypes, IL-17 may be considered a new marker to identify neutrophilic-dominant diseases [8,10]

Subcutaneous immunotherapy (SCIT) of respiratory allergy achieves a hyposensitization and reduces both early and late responses occurring during the natural allergen exposure. Successful SCIT is associated with the reduction of symptoms and medication scores and the improvement of quality of life [1,11[■]]. After discontinuation, SCIT confers long-term remission of symptoms and prevents the onset of new sensitizations in children. SCIT suppresses the allergen-induced late response in the lung and nose, as it reduces infiltrating T cells, eosinophils, basophils, mast cells and neutrophils [11[■],12[■]].

Sublingual immunotherapy (SLIT) was introduced 30 years ago and its efficacy was confirmed by meta-analyses of controlled trials in adults with respiratory allergy [13,14]. WHO (1998) and ARIA (2001) documents indicate SLIT as a valid alternative to SCIT and its use is justified in adults [15]. SLIT efficacy in children is less convincing, because subgroup analyses for seasonal and perennial allergens provide controversial data [16,17]. Evidence of efficacy for SLIT on patients (adults and children) with perennial asthma or allergic conjunctivitis has been provided [18,19[■]].

T-CELL RESPONSE DURING SUBCUTANEOUS IMMUNOTHERAPY

The reduced allergen-specific T-cell proliferation observed in SCIT is consistent with a mechanism

of peripheral tolerance [20,21]. High-dose tolerance has been proposed for SCIT, as cumulative doses are much higher than those encountered naturally [22]. Anergy was shown in studies of SCIT for bee venoms and the decreased T-cell response to phospholipase A2 was associated with increased IL-10 [23[■]]. High-dose antigen can also trigger apoptosis of Th2 cells in allergen-stimulated peripheral blood mononuclear cells (PBMCs) from bee venom-treated patients [24].

With the disclosure of the Th1–Th2 paradigm, initial results indicated that SCIT skewed allergen-specific responses from Th2 to a more protective Th1 phenotype (immunodeviation) [1]. Even though Th1 redirection induced by SCIT with allergen extracts or peptides continues to be reported in more recent reports [25,26,27[■]], other groups, however, did not reproduce these data [28].

Some studies described a Th1 redirection also in respiratory mucosa in SCIT-treated patients after pollen challenge [11[■]]. The proportions of mucosal IFN- γ ⁺ T cells associated with the decrease in type 2 cytokines, inversely correlated with symptom scores and medication scores during the pollen exposure [29].

SCIT has been shown to inhibit allergen-induced late cutaneous responses by increasing IL-12 mRNA expression in skin macrophages, and this directly correlates with the presence of Th1 cells and, inversely, with the number of Th2 cells [30,31]. Even though few data are available at present on the effect of SCIT on the IL-23/IL-17 axis, it is likely that the local excess of IL-12 can also shift the Th17 response to Th1 response [6]. Thus, much evidence indicates that immunodeviation occurs during SCIT at both local and systemic levels.

With the discovery of adaptive T regulatory (aTreg) cells, their increased activity (immunoregulation) was emphasized as the main or even unique explanatory mechanism for the clinical efficacy of SCIT. aTreg cells are extremely heterogeneous, initially including Th3, Tr1 and CD25^{high}CD127^{low}Foxp3⁺ T cells. Several other T-cell subsets have been shown to exhibit regulatory activity [32] which is mediated by both cytokines (IL-10, TGF- β , IL-35) and cell-to-cell contact signals [33[■]].

CD4⁺CD25^{high} Foxp3⁺ Treg cells from grass-sensitive patients are able to impair IL-13 and IL-5 less than those of healthy controls [34]. By using Treg markers (Foxp3 and CD127^{low}), however, some reports showed that the Treg proportion remained unchanged in SCIT [35[■]]. Moreover, the allergen (Der p 1) specific CD25^{high}Foxp3⁺ Treg cells from the PBMCs of healthy donors were similar in number and functions to those derived from house dust

mite (HDM) allergic donors [36]. Even though the suppression of allergen-specific responses by Treg cells may represent the normal response to these molecules [23[■]], some recent reports show that allergen-specific T cells also in newborns can exert a Th1 profile [37]. Purified Bet v 1-specific CD4⁺ T cells from healthy individuals usually secrete much more IFN- γ than IL-10 upon allergen stimulation [38]. This suggests that healthy and allergic patients show differently polarized effector T cells recognizing allergens, rather than different mechanisms of homeostasis [39].

A high proportion of nonallergen-specific aTreg cells producing IL-10 and TGF- β has been described under SCIT regimen [1], likely suggesting their in-vivo role in successful therapy. IL-10 inhibits major histocompatibility complex class II expression on dendritic cell and tyrosine phosphorylation of CD28 in T cells, preventing downstream signaling events [1,23[■]]. IL-10 downregulates Th1, Th2 and Th17 cells *in vitro* and induces anergy of these cells [40]. The upregulation of regulatory cytokines during SCIT was described in PBMCs of patients treated for hymenoptera venom, in their skin after intradermal challenge and in nasal mucosa under SCIT for respiratory allergy [23[■],29]. Increased mucosal Tr1 cells have been associated with elevated serum IgG4 and blocking activity for IgE-facilitated allergen presentation [23[■]]. aTreg cells have also been related to the overproduction of TGF- β , a molecule promoting Treg cell upregulation of cutaneous T-lymphocyte antigen (CTLA)-4 [41], regulating antigen presentation, co-stimulatory molecules expression and T-cell proliferation [23[■],34]. TGF- β modulates IgE, Fc ϵ RI expression on Langerhans cells and induces noninflammatory IgA switch in humans. Increased allergen-specific IgA2 in the sera have been associated with increased aTreg cells and successful SCIT [42].

T-CELL RESPONSE DURING SUBLINGUAL IMMUNOTHERAPY

Studies on the immunological changes induced by SLIT indicate that both immunoredirection and regulation can occur. However, it is emerging that oral mucosa is a site of natural immune tolerance. Indeed, sublingual allergen is captured within the oral mucosa by Langerhans-like dendritic cell which are regulatory cells in nature. They express Fc ϵ RI which is triggered by allergen inducing IL-10, TGF- β and indoleamine 2–3 acetate dioxygenase (IDO) [43[■],44]. Oral mucosa contains limited numbers of mast cells, thus explaining the safety profile of SLIT [44]. During the pollen season, increased expression of programmed cell death ligand (PDL1)

on B cells and monocytes and the parallel reduction of IL-4 were observed in PBMCs of SLIT-treated patients [45[■]]. By comparing the oral mucosa and skin, it has been shown that vestibular/buccal and sublingual regions represent a pro-tolerogenic microenvironment with IL-10 and TGF- β -producing T cells. Both regions form a Th1 cytokine-predominated microenvironment, but also express mRNA for Th17 cytokines. Finally, TLRs detectable in oral mucosa might serve as target structures for adjuvants, thus implying that suppressive mediators of Th2 responses are constitutively expressed in oral mucosa [43[■]].

A clinical study [21] performed in HDM-sensitive children indicated that, despite similar clinical improvement, SCIT and SLIT exerted different and variable immunological responses. Some studies did not show alterations in T-cell response after SLIT, whereas others described alterations of T-cell proliferation, cytokine production and serum IgG1/IgG4 ratio [46]. Even though changes were more evident in SCIT, the immunological alterations of SLIT and its efficacy seems to be dependent on the doses and duration of treatment [21]. Indeed, cytokine production by allergen-specific T cells varied during the treatment. SLIT increases IL-10 or TGF- β in the first months of therapy but upregulates IFN- γ or respectively IFN- γ /IL-10 production after 24 months of treatment [28,47,48]. A parallel increase in Treg cells and in IL-10 and TGF- β mRNA expression was found in a 2-year trial of SLIT for pollens in children [49[■]]. Notably, in the latter study, the increased IL-17 synthesis by allergen-stimulated PBMCs correlated with poor effects of SLIT, whereas in another report, the reduced IL-17 production was associated with successful therapy [50]. These data provide evidence that at least qualitatively SLIT induces similar alterations of SCIT [42].

THE IMMUNODEVIATION AND REGULATION INDUCED BY SUBCUTANEOUS IMMUNOTHERAPY/ SUBLINGUAL IMMUNOTHERAPY ARE NOT ALTERNATIVE MECHANISMS

The mechanisms of immunodeviation and immunoregulation have been usually considered as alternative, mutually exclusive events. Some recent data, however, indicate that SCIT-driven alterations involve both allergen-specific Th1 and Tr1 cells and that, likely, this dualism may be re-considered as two aspects of the same phenomenon.

By using tolerogenic Fel d1 peptides as vaccine, it was shown that two major epitopes were able to selectively induce IL-10 or IFN- γ and fulfil a tolerogenic role in SCIT-treated patients or healthy

subjects [51]. By using a low-dose regimen of Fel d1, the reduction of the cutaneous late-phase reaction paralleled the accumulation of Tr1 cells and Th1 cells in allergen-challenged skin sites [31]. Single selected peptides of Fel d1 induce *in vitro* similar amounts of IFN- γ and IL-10, and the *in vivo* effects of the immunotherapy with these peptides are related to the induction of adaptive allergen-specific Tr1 cells [52,53].

The link between IFN- γ and IL-10 was initially described in experimental models in which CXCL10 shifted mucosal dendritic cells able to expand Treg cells *in vitro* [54]. Other reports indicate that chronic stimulation of dendritic cells by IFN- γ induced the production of IDO, whose metabolites decrease T-cell proliferation and expand Tr1 cells [55].

In rush immunotherapy for bee venom, a shift from Th2 to Th1 profile was described, associated with a parallel increase in naturally or adaptive, IL-10⁺ or IL-10⁻ Treg cells [56]. Some authors suggested that the predominance of different mechanisms of tolerance is time-dependent and dose-dependent. Indeed, SLIT has been shown to induce Tr1 cells in the early (1 month) phase of treatment, followed by a Th1 skewing after 1 year of therapy [47] and this result has been recently confirmed [27[•],35^{••}]. The previous study with HDM-sensitive patients provided evidence that successful SLIT associated with downregulation of allergen-specific T-cell response, early (12 months) increase in TGF- β and late (24 months) upregulation of IFN- γ and IL-10. Notably, IL-10 was found to be exclusively produced by T cells [48]. Similarly, high cumulative doses of SCIT promoted the expansion of IFN- γ -producing T cells, the apoptosis of Th2 cells and T-cell anergy *in vitro* [57]. Also, in some reports showing the increase in CD25^{high}Foxp3⁺IL-10⁺ T cells in the blood or in nasal mucosa, a parallel increase in IFN- γ -producing T cells was underestimated [29,57].

After 6 months of SLIT, we showed that PBMCs produced higher amounts of IL-10 and of IFN- γ *in vitro* upon allergen (HDM) stimulation. Notably, the coexpression of both cytokines by allergen-specific CD4⁺ T cells was described in patients undergoing immunotherapy, but not before treatment [58]. The coexpression of IL-10 and IFN- γ in Th cells during chronic antigen stimulation has been reported [59,60].

Regulatory IFN- γ /IL-10 double positive T cells (called Th1-like Tr1 cells) have been described in mice undergoing chronic antigen stimulation. In humans, they contribute to the protection to pathogens as leishmania, borrelia and mycobacteria in which CD4⁺CD25-Foxp3-Th1 cells were the principal source of IL-10 [60]. Even though the IL-10 gene

promoter is silenced in Th1 cells, the IL-10 locus is in a reversible histone deacetylase-responsive state, which can be reactivated with a prolonged antigenic stimulus in the presence of high IL-12 levels [61]. The production of both IFN- γ and IL-10 by effector T cells probably limits the collateral damage caused by exaggerated inflammation [62]. Notch1 triggering through different Notch ligands is the major signal to elicit IL-10 production in newly developing and already established Th1 cells via a signal transducers and activators of transcription 4-dependent pathway. This latter requires the triggering of IL-12 and IL-27 which favour Th1 cells to synthesize IL-10 [63]. Notably, IL-27 has been shown to induce IL-10 not only by Th1, but also Th2 and Th17 effector cells [64].

Regulatory IL-10-producing Th1 cells is the best candidate to explain the complex changes induced by SCIT and SLIT. Allergens directly or indirectly trigger TLRs on dendritic cells, creating microenvironmental conditions to elicit Th1-like Tr1 cells. The chronic allergen administration favours the conditions useful to expand Th1-like Tr1 cells (delta 4 expression on APCs and IL-12 and IL-27 synthesis). They are recruited into inflamed tissues which initiate an amplification loop involving the IFN- γ -driven activation of dendritic cells with expansion of additional Th1-like Tr1 cells and increase in microenvironmental IL-12, IL-10 and TGF- β . IL-12 skews allergen-specific Th2 and Th17 into Th1 cells and expands NK and NK T cells which further increase IFN- γ , directly inducing the reduction in IgE and the IgG1 switch. IL-10 suppresses allergen-specific Th2 and Th17 responses, limits Th1, NK T cells and NK cells and their cytolytic activity, induces IgG4 and inhibits mast cells, basophils and eosinophils recruitment. Finally, TGF- β decreases mast cells and eosinophil activation, blocks Th2 (and the excess of Th1) response and contributes to IgA2 switch.

CONCLUSION

Allergen-specific Th2 and Th17 responses are under the double control of Th1-polarizing cytokines and immunosuppressive cytokines, both of which increased under chronic antigen stimulation. Successful SCIT or SLIT is likely the result of the upregulation of the two mechanisms and in particular of Th1-like Tr1 cells. This view gives insights into new strategies to improve SCIT with adjuvant molecules as synthetic TLR ligands able to stimulate balanced levels of endogenous IL-12, IL-27 and IL-10. TLR2 agonists enhance CD8⁺Foxp3⁺IL-10⁺ regulatory T cells and suppress Th2 immune responses during SCIT [65^{••}], whereas some TLR7

ligands (modified adenines) *in vivo* redirect Th2 response and Th17 response upregulating a balanced IFN- γ and IL-10 response [66]. When covalently linked to allergens, TLR2, TLR5 and TLR7 agonists induce IL-12/IFN- γ and IL-10 in human and mouse models ([67,68]; L. Fili, *et al.*, unpublished data), as well as in-vivo TLR9 agonists (bound to ragweed pollen) upregulated human T cells with Th1/Tr1 features [69]. The precise mechanism through which SCIT/SLIT forces dendritic cells to induce a tolerogenic micromilieu favouring the expansion of allergen-specific Th1-like Tr1 cells and influencing the efficacy of therapy remains to be elucidated.

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Conflicts of interest

There are no conflicts of interest.

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