Immunology of allergen immunotherapy

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Summary
Allergen immunotherapy (AIT) is the only disease-modifying therapy for allergic disease. Through repeated inoculations of low doses of allergen—either as whole proteins or peptides—patients can achieve a homeostatic balance between inflammatory effectors induced and/or associated with allergen contact, and mediators of immunologic non-responsiveness, potentially leading to sustained clinical improvements. AIT for airborne/respiratory tract allergens and insect venoms have traditionally been supplied subcutaneously, but other routes and modalities of administration can also be effective. Despite differences of allergen administration, there are some similarities of immunologic responses across platforms, with a general theme involving the restructuring and polarization of adaptive and innate immune effector cells. Here we review the immunology of AIT across various delivery platforms, including subcutaneous, sublingual, epicutaneous, intradermal, and intralymphatic approaches, emphasizing shared mechanisms associated with achieving immunologic non-responsiveness to allergen.

Keywords: epicutaneous immunotherapy, intradermal immunotherapy, intralymphatic immunotherapy

Abbreviations: AIT: allergen immunotherapy; APCs: antigen-presenting cells; BAL: bronchoalveolar lavage; Breg: regulatory B cell; cTfh: circulating follicular helper T cell; cTfr: circulating follicular regulatory T cell; CSMS: combined symptoms and medications score; DCreg: regulatory dendritic cell; EPIT: epicutaneous immunotherapy; HDM: house dust mite; IDIT: intradermal immunotherapy; ILC: innate lymphoid cell; ILIT: intralymphatic immunotherapy; rTr35: IL-35–induced regulatory T cells; LAP: latency-associated peptide; LC: Langerhans cell; LN: lymph node; MS: medication scores; MSC: musculin; NPT: nasal provocation testing; OIT: oral immunotherapy; OVA: ovalbumin; PBMC: peripheral blood mononuclear cell; RCT: randomized controlled trial; SCIT: subcutaneous immunotherapy; sIg: regulatory T cells; LAP: latency-associated peptide; Th2A: allergen-specific Th2 cell; tIg: total immunoglobulin; Treg: regulatory T cell.

Introduction
Allergic disease is a growing global problem driven by a pathophysiology that is not yet fully understood. Allergen immunotherapy (AIT) is a process of supplying the allergen to which the patient is sensitized (i.e. allergic) in small doses, escalating over time until reaching a maintenance dose that continues to be provided for an extended period. While patients are receiving AIT, they may become ‘desensitized’ to the allergen; that is, they become transiently non-responsive upon exposure to allergen [1]. The ideal outcome of AIT is to achieve a state of sustained non-responsiveness that may persist even after discontinuation of therapy, reflecting a persistent restructuring of the immune system; however, this has only been demonstrated in a few instances.

Allergens used in AIT have traditionally been aeroallergens, such as plant pollens, dust, and animal dander, as well as insect venom allergens, supplied via the subcutaneous route. Other routes of entry have been studied as well, particularly for food allergens via the direct oral route. Different routes of AIT also include sublingual, epicutaneous, intradermal, and intralymphatic.

Mechanisms of allergic sensitization in humans remain largely mysterious, in part because it often occurs at some undetermined time and place before the onset of symptoms. While it is unlikely that desensitization is simply the reverse of sensitization, examination of how different routes of allergen administration influences response may provide some hints into long-standing questions about how sensitized states are established and maintained. In addition, understanding how route of administration influences AIT outcomes could shed light on optimal methods for achieving immunologic non-responsiveness and provide practical options for patient care.

Pathophysiology of allergic inflammation
In allergic disease, various allergens including pollens, dust mites, animal dander, insect venoms, and foods trigger an IgE-mediated inflammatory response [2]. The route of exposure, such as inhalation in the case of aeroallergens or skin contact or consumption in the case of oral allergens, determine which tissues initially encounter the allergen and influence how symptoms manifest.

