



Allergen immunotherapy, routes of administration and cytokine networks: an update

Allergen immunotherapy is a disease-modifying therapy, effective for the treatment of allergic rhinitis, allergic asthma, conjunctivitis or stinging insect allergy. Allergen immunotherapy involves the administration of increasing doses of allergens with the aim of ameliorating the allergic response. Although precise underlying mechanisms of the induction of immune tolerance remain unclear, immunotherapy has been associated with the induction of distinct subsets of Tregs that eventually lead to peripheral tolerance by inducing a deviation from Th2 to Th1 immune responses. This review focuses on the current knowledge of the mechanisms of immunotherapy in relationship to different routes of administration and also provides a unifying view.

Keywords: cytokine network • immunotherapy • intradermal route • intralymphatic route • route of administration • subcutaneous route • sublingual route

Background

Allergen immunotherapy (AIT) is recognized as a highly effective practice for the treatment of patients with severe allergic rhinitis and/or asthma, currently recommended by the WHO [1] as the only treatment able to modify the natural course of the allergic disease [2,3]. The success of AIT is characterized by a marked decrease in symptoms during allergen exposure, a reduced use of antiallergic drugs, a decrease in absenteeism from school or work and improvement in patients' quality of life [4–6]. These clinical benefits have been shown to persist for several years following discontinuation of immunotherapy, confirming immunological tolerance against allergens.

At present, there are two commonly used routes of immunotherapy administration, subcutaneous (SCIT) and sublingual (SLIT), which efficacy has been confirmed by a number of recent meta-analyses [7–9]. The SLIT approach has gained considerable interest as a valid alternative to SCIT [10–12] owing to safety, ease of administration, reduction of severe adverse reactions and equal efficacy [13–15].

If correctly used, both routes of administration are effective in the treatment of allergic rhinitis and asthma, although it seems that SLIT more consistently reduces the need of further antiallergic drug use compared with SCIT [16].

On the contrary, side effects of SLIT and SCIT are different. In fact, SCIT appears to be associated with more systemic allergic reactions than SLIT, while SLIT appears to have a higher rate of local side effects [17]. Main risks factors for a severe adverse reaction during SCIT are: coexisting asthma or poor control of asthma; previous adverse effects to immunotherapy; dosing errors; and change-over of allergen [18]. The indications for both treatments are similar, and often, the route of administration is a patient's choice [19].

Current research on AIT is focused on enhancing its efficacy, safety and patients' convenience with the goal of offering a diffusely accepted treatment option for all patients. Accordingly, there is a growing interest in alternate routes of administration such as intralymphatic allergen-specific immunotherapy (ILIT) and intradermal allergen-specific immunotherapy (IDT) [20].

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Finally, the long-standing experience with the two common routes of administration, SCIT and SLIT, has given rise to some critical points and has revealed the need to define a new method of administration resulting in a faster reaction time and a lower risk of adverse reactions. SCIT shows efficacy in approximately 3–5 years and after 30–80 injections of allergen, which may be eventually associated with local reactions at the site of inoculation. SLIT is certainly patient friendlier than SCIT, but it is still burdened by a long latency before benefits occur and this may impair patients' compliance [18].

The induction of peripheral T-cell tolerance represents an essential target of AIT. This occurs by a complex interaction between the innate and adaptive immunity responses that involve cells, cytokines and chemokines (Figure 1) [21]. The putative mechanisms of action of AIT have been summarized in (Box 1) [21]. This review will focus on the modifications of cytokine networks during AIT administered by different routes.

Allergic inflammation

The allergic cascade starts with the recognition of allergens by the antigen-presenting cells, mainly dendritic cells (DCs), leading to Th2 polarization, switching to IgE production by B cells, culminating in mast cell sensitization and triggering. DCs have been demonstrated to play a crucial role in orchestrating allergic disorders. An allergic reaction is characterized by the synthesis of allergen-specific immunoglobulins of the IgE class and Th2 cytokines (e.g., IL-4, IL-5 and IL-13), which lead to the recruitment and sensitization of effector cells such as eosinophils, basophils and mast cells [22,23]. During allergen re-exposure, the crosslinking of IgE molecules bounded to high-affinity Fcε receptors on the surface of mast cells and basophils results in an immediate release of soluble mediators, such as histamine, leukotriene and prostaglandins, which are responsible for the allergic reaction [24,25]. Antigen recognition and uptake by innate immune cells is the first step in the process of antigen presentation that could lead to initiation of adaptive immune responses. During the development of allergic diseases, effector Th2 cells produce traditional Th2 cytokines such as IL-4, IL-5 and IL-13, and newly discovered Th2 cytokines with proinflammatory functions, such as IL-25, IL-31, IL-33 and IL-9 [26,27]. In addition to cytokines inducing allergen-specific IgE production, eosinophilia and recruitment of inflammatory cells to inflamed tissues [28]; IL-9, derived primarily from Th9 lymphocytes, promotes expansion of the Th2 subset and enhances the mechanisms of allergic diseases [29].

Besides the Th2/Th1 schema, other T subpopulations, such as the Tregs and Th17 cells, play a role in atopic diseases [30]. Tregs may play a critical role in

controlling the development of atopic diseases, as they can suppress a potentially harmful immune response. There is evidence that the number and function of two major subsets of Tregs, namely CD4⁺CD25⁺Foxp3⁺ Tregs and IL-10-producing Tregs, are impaired or altered in patients with atopic asthma compared with healthy individuals [31,32].

Moreover, Th17, a Th cell lineage distinct from Th1 and Th2 cells, has been recognized as a novel proinflammatory CD4⁺ T effector cell and is negatively regulated by IFN-γ and IL-4 [33–36]. Consistently, Th17 cells have been shown to play a key role in many autoimmune disorders, such as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and inflammatory bowel disease [37]. Furthermore, recent data suggest that Th17 cells are also implicated in allergy, asthma and rhinitis [38–40], even if the mechanisms underlying the enhanced Th17 immunity in allergic diseases still remain unclear [41].

Th17 cells are defined by IL-17A, IL-17F, IL-6, IL-21, IL-22 and TNF-α. Th17 cells are also regulated by IL-23, a novel member of the IL-12 heterodimeric cytokine family. IL-23 is a heterodimer comprising the p19 and p40 subunits shared with IL-12p70 [42]. It is secreted by DCs in response to immune danger [42]. Moreover, IL-23 may contribute to the differentiation of macrophages. It has been shown that the enforced expression of IL-23 in the lung enhances not only antigen-induced IL-17A production and neutrophil recruitment, but also antigen-induced Th2 cytokine production and eosinophil recruitment in the airways [43–45]. Furthermore, IL-23 promotes IL-17 synthesis and activates the transcription factor signal transducer and activator of transcription 3 to maintain a Th17 phenotype of CD4⁺ T cells [46]. IL-17 in turn induces IL-1β and IL-6. On the other hand, in the presence of IL-6, TGF-β further promotes the generation of Th17 from naive T cells [47].

Recent data reported that cytokine-stimulated B lymphocytes could be a significant source of IL-17A/F, participating, via alternative mechanisms, in immune responses [48]. Li *et al.* demonstrated that specific immunotherapy (SIT) could change the production of serum Th17-related gene levels (decrease of IL-6, IL-17 and IL-23, but increase of IL-27) from subjects affected by allergic rhinitis. Otherwise, after SIT, Th17 cells and the Th2/Th1 ratio were suppressed, while IL-10-producing CD4⁺ T cells were elevated [40]. Therefore, IL-17 may be a useful biomarker for disease severity and SIT response.

Cytokines & AIT

Serum cytokine levels reflect the activity of the immune system. Therefore, modulation of cytokine

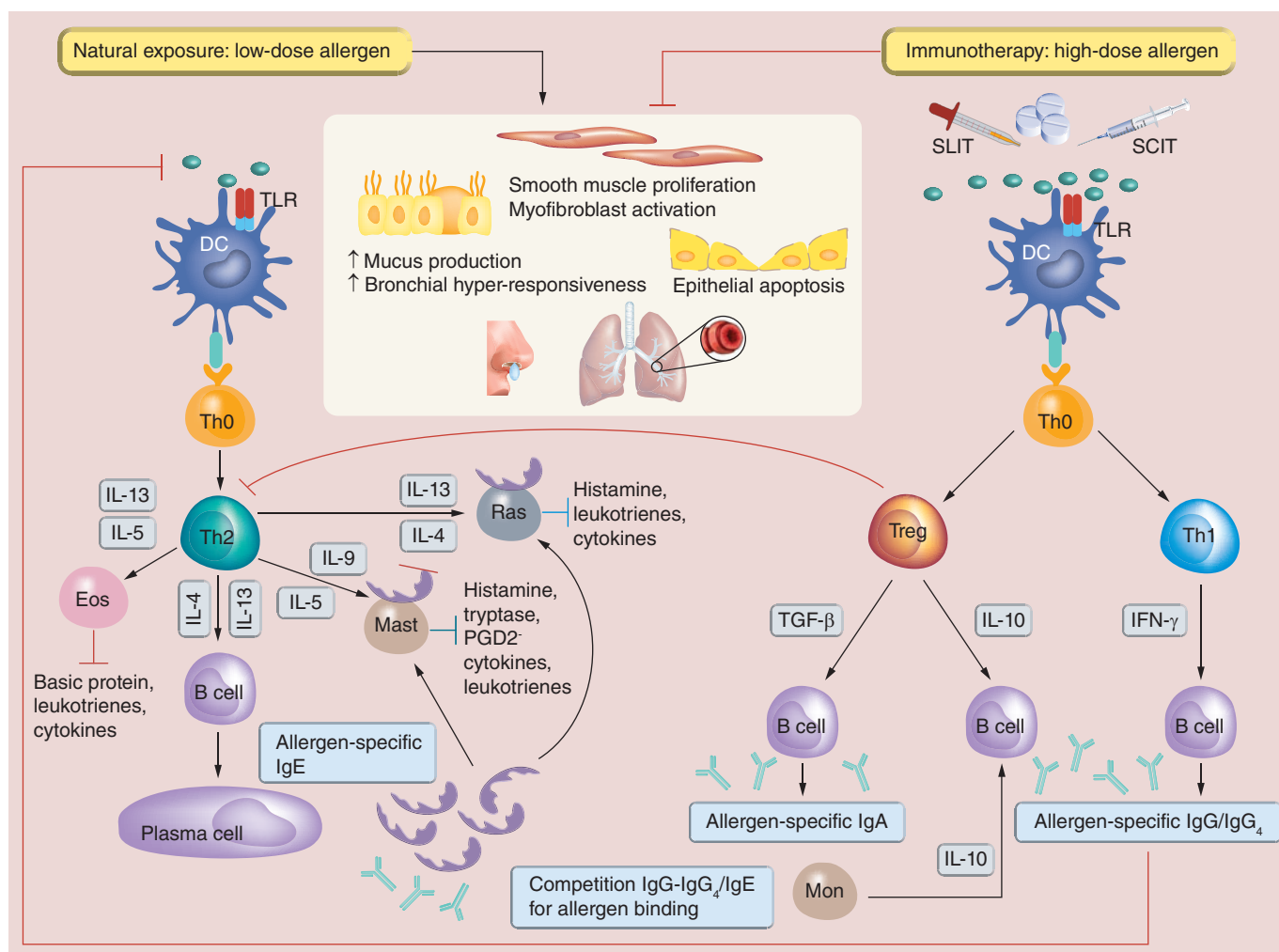


Figure 1. Mechanisms of immunological tolerance in allergen immunotherapy. Low-dose and repeated allergen exposure in atopic patients drives Th2, which produce interleukins that promote IgE production by B cells, and activate mast cells, eosinophils and basophils that release inflammatory mediators. High-dose allergen administered by SLIT or SCIT leads to immune deviation from a Th2 to Th1 response. This is characterized by an increase of Th1 cytokines (IFN- γ and IL-12). It was also shown that the induction of Tregs suppresses the activity of Th2 cells and promotes IgG and IgA production, rather than IgE. The main cytokines involved appear to be IL-10 and TGF- β .

DC: Dendritic cell; Eos: Eosinophil; Mast: Mast cell; Mon: Monocyte; SCIT: Subcutaneous immunotherapy; SLIT: Sublingual immunotherapy; TCR: T-cell receptor.

environment and balance of Th1/Th2 are critical points to achieve tolerance and immune suppression. A long-term AIT can influence specific Th cell subsets and their cytokine production, leading to successful or unsuccessful treatment.

IL-4

Suárez-Fueyo and coworkers demonstrated that IL-4 shows a double kinetic change in the SLIT response [49]. Initially, in addition to enhanced Th2, allergen-specific IgE and IgG₄ response, an increase in IL-4-producing cells was recorded [49]. This also suggests that IL-4 promotes Th2 cells to become refractory to Treg suppression. Therefore, Th2 cell depletion is a critical first step in the induction of immune tolerance [50]. This phase

leads to downregulation of the Th2 response with a shift towards a Th1 cytokine profile and CD4⁺ T cells with a regulatory phenotype [51]. These data were also confirmed in patients treated with SIT. The levels of IL-4 and total IgE were significantly lower in post- than pre-SIT [52].

IL-9

In addition to cytokines inducing allergen-specific IgE production, eosinophilia and recruitment of inflammatory cells to inflamed tissues [28]; IL-9, derived primarily from Th9 lymphocytes, promoted expansion of the Th2 subset and enhances the mechanisms and symptom severity of allergic diseases [29]. Recently, Ciprandi *et al.* reported that a SLIT course could

Box 1. Putative mechanisms of action of allergen immunotherapy.**Early events**

- Mast cells
 - Reduction of tissue numbers
 - Decrease in mediator release
 - Decrease in proinflammatory cytokine production
- Basophils
 - Decrease in mediator release
 - Decrease in proinflammatory cytokine production
- Eosinophils
 - Reduction of tissue numbers
 - Decrease in mediator release

Late events

- T cells
 - Decreased allergen-induced proliferation
 - Induction of Tregs
 - Increased secretion of IL-10 and TGF- β
 - Suppression of Th2 cells and cytokines (IL-5, IL-4, IL-9 and IL-13)
 - Decreased T-cell numbers in late-phase response
- B cells
 - Decreased specific IgE production
 - Increased specific IgG₄ production
 - Increased specific IgA production
 - Suppressed IgE-facilitated antigen presentation
- Dendritic cells
 - Suppressed IgE-facilitated antigen presentation

modulate serum IL-9 levels [53]. In fact, SLIT-treated patients showed significantly lower serum IL-9 levels than untreated patients [53]. IL-9 may be considered as another marker of immune response.

IL-10

IL-10 plays a critical and anti-inflammatory role in the induction in both Th1 and Th2 tolerance. IL-10 acts by the activation of the receptor-associated JAK family (Jak1 and Tyk2), and STAT1, STAT3 and STAT5 [54]. IL-10 is produced by a variety of immune cells such as Th0, Th1, Th2 as well as B cells, keratinocytes and monocytes, drivers of systemic inflammation [55]. In addition to repeated exposure to high- and/or low-dose antigen, IL-10 overproduction modulates immunological response and influences activity of several immune cells [56], promoting peripheral T-cell tolerance [57], and induction and maintenance of an anergic state [58].

During the course of SIT, Wambre *et al.* also showed that IL-10-producing, allergen-specific, Th1-like Tr1 cells are increased [50]. Otherwise, a Th2 cell and Th2 cytokine depletion was reported [50]. Moreover, during both bee venom SIT and phospholipase A peptide immunotherapy, it has been demonstrated that the time to establish a peripheral tolerance is between 7 and 28 days [59]. In this period, an increased parallel IL-10 release is also noted. Precisely, intracellular IL-10 synthesis is significantly greater in

CD4⁺ T lymphocytes, monocytes and B cells. These data seem to suggest that an autocrine production may play a critical role in the induction and maintenance of peripheral tolerance [60]. IL-10 further acts on costimulation pathways. Interestingly, neutralization of endogenous IL-10 also causes the abrogation of these regulatory effects [61]. Moreover, healthy subjects show higher serum IL-10 levels. However, these data have not always been confirmed. In experimental allergy models, IL-10 is associated with atopic disease, enhancing eosinophilia [62] and airway hyper-responsiveness [63].

IL-17

IL-17, derived from Th17, has a proinflammatory and chemotactic bioactivity, also involved in atopic disorders [64]. Qui *et al.* reported a greater reduction in symptoms and IL-17 expression at 2 years of SIT than at 1 year [65]. This suggests that immunotherapy could downregulate release of IL-17, especially where AIT is administered for the long term [65].

IL-35

IL-35 is a recently described regulatory cytokine whose functions in allergic inflammation are still unclear. IL-35 belongs to the IL-12 cytokine family. It is produced by a specific Treg subset, also known as Tr35, that mediated suppression only via IL-35 but not IL-10 and/or TGF- β [66]. In mice, FOXP3⁺ Tregs also promote the release of IL-35, which in turn enhances the suppressive functions of Tregs [67], decreases airway inflammation and serum IgE, Th17 and IL-17 levels [68].

TGF- β

TGF- β has been also shown to induce T-cell suppression through a dependent and/or independent Smad pathway during SIT and in normal patients [69]. However, the effects of TGF- β are also mediated by the costimulatory CD28 molecule. In fact, in the absence of CD28, TGF- β can inhibit T-cell receptor-stimulated proliferation of naive T cells, otherwise in its presence. Moreover, CD28/TGF- β axis can downregulate serum IL-2, IL-12 and IFN- γ , and decrease T-cell proliferation by apoptotic cell death [70]. AIT can modulate TGF- β production. Ciprandi *et al.* demonstrated that TGF- β significantly increased after both the first and the second SLIT course [71]. In addition, they also reported an analogous and parallel trend of IgA serum levels, which in turn influences TGF- β synthesis [71].

IFN- γ

IFN- γ is a Th1 cytokine. AIT may induce a significantly increased production of this cytokine. Therefore, IFN- γ can be considered as an early marker of AIT

response. However, literature data are still controversial. Li and Li showed that levels of IFN- γ were significantly higher in pre- than post-SIT [52]. Conversely, Boghdadi *et al.* did not report a significant increase in IFN- γ levels at the end of immunotherapy [72].

Subcutaneous immunotherapy

SCIT has been shown to be an effective treatment for IgE-mediated disorders such as allergic rhinitis, bee venom allergy and asthma [8,73]. SCIT acts in a similar manner to SLIT, although cellular and humoral changes are more pronounced during SCIT [74]. Akdis *et al.* have demonstrated that successful SCIT is associated with an induction of peripheral T-cell tolerance [75]. SCIT induces a switch from the disease-eliciting Th2-like phenotype towards a less pathogenic Th0/Th1-like phenotype [76] and, in addition, it promotes the reduction of allergic inflammation cells such as basophils, eosinophils, T cells, mast cells and neutrophils in the skin, nose, eye and bronchial mucosa [76]. Moreover, SCIT generally inhibits allergen-induced late cutaneous responses and this correlates with increased IL-12 mRNA expression in skin macrophages [77]. IL-12 levels also correlate directly with IFN- γ and, inversely, with IL-4 levels [78]. On the other hand, few data are available on the effect of SCIT on the IL-23/IL-17 axis. It has been recently shown that high IL-12 levels can switch a Th17 towards a Th1 response [11]. SCIT also exerts its modulating action through Tregs. Tregs (CD4⁺ and CD25⁺) play a key role in allergen tolerance through secretion of soluble factors including IL-10 and TGF- β [11]. Tregs, producing IL-10 and TGF- β , can modulate the synthesis of Th2 cytokines, such as IL-4, IL-5 and IL-13. The latter can in turn induce B cells. IL-10 and TGF- β lead to immunoglobulin switching in favor of IgG over IgE, and in a few reported cases, IgA responses. Moreover, the presentation of the allergen via a subcutaneous route may have the additional benefit of restoring DC TLR9-mediated IFN- α production [79–82].

Sublingual immunotherapy

Similarly to SCIT, SLIT hinders the expression of intercellular adhesion molecule-1, the recruitment of eosinophils in the eye, as well as of neutrophils and eosinophils in the nasal mucosa [83]. However, SLIT does not influence the numbers of DCs or T lymphocytes in the epithelium or lamina propria of the oral mucosa [78]. Moreover, SLIT seems to increase only allergen-specific IgG₄ levels resulting therefore in a limited immune-modulatory effect compared with SCIT [11].

Studies of local and systemic immune responses to immunotherapy have proposed several mechanisms to

explain its effect, including the regulation of proallergic Th2 responses, the suppression of innate effector cells of allergic inflammation (mast cells and basophils) and the impaired antibody-production inhibition, specifically through the IgG blockade of IgE-mediated responses [84,85].

Recent studies have proposed that successful immunotherapy modifies the T-cell response to the allergen through the induction of regulatory mechanisms [85]. The allergen-tolerant state is associated with local and systemic induction of distinct populations of allergen-specific Tregs that are able to produce anti-inflammatory cytokines such as IL-10 and TGF- β . There are different Treg subsets associated with distinct phenotypes and mechanisms of action. These include: IL-10⁺ Tregs (Tr1 cells), TGF- β ⁺ Tregs and FoxP3⁺ memory Tregs [86–88]. These cells play a key role in allergen tolerance and they can be induced by AIT in humans [89].

More studies have demonstrated that, after the beginning of AIT, there is a shift of CD4⁺ Th cells from a Th2 (IL-4 and IL-5 production) to a Th1 profile (IFN- γ and IL-10 production) upon stimulation with allergen. Early production of IL-10 and maintained levels of TGF- β are related to its efficacy [90].

Tregs not only diminish Th2 immune responses, but also target other cell types such as DCs, mast cells, basophils and eosinophils. The effects of Tregs include, among others, allergen-specific IgE regulation, IgG and IgA induction, and specific inhibition of mast cell degranulation [79].

Induction of allergen-specific, IL-10- and/or TGF- β -producing FOXP3⁺ Tregs is considered as one of the key mechanisms of AIT success. Increased numbers of FOXP3-expressing CD4⁺CD25⁺ Tregs have been found in nasal mucosa after grass pollen AIT. The increase correlates with the clinical efficacy as well as the suppression of local allergic inflammation, involving a reduction in the numbers of mucosal IL-5 mRNA⁺ cells and eosinophils [91]. The capacity of these cells to produce IL-10 and TGF- β , essential for the induction of immunologic tolerance, has been reported by several studies [90].

IL-10 is a general inhibitor of proliferative response in T cells and may reduce the Th2 profile of cytokine production (IL-4, IL-5 and IL-13). IL-10 is also produced by different cell types such as phagocytes, NK cells, B cells, and both Th1 and Th2 cells [92]. High levels of IL-10 have been found in patients treated with AIT compared with placebo, underscoring its role in immune tolerance.

TGF- β is a pleiotropic cytokine with several regulatory functions in the immune system such as down-regulation of naive T-cell differentiation into effector cells and blockade of the differentiation of Th1 and

Th2 cells [93,94]. It has been suggested that the regulation of both Th1 and Th2 polarization may at least in part be due to the effects of Tregs. SLIT also induces IL-10 production not only in Tregs, but also in B cells, monocytes and DCs that act as antigen-presenting cells. IL-10 suppresses either total IgE or allergen-specific IgE secretion. IL-10 induces a switch from IgE to IgG production, acting on allergen-specific B cells, leading to IgG₁ and IgG₄ secretion [81]. The increase in allergen specific IgG₄ is more pronounced than IgG₁.

In AIT-treated patients, B cells are induced to produce IgG (particularly IgG₄) and IgA antibodies that possess blocking activity for IgE-dependent events, including basophil and mast cell activation and IgE-facilitated allergen binding to B cells with the subsequent inhibition of the release of inflammatory mediators [86,95]. These allergen-specific IgG₄ antibodies may compete for allergen with IgE bound to mast cells and therefore reduce the sensitivity of antigen-presenting B cells and thus T cells to the allergen [96]. IL-10 has also been implicated in the induction of specific secretory IgA that exert a protective action at the mucosal surfaces [82].

DCs play a significant role in stimulating IL-10-producing Tregs. The DC subtype DC1 directs Th1 responses and DC2 stimulates the Th2 pathway. It has been proposed that allergen extracts used for SLIT could act directly on DCs to induce a phenotype with an immune tolerance [97].

Recently, a novel subset of Th cells, namely Th17 cells, secreting predominantly IL-17, has been identified. Experimental data suggest that Th17 cells play a proinflammatory role in various forms of acute and chronic inflammation [98]. In several experimental models of allergic asthma, IL-17 and the Th17 cells have also been shown to contribute to the pathogenesis of allergic airway inflammation [39–41]. The expressions of IL-17 or the Th17-regulating cytokines IL-23 and IL-27 during SLIT remain currently unexplored.

Intradermal immunotherapy

Since 1921, when the first study describing the administration of allergens via the skin was reported, the intradermal route was well established in prophylactic vaccination for infectious diseases such as influenza, rabies, tuberculosis, hepatitis B virus and HIV [99–103]. Moreover, it appears an effective route for revaccination of nonresponders to the intramuscular route [103,104].

The skin and its associated lymphoid tissue is considered an immunologically active environment. Whole-protein antigens intradermally administered [105] are capable of penetrating the stratum corneum, and from here, they diffuse down to immune cells of the epidermis [106].

The dermis includes inflammatory and immune cells of myeloid or lymphoid origin, fibroblasts, macrophages, mast cells, as well as DCs. The latter promote B-cell class switching and a Th1-type response [107]. Furthermore, DCs can also upregulate high-affinity IgE receptor FcεRI levels, which facilitate allergen uptake and processing at low concentrations [108]. Therefore, the higher abundance of DCs in the dermis and epidermis than in the connective tissue could also explain the potency of IDT in comparison with SCIT. Conversely, the epidermis contains specialized cells such as keratinocytes, pigment-producing melanocytes and antigen-presenting Langerhans cells [109]. During stressful conditions, keratinocytes may trigger expression of IL-7, cytokine thymic stromal lymphopoietin, IL-25 and IL-33. Therefore, epithelial changes may increase the expression of other molecules such as IL-1α, IL-6 and TNF-α, promoting Th1-type responses [105]. It has also been described that keratinocytes enhance the adaptive immune responses [105].

Otherwise, Langerhans cells play a key role in the induction of CD8⁺T-cell responses and in the switch from Th2 to Tregs [109], participating in the development of adaptive immunity or tolerance.

Murine studies also demonstrated that IDT prevents allergic disease, such as grass pollen allergy [110]. In 1911, the use of IDT with grass pollen for treatment of season allergic rhinitis was first described [111]. Despite poor effects on early response [112], intradermal allergen injection causes a 'late response' characterized by local swelling due to infiltration of inflammatory cells (Th2 cells, eosinophils and basophils) within 6 h that persisted for 24–36 h [102]. The conventional approach only involves the subcutaneous administration of doses of allergen. In addition to release of allergen-specific 'blocking' IgG antibodies, this protocol promoted induction of Tregs through interaction between DCs and CD4⁺ T cells [113]. It has been demonstrated that repeated high-dose antigen stimulation, by preferential allergen-specific Th2 cell deletion, can be another independent mechanism to restore immune tolerance. Wambre *et al.* used a specific model for studying grass pollen allergies. They also noted that AIT was not correlated with change in the frequency of Th1/Tregs, but with deletion-specific Th2 cells [114].

Unfortunately, high-dose SCIT was associated with additional expense or/and limitations. Therefore, it was hypothesized that low-dose intradermal allergen administration is as effective as high-dose IDT. This new protocol permitted similar systemic immunological effects on serum-specific IgE and IgG levels, and basophil response [115].

Moreover, after its treatment discontinuation, low-dose IDT exerts long-term actions [115]. IDT is also helpful in the treatment of food allergy as measured by the prevention of mast cell degranulation upon oral allergen challenge [110]. Agostinis *et al.* recently demonstrated that the treatment was well tolerated, with no serious systemic allergic reaction, although a significant increase in local eczematous reactions, even on intact skin, was observed [116].

In conclusion, these studies invite a reconsideration of low-dose intradermal allergen as a potentially effective alternative to the usual SCIT.

Intralymphatic immunotherapy

The lymph node, due to its structural and immunological characteristics, allows for more efficient responses than other tissues such as the skin tissue. The lymph nodal tissue is characterized by a high number of DCs and a low density of mast cells and basophils (cells responsible for adverse reactions), and structurally is neither vascularized, which reduces the probability of adverse reactions, nor innervated with a significant reduction in pain during the ILIT compared with the SCIT [117]. For these reasons, it represents a new target for the administration of allergens and the ILIT arises as a route able to overcome the weak points presented by the conventional methods of immunotherapy. In humans, this technique has been used through ultrasound-guided injection of allergen, at the level of the inguinal lymph nodes (a chain of 10–15 lymph nodes) via small hypodermic syringes similar to those used for insulin administration.

In addition, the antigenic doses are not particulate (larger than viruses and bacteria), and this makes it difficult to drain from the skin tissue to the lymph nodes (<1% in SCIT) [118] with a progressive reduction in the quantity of allergen that actually reaches the lymph nodes. In ILIT, the direct introduction of the allergen in the lymph nodes allows great stimulation of the immune response. It has been shown that the ILIT required 1000-fold less allergens for a response ten-times higher than SCIT [110]. ILIT has been implemented with different types of allergen such as DNA, RNA, oligosaccharides, proteins and adjuvant, and in all these cases, there has been an increase in the immune response [119].

In a double-blind trial, the use of a recombinant allergen in patients with allergy to cat dander induced an increase in nasal tolerance 74-fold higher compared with placebo ($p < 0.001$), with a significant increase, after three injections, of the T-cell response, increase in serum IL-10 levels IgG₄ levels and suppression of IgE production [120].

In a clinical trial, the ILIT was tested in 165 patients with allergy to dust mites. A group received for 3 years a vaccine subcutaneously (SCIT) for a total of 54 injections and a maintenance dose of 100,000 SQ units each. The other group received the vaccine via ILIT for a total of three injections of 1000 units each. Considering the nasal provocation as the first outcome, the ILIT group presented a rise in the threshold of eight times after only 3 months. By contrast, the SCIT group, at 3 months, did not present any effect. At follow-up after 3 years, both groups presented the same results confirming that both SCIT and ILIT are able to reach the same results, although ILIT elicits responses earlier in a more patient-friendly manner [117].

Compared with SCIT, in which the encounter between allergen and antigen-specific lymphocytes is late and with low frequency, the ILIT is characterized by an instantaneous match between allergen and T and B cells. Moreover, different experimental settings have demonstrated a marked and rapid increase in the levels of IL-2, IL-4, IFN- γ and IL-10, and consequently IgG₄ [121].

Witten *et al.* in part confirmed these data [122]. They enrolled 45 adult patients affected by grass pollen-induced rhinoconjunctivitis. In comparison to placebo, treated patients showed significantly lower IFN- γ and higher IL-10 levels. There was no differences in IL-4 and FoxP3 expression [122].

The reason for these controversial data may be related to disease severity and the different amount of allergen administered [123]. Therefore, it has been hypothesized to increase the amount of administered allergen. However, several systemic side effects were reported. Probably, ILIT may be effective in association to hypoallergenic recombinant allergens. Further investigations are needed to better understand and translate the results obtained experimentally in daily clinical practice.

Conclusion

Current research on AIT has confirmed the ability of this approach to modify the natural history of allergic diseases since it is able to induce long-lasting immunological and clinical tolerance for several years following cessation of treatment.

AIT is associated with the suppression of allergic inflammatory cascade, the increase of IgG₄ and IgA₂-associated blocking antibodies and the suppression of allergen-specific Th2 cell responses. This suppression is mediated by the induction of Tregs that exert their effects by cell–cell contact, and by release of immunomodulatory cytokines such as IL-10 and TGF- β . New routes of administration such as IDT and ILIT appear promising in increasing the efficacy of specific

immunotherapy, and reducing allergen dosage and side effects. Further studies are warranted to better explore the efficacy and applicability of these approaches in clinical practice.

Future perspective

Future studies identifying the targets and mechanisms by which AIT acts in specific allergic conditions will hopefully provide new tools for optimizing therapy in individual cases, and may yield insights into whether the different routes of immunotherapy result in different long-term outcomes. Further studies are needed to better understand the precise

immunological mechanisms of AIT in relationship to these new different routes of administration.

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Executive summary

- The prevalence of allergic diseases has strongly increased worldwide. Allergen immunotherapy (AIT) has been successfully used for the treatment of allergic disorders. AIT provides not only symptomatic relief, but it is potentially curative.
- Immunotherapy has been associated with the induction of Tregs whose suppressor cytokine (IL-10 and TGF- β) spectrum has a important impact on whether Th cells differentiate into Th1 or Th2 cells, on increasing titer of specific IgG₄ and gradual decline in the number and function of IgE, basophils and mast cells. Therefore, AIT contributes to modifying peripheral tolerance.
- Subcutaneous immunotherapy (SCIT) has been shown to be effective in treatment for IgE-mediated disorders such as allergic rhinitis, bee venom allergy and asthma. The presentation of the allergen via the subcutaneous route may have an added benefit of restoring dendritic cell TLR9-mediated IFN- α production.
- Sublingual immunotherapy acts in a similar way to SCIT, although it does not influence the numbers of dendritic cells. Moreover, sublingual immunotherapy seems to increase only allergen-specific IgG₄ levels, which are more limited than that induced by SCIT.
- Several studies invite a reconsideration of low-dose intradermal allergen as a potentially effective alternative to the usual SCIT because intradermal allergen-specific immunotherapy seems to amplify cytokine networks related to allergen administration at a lower dosage.
- Intralymphatic immunotherapy is characterized by an instantaneous match between allergen and T and B cells. However, more studies on a larger sample of subjects are needed to be able for this to be used in daily clinical practice.

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