Allergic inflammation relies on an initial sensitization event whereby allergens enter and are subsequently taken up by local antigen-presenting cells (APCs) which migrate to regional lymph nodes (LNs) and activate CD4+ T lymphocytes to elicit a T helper cell (Th) type 2–response [3, 4]. In Th2 inflammation, IL-4 and IL-13 stimulate B cell class switching to IgE in a STAT6-dependent mechanism [5, 6]. Following initial sensitization, re-exposure of allergen can induce allergic inflammation.
Within seconds to minutes, allergens interact with and crosslink the high-affinity IgE receptor (FceRI), initiating a signaling cascade leading to the degranulation of mast cells and basophils with release of histamines, leukotrienes, and prostaglandins [7, 8]. These inflammatory mediators can facilitate vasodilatation and vascular leak, causing mucosal edema that can manifest clinically as nasal congestion and rhinorrhea in the case of inhaled allergens or gastrointestinal upset and diarrhea in the setting of food allergens [2]. Between 4 and 12 hours after initial exposure, a later phase response may potentially ensue with altered profiles of cellular adhesion markers including E-selectin and vascular cell adhesion molecules as well as chemotactants including IL-5 facilitating the recruitment of eosinophils, basophils, T cells, and monocytes into the mucosa, leading to more persistent inflammation, epithelial damage, and mucosal edema [9, 10].

Immunology of subcutaneous and sublingual immunotherapy

Much of our understanding of the mechanisms of AIT comes from studies in subcutaneous immunotherapy (SCIT), which has been in practice for over a century [11]. AIT influences allergen sensitivity by modulating aspects of the early and late phase responses of allergic inflammation [12]. SCIT has seen wide usage in management of airborne and insect venom allergies. However, use of SCIT in oral allergen therapy has been limited due to side effects [13].

During the repetitive allergen exposures of SCIT, APCs up-take allergen and travel to draining LNs where they induce regulatory T cells (Tregs), which secrete IL-10 and TGF-β, which tend to curtail Th2-mediated immunity in allergic inflammation [14–17]. SCIT can abrogate allergen-specific Th2 cells (Th2A), downregulate Th2 cytokines including IL-4, IL-5, and IL-13, and skew the immune response to Th1 cells [7, 18, 19].

The impact of SCIT on skewing T cell fates towards a regulatory phenotype facilitates the induction of B cell class switching with consequent production of allergen-specific IgG and IgA. These antibodies function to, among other possible mechanisms, compete with IgE for binding to allergen, preventing downstream FceRI-mediated degranulation of mast cells and basophils and FcεRII (CD23)-mediated allergen presentation [12, 20, 21]. Generation of allergen-specific IgG with IgE inhibitory activity in the nasal mucosa has been demonstrated with strong associations to clinical response [22]. AIT is accompanied by an initial increase in allergen-specific IgE followed by a return to baseline levels or a drop below baseline over years [23–25]. SCIT can induce allergen-specific B cells, plasmablasts, and IL-10 and IL-1 receptor antagonist-producing B cells [22, 26]. Recent research has also demonstrated that SCIT can upregulate circulating follicular regulatory T cells (cTfrs) and downregulate circulating follicular helper T cells (cTfs), which may ultimately contribute to suppression of IL-4 and IgE production [27, 28].

With regard to innate immune system modulation, SCIT has been implicated in curtailing the recruitment of basophils and eosinophils into the nasal epithelium [29, 30]. Notably, symptomatic improvement following SCIT was associated with diminished IL-5 expression and reduced recruitment of eosinophils into the nasal mucosa, likely as a reflection of diminished late phase reactions [31]. Furthermore, ex vivo studies of patients receiving SCIT have demonstrated suppression of basophil FcεRI-mediated activation and cytokine release, occurring in association with the upregulation of histamine receptor-2 [32].

In addition to its effects of basophils and eosinophils, SCIT was found to reduce skin mast cell populations, which in turn correlated with clinical response [33]. Furthermore, the induction of IL-10 in SCIT may mechanistically inhibit mast cell release of TNF-α, IL-8, and histamine [34].

More recent studies have uncovered an effect of SCIT on type 2 innate lymphoid cells (IIC2s). ILC2s, which secrete IL-5 and IL-13 to polarize naive T cells into Th2 cells [35, 36], appear to be downregulated by SCIT with concomitant expansion of Type 1 ILCs [37, 38].

Another mechanism by which SCIT elicits immunologic non-responsiveness may be through modulations in the composition of APCs. Specifically, SCIT can upregulate tolerogenic plasmacytoid dendritic cells (DCs) and CD14+ myeloid DCs and downregulate CD1c+ myeloid DCs [38]. SCIT has also been implicated in the induction of anti-inflammatory intermediate monocytes and downregulation of pro-inflammatory non-classical monocytes [38]. Taken as a whole, SCIT has been associated with restructuring of multiple compartments of the innate and adaptive immune systems, spanning initial antigen acquisition to the ultimate effectors of allergic inflammation.

As the immunologic mechanisms of SCIT have become clearer with recent studies, a key unknown that remains is the durability of protection conferred by SCIT. The ability of SCIT to induce a state of non-responsiveness to allergen even after the cessation of therapy has been demonstrated on some instances at least for a few years. A foundational randomized controlled trial (RCT) of participants treated with 3–4 years of grass SCIT demonstrated sustained improvements in allergic symptoms over at least 3 years after treatment discontinuation [39]. Furthermore, the participants who had discontinued SCIT had similar improvements in allergic symptoms compared to those who had continued SCIT. Immunologically, the participants who had discontinued SCIT exhibited diminished late skin responses and reductions in CD3+ T cell infiltration and IL-4 expression on allergen challenge.

The duration of SCIT therapy appears to be an important factor in whether sustained non-responsiveness can be achieved [40]. Specifically, a 3-year duration of SCIT may be required to achieve some degree of sustained non-responsiveness after discontinuation of therapy [41]. Exploratory analyses of the GRASS RCT demonstrated that 2-years of SCIT therapy was unable to confer a sustained improvement in nasal symptom scores on allergen challenge at one year after SCIT discontinuation as compared to placebo [42]. Furthermore, differing allergens may be variably amenable to achieving sustained non-responsiveness. An RCT of 3-years of SCIT in ragweed allergy demonstrated a partial return of nasal reactivity on allergen challenge, suggesting that there may be some waning in protection over time [43]. Future studies exploring the persistence of key immunologic mechanisms of SCIT after discontinuation of therapy may elucidate the critical mediators of immunologic non-responsiveness.

Allergen provided by the sublingual route has been shown to be efficacious in reducing symptomatic rhinitis since the 1980s [44] and shares many mechanistic features with SCIT [45–50]. Sublingual immunotherapy (SLIT) has been widely used in therapy for aeroallergens including pollens and dust...
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Slit relies on initial antigen capture by Langerhans cells (LCS) in the oral mucosa, followed by migration to draining LNs, including the submandibular and cervical chains, for antigen presentation to T cells [52–54]. This interaction stimulates generation of Th1 cells and Tregs while downregulating Th2 and Th2A cells, analogous to the mechanisms observed in SCIT [18]. Single-cell immunophenotyping implicated increased expression of muscle (MSC), a transcription factor that represses the activity of Th2 cells; in pathogenic Th2 cells, among SLIT-treated patients one year into therapy [55]. Patients who had a favorable response to SLIT exhibited increases in MSC levels, raising its candidacy as a potential biomarker for clinical efficacy. Furthermore, SLIT is associated with regulatory changes at the epigenetic level with a possible induction of CD4+ CD25high CD127low CD45RO− FOXP3+ memory Tregs with hypomethylation and increased expression of Foxp3 [56]. SLIT has also been demonstrated to elicit a newly described subset of Tregs, namely IL-35–induced regulatory T cells (iTr35s), which further contribute to suppression of Th2 effector cells and cytokine production [57], although it is unclear if this mechanism is absent in SCIT.

Ex vivo analysis showed that both SCIT and SLIT can be associated with downregulation of cTfhs and upregulation of cTfrs, which in turn may prevent the induction of IL-4, IL-21, and IL-6 in the nasal mucosa after allergen exposure (Table 1) [58]. IL-4 is known to stimulate B cell class switching to IgE and IL-21 has more nuanced role in the regulation of IgE production [59, 60]. Ex vivo analysis further demonstrated significant differences in chromatin accessibility in cTfhs and cTfrs between SCIT and SLIT [58], the clinical impact of which remains to be elucidated.

In regards to the humoral response, clinical studies have demonstrated that SLIT can induce allergen-specific IgG4 and IgG2 production with corresponding increases in allergen-specific IgG4 and IgG2 memory B cells [46]. A recent RCT demonstrated that induction of allergen-specific IgG2 by SLIT was associated with favorable clinical responses [71]. An RCT comparing SCIT and SLIT demonstrated that SLIT generates more robust allergen-specific IgA responses, most prominently in nasal fluid, which may be expected based upon the mucosal route of delivery (Table 1) [69]. In contrast, SCIT generated a stronger allergen-specific IgG4 response than SLIT. Furthermore, in both SCIT and SLIT, the induction of a population of IL-10 secreting ILCs was associated with improved clinical response, possibly acting mechanistically through Th2 suppression and the maintenance of epithelial barriers against allergens [70]. A recent ex vivo analysis of patients with lipid transfer protein allergy has additionally demonstrated ILC modulation by SLIT [72].

Additionally, SLIT can have immunomodulatory effects at the level of APCs [73]. Specifically, SLIT can downregulate the costimulatory receptor CD86 and induce a subset of regulatory DCs, characterized by increased expression of complement component 1Q and stabilin-1 [74, 75]. Furthermore, the expression of complement 1Q and stabilin-1 in DCs was increased among patients who demonstrated a clinical response to SLIT as compared to nonresponders or placebo-treated patients, suggesting potential as a clinical biomarker.

Akin to SCIT, several studies have suggested that SLIT may be able to induce a persistent state of non-responsiveness to allergen, even after years of discontinuation of therapy. A number of RCTs analyzing 3-year courses of SLIT in grass pollen allergy have demonstrated sustained symptomatic control in the 1-2 year period after discontinuation of therapy [76–79]. In association with these clinical findings, SLIT-treated patients had persistent reductions in total IgE, allergen-specific IgE, and diminished skin test reactivity 2 years after treatment was discontinued [76]. Furthermore, another RCT evaluating patients at two years post-treatment demonstrated sustained increases in both allergen-specific IgG4 and in IgE-blocking activity [79]. As with SCIT, the duration of SLIT therapy appears to be critical in whether sustained non-responsiveness can be achieved. The GRASS RCT demonstrated that a 2-year course of SLIT for grass allergy was insufficient in improving nasal symptoms upon allergen challenge at 1-year post-therapy as compared to placebo [42]. Longer studies will be needed to ascertain the persistence of these effects as well as to explore the duration of protection in other systems beyond grass allergens.

Other methods of AIT were conceived to maximize practical aspects of treatment delivery and therapeutic longevity by shortening the duration of intervention while minimizing systemic allergic side effects [80]. The rationale for these approaches would be to deliver allergen to tissue containing abundant APCs to maximize immunogenicity while also containing few mast cells and limited vasculature to minimize both local and systemic reactions.

Epicutaneous immunotherapy

The precedent for epicutaneous immunotherapy (EPIT) was set in the 1950s among patients who underwent needle scarification of the forearm with pollen extract, resulting in improvement in hay fever outcomes [81]. EPIT was revisited in the 2000s as an AIT modality that would capitalize on the high density of antigen-presenting LCs in the epidermis while minimizing risk of systemic reactions due to poor vascularity. Initial studies had utilized an adhesive tape stripping approach in EPIT to remove layers of stratum corneum, generating an inflammatory response from keratinocytes with increased LC expression of MHC class II, CD86, CD40, CD54, and CD11c, accompanied by migration to draining LNs [82]. The initial RCT of tape stripping EPIT for grass pollen allergy demonstrated significant symptomatic improvement [83]

The Epicutaneous Viaskin Patch is a more recent mode of delivery in EPIT which consists of a transparent plastic membrane loaded with powdered allergen, creating an occlusive chamber and utilizing transepidermal water loss to increase permeability of the stratum corneum to solubilize allergens and facilitate accessibility to APCs [84, 85]. Viaskin patch EPIT has shown significant promise in the treatment of peanut allergy [86]. However, Viaskin patches continue to have low rates of allergen delivery into the epidermis, suggesting potential for further optimization [85]. More recently, a powder-laden, dissolvable microneedle array (PLD-MNA) has emerged as an EPIT platform to optimize allergen delivery [87]. In this modality, microneedles loaded with powdered allergens are inserted into the skin. The shafts of the microneedles dissolve, releasing the powdered allergen, which is retained in the epidermis with minimal escape into the circulation [88].

In contrast to SCIT, EPIT has seen use in both oral allergens and aeroallergens (Table 2) [89]. A recent systematic review
Table 1. Clinical studies comparing immunologic correlates in SCIT and SLIT

| Allergen | Study system | Immunologic correlates | Clinical response | References |
|----------|--------------|------------------------|-------------------|------------|
|          |              | Humoral | Cell-mediated | Other |                   |                     |
| HDM      | RCT          | ↑ In sIgG, in SCIT     |                   | Improvement in symptom scores for rhinitis and asthma and MS in SCIT group | Mungan [61] |
|          |              | and SLIT groups (as compared to baseline) |       | Improvement in rhinitis symptom scores and MS in SLIT group |       |
| Birch    | Non-randomized, no control | = sIgE after pollen season in SCIT and SLIT groups |       | No difference in CSMS between SCIT and SLIT groups | Mauro [62] |
| HDM      | Non-randomized, no control | ↑ In sIgE and sIgG, in only SCIT group |       | Improvement in subjective evaluations in SCIT and SLIT groups | Antúnez [63] |
| HDM      | RCT          | ↓ In sIgE in SCIT and SLIT groups |       | Improvement in total symptom and MS in SCIT and SLIT groups | Eifan [64] |
| HDM      | RCT          | ↓ In sIgE in SCIT and SLIT groups | ↑ In IL-10 levels in SCIT and SLIT groups | Improvement in symptom scores and MS for rhinitis and asthma in SCIT group | Yukselen [65] |
| HDM      | RCT          | Greater ↑ in sIgG, in the SCIT group compared with SLIT group | ↓ In nasal eosinophils after nasal provocation in SCIT and SLIT groups | Improvement in total symptom scores in both SCIT and SLIT groups | Karakoc-Aydiner [66] |
| Grass    | RCT          | ↓ In frequencies of allergen-specific CD4⁺ T cells in SCIT and SLIT groups (though reversed 1 year after discontinuation) |       | = Total nasal symptom scores after allergen test in SCIT and SLIT groups (1 year after discontinuation of therapy, as reported in [42]) | Renand [18] |
| Mono- or poly-sensitized to different allergens | Prospective comparative case series | ↓ In tIgE in SCIT and SLIT groups |       | Improvement in total ocular symptom scores and MS in SCIT and SLIT groups | Sayed [67] |
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Table 1. Continued

| Allergen | Study system | Immunologic correlates | Clinical response | References |
|----------|--------------|------------------------|-------------------|------------|
| HDM      | RCT          | ↑ In sIgG in SCIT and SLIT groups (higher levels in SCIT group) | Improvement of total rhinitis score and M5 in SCIT and SLIT groups | Xian [68] |
| Grass    | RCT          | Greater ↑ in sIgA1/2 in SLIT compared to SCIT | Improvement in total symptom scores in SCIT and SLIT groups | Shamji [69] |
| Grass    | Cross-sectional study | ↓ In cTfh cells in SCIT and SLIT groups | Improvement in total symptom scores in SCIT and SLIT groups | Sharif [58] |
| Grass    | SCIT and SLIT | Restoration of IL-10+ KLRG1+ ILC2s in both SCIT and SLIT with correlation of frequencies of IL-10+ ILC2s with symptomatic improvement | Improvement in total symptom scores in SLIT group (SCIT evaluation was cross-sectional) | Golebski [70] |

concluded that EPIT was likely efficacious in inducing non-responsiveness in peanut allergy with uncertain benefit for cow milk or grass pollen [90]. In EPIT using the Viaskin Patch, initial antigen acquisition relies on both CD11b+ CD64+ classical dendritic cells type 2 (cDC2s) and LCs [91, 92]. One murine model study demonstrated that PLD-MNA may induce classical dendritic cells type 2 (cDC2s) and LCs [91, 92]. One murine model study demonstrated that PLD-MNA may induce skin-resident CD11b+ CD64+ macrophages to produce IL-10 [93].

Grass RCT Greater ↑ in sIgA1/2 in SLIT compared to SCIT Greater ↑ in sIgG and sIgG2 in SCIT compared to SLIT

In hallmarking a shift towards a skin-resident CD11b+ CD169+ macrophages to produce IL-10, a recent murine model study demonstrated that PLD-MNA may induce classical dendritic cells type 2 (cDC2s) and LCs [91, 92]. One murine model study demonstrated that PLD-MNA may induce skin-resident CD11b+ CD169+ macrophages to produce IL-10 [93].

Another murine model study highlighted the significance of induction of CD4+ CD25+ Foxp3+ CD62L+ Tregs in the ‘bystander effect’ whereby transfer of CD62L+ Tregs induced by milk-EPIT allowed for protection against sensitization to a separate antigen, peanut [127]. However, only EPIT was able to additionally induce naïve (Foxp3+ CD44hi CD62L+ Tregs) Tregs. Underlying the significance of this mechanism, 8 weeks after discontinuation of AIT, only the Tregs induced by EPIT demonstrated persistent immunosuppressive activity in contrast to those induced by SLIT or OIT. This long-lasting protection may have clinical correlates in establishing a state of non-responsiveness to allergens, whereby patients remain desensitized despite discontinuation of AIT. Promisingly, two initial studies of patients with peanut allergy who had been treated with long-term EPIT and then discontinued for 2 months demonstrated transient non-responsiveness of peanut after treatment [97, 99]. Of note, the duration of protection after discontinuation of therapy demonstrated in this setting is limited in comparison to the long-term protection conferred by SCIT and SLIT in grass pollen allergy.

Another murine model study highlighted the significance of induction of CD4+ CD25+ Foxp3+ CD62L+ Tregs in the ‘bystander effect’ whereby transfer of CD62L+ Tregs induced by milk-EPIT allowed for protection against sensitization to a separate antigen, peanut [128]. The bystander effect was hypothesized to be linked to the hypermethylation of the Gata3 promoter, involved in Th2 differentiation, with consequent downregulation of Th2 cytokines including IL-4, IL-5, and IL-13, as well as hypomethylation and upregulation of Foxp3. These epigenetic modulations are also hypothesized to mechanistically influence the durable clinical protection conferred by EPIT [99].
| Modality | Allergen | Study system | Immunologic correlates | Clinical response | References |
|----------|----------|--------------|------------------------|-------------------|------------|
|          |          |              | Humoral                |                   |            |
| 1. EPIT  | Cow milk | RCT          | $= \text{sIgE}$         |                   | Dupont [93]|
| Peanut   | RCT      |              | $= \text{sIgE}$         |                   |            |
| Peanut   | RCT      | $\uparrow \text{sIgG}_4$, $\frac{\text{sIgG}_4}{\text{sIgE}}$ | Improvement in successfully consumed dose on food challenge | Jones [94]|
| Peanut   | RCT      | $\uparrow \text{sIgE}$ followed by return to baseline $\uparrow \text{sIgG}_4$ | Improvement in eliciting dose on food challenge | Jones [95]|
| Peanut   | RCT      | $\uparrow \text{sIgE}$ followed by return to baseline $\uparrow \text{sIgG}_4$ | Improvement in eliciting dose on food challenge | Sampson [96]|
| Peanut   | RCT      | $\uparrow \text{sIgE}$ followed by return to baseline $\uparrow \text{sIgG}_4$ | Improvement in eliciting dose on food challenge | Fleischer [86]|
| Peanut   | RCT      | $\uparrow \text{sIgG}_4$ | Improvement in successfully consumed dose on food challenge | Fleischer [97]|
| Peanut   | RCT      | $\uparrow \text{sIgG}_4$ | Continued non-responsiveness on food challenge | [99] Brown-Whitehorn 2021 |
| Peanut   | Non-randomized, no control (open label extension) | $\uparrow \text{sIgG}_4$, $\uparrow \text{sIgE}$ followed by return to baseline $\uparrow \text{sIgG}_4$ | Improvement in eliciting dose on food challenge | Scurlock [98]|
| Peanut   | Non-randomized, no control (2 months of discontinuation of EPIT) | $\uparrow \text{sIgG}_4$ | Positive association between proportion of allergen-specific type 2 cells after allergen stimulation and sIgE levels $\downarrow$ in CD154+ IL-4+ and CD154+ IL-13+ cells after allergen stimulation $\downarrow$ in CD154+ IL-10+ cells after allergen stimulation Negative association between proportion of allergen-specific type 2 cell and successfully completed dose on oral food challenge Positive association between proportion of allergen-specific type 2 cells after allergen stimulation and basophil activation tests | As per Jones [95]|
| 2. Intradermal | Grass | Randomized, no placebo $\uparrow \text{sIgG}$ | $\downarrow$ Cutaneous late phase responses | Not evaluated | Rotiroti [101]|
| Grass    | RCT      | $\uparrow \text{sIgE}$ | Skin biopsy with $\uparrow$ T cell expression of Th2 surface marker CRTH2 and decreased expression of Th1 marker CXCR3 Microarray of cultured T cells demonstrating increased IL-5 expression. | No effect on CSMS | Slovick [102]|
| HDM      | Non-randomized, no control $\uparrow \text{sIgG}_4$ | $\uparrow \text{sIgG}_4$ | Improvement in total nasal symptom scores | Vieira-Hernández [103] |
| Modality          | Allergen | Study system | Immunologic correlates                                                                 | Clinical response                        | References         |
|-------------------|----------|--------------|----------------------------------------------------------------------------------------|------------------------------------------|--------------------|
|                    |          |              | Humoral \[ \text{lgG}_4 \downarrow \text{lgE} \] Cell-mediated \[ \text{lgG}_4 \uparrow \] Other \[ IL-10 \uparrow \] |                           |                    |
| Grass RCT          | Grass    |              | = \text{lgG}_4, \text{lgE} \downarrow, \text{lgG}_4, \text{lgE} \uparrow, \text{lgG}_4 \uparrow | Improvement in CSMS                       | Sola Martínez [104]|
| HDM Non-randomized, no control | HDM     |              | \[ \text{lgG}_4 \uparrow \] \[ IL-10 \uparrow \] | Improvement in total nasal symptom scores | Rondon [105]       |
| 3. Intralymphatic Grass RCT | Grass    |              | \[ \text{lgE} \downarrow \] \[ IL-10 \downarrow \] | Improvement on NPT and subjective symptom scores | Senti [106]        |
|                    | Cat      | RCT          | = \text{lgG}_4, \text{lgE} \downarrow, \text{lgG}_4, \text{lgE} \uparrow, \text{lgG}_4 \uparrow | Improvement on NPT                        | Senti [107]        |
|                    | Grass    | RCT          | \[ \text{lgE} \downarrow, \text{lgG}_4 \uparrow \] \[ IFN-\gamma \downarrow \] \[ IL-10 \uparrow \] | No effect on CSMS                         | Witten [108]       |
| Grass, birch RCT   | Grass,   |              | \[ \text{lgE} \downarrow, \text{lgG}_4 \uparrow \] \[ Serum expression of activation markers CD69 and CD98 on CD4+ T cells \[ Allergen-stimulated IL-10 secretion \] | Improvement in symptom scores            | Hylander [109]     |
|                    | birch    |              | = CD4+ CD25+ FoxP3+ cells \[ Pollen-induced histamine release \| In number of living leukocytes and epithelial cells in nasal lavage Tendency towards \[ in IL-8 in nasal lavage \] | Improvement in symptom scores            |                    |
|                    | Cat      | RCT          | \[ \text{lgE} \downarrow, \text{lgG}_4 \uparrow \] \[ IFN-\gamma \downarrow \] \[ IL-10 \uparrow \] | As reported in Senti [107]                 | Freiberger [111]   |
|                    | Grass    | RCT          | \[ \text{lgE} \downarrow, \text{lgG}_4 \uparrow \] \[ Memory T cells with increased proportion of CCR7 effector memory T cells in LNs \[ Increase in CCR5+Th1 cells and CD4+ CD25+ Tregs in blood \| Number of FoxP3+ Tregs or Bregs between AIT and placebo \] | Improvement on NPT                        | Hellkvist [112]    |
|                    | Japanese | cedar RCT    | \[ \text{lgE} \downarrow, \text{lgG}_4 \uparrow \] \[ Memory T cells with increased proportion of CCR7 effector memory T cells in LNs \[ Increase in CCR5+Th1 cells and CD4+ CD25+ Tregs in blood \| Number of FoxP3+ Tregs or Bregs between AIT and placebo \] | Improvement on NPT                        | Terada [113]       |
|                    | Birch    | timothy RCT  | \[ \text{lgE} \downarrow, \text{lgG}_4 \uparrow \] \[ Memory T cells with increased proportion of CCR7 effector memory T cells in LNs \[ Increase in CCR5+Th1 cells and CD4+ CD25+ Tregs in blood \| Number of FoxP3+ Tregs or Bregs between AIT and placebo \] | Improvement on NPT                        | Konradsen [114]    |

Table 2. Continued
Induction of intestinal non-responsiveness to food allergens may require Tregs with the capacity to home to intestinal tissue to undergo local proliferation in the intestinal lamina propria [129]. Certain LN distributions within the gut may be predisposed to generate tolerogenic versus pro-inflammatory immune responses [130]. In a murine model, drainage of dietary antigens into the proximal intestine was associated with FOXP3+ Treg-mediated immunity, whereas delivery of antigen to the LNs in the distal GI tract resulted in generation of effector T cells. These findings suggested that compartmentalized delivery of antigens may be a potential strategy to influence tolerogenic immunity.

Insight into how EPIT can induce gut-specific non-responsiveness to oral allergens was elucidated in a set of recent studies [91, 92, 131]. Firstly, in a comparison of EPIT and OIT in the prevention of ovalbumin (OVA)-induced anaphylaxis in mice models, only EPIT demonstrated protection four weeks after discontinuation of therapy [131]. EPIT induced a special population of Tregs, namely Foxp3 - latency-associated peptide (LAP)+ Tregs, which functioned to directly inhibit mast cell function in a TGF-β dependent mechanism. Furthermore, while T cells activated in cutaneous LNs generally exhibit homing towards cutaneous tissue, the LAP+ Tregs induced by EPIT demonstrate homing capacity for both the skin (CCR4) and gut (CCR6, CCR9) [131, 132]. The LAP+ Tregs appear to be transiently activated, appearing after 2 weeks of EPIT though not persisting after 8 weeks [92]. These findings led to the suggestion that induction of LAP+ Tregs may be part

| Table 2. Continued |
|-------------------|
| **Modality** | **Allergen** | **Study system** | **Immunologic correlates** | **Clinical response** | **References** |
|-----------------|-------------|-----------------|-----------------------------|----------------------|----------------|
| Humoral | Cell-mediated | Other |                |                      |                |
|Mountain cedar | RCT | ↑ sIgE (though similar ↑ in placebo) |                | Improvement in CSMS | Thompson [115] |
|Grass | RCT | ↑ sIgG4 |                | No significant change in symptom scores | Weinfeld [116] |
|Human dust mite, cat, dog | RCT | ↑ sIgE | ↑ Intralymphatic DC expression of costimulatory molecules CD80 and CD86 = CCR5+ central memory T cells or CD25+ CD4+ effector memory T cells in blood | Trend towards ↓ percentages of activated CD63+ basophils on basophil reactivity test | No significant change in symptom scores or MS | Park [117] |
|Grass | RCT | ↑ sIgG4 | ↑ sIgE | Improvement in CSMS | Skaarup [118] |
|Grass pollen | Study 1: RCT (previous grass SCIT) | ↑ sIgG4 | ↑ sIgE | Study 1: improvement in CSMS | Hellkvist [119] |
|Study 2: RCT (no previous AIT) | | | | Study 2: no improvement in CSMS | |
|Birch, grass pollen | RCT | ↓ sIgE = sIgG4 | ↑ Allergen-induced IL-10 secretion ↑ CD44+ CD25+ Foxp3+ Tregs and activated CD3+ CD4+ CD45RA+ Foxp3+ Tregs = resting CD3+ CD4+ CD45RA+ Foxp3+ Tregs | Improvement in symptom scores and MS | Ahlbeck [120] |
|Bee venom | Study 1: non-randomized, no control | ↑ sIgG1 = sIgG4, sIgE | Study 2: randomized, no control | Study 1: protection on insect venom challenge | |
|Study 1: randomized, no control | ↑ sIgE, sIgG1 | ↑ sIgE | Study 2: several serious adverse effects on insect venom challenge | Study 2: several serious adverse effects on insect venom challenge | Chabot [121] |
|Birch, grass | RCT | ↑ sIgG4 | ↑ sIgE | ↓ CD4+ memory T cells in LNs ↓ FcrR1 on basophils | Improvement in CSMS and MS | Hjalmarsson [122] |

| References |
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| Thompson [115] |
| Weinfeld [116] |
| Park [117] |
| Skaarup [118] |
| Hellkvist [119] |
| Ahlbeck [120] |
| Chabot [121] |
| Hjalmarsson [122] |
of the initial mechanistic induction of EPIT, followed by a more persistent response mediated by CD62+ Tregs [123]. The efficacy of EPIT in oral allergy therapy highlights the interconnectedness of the immune system, whereby antigen exposure in the skin can induce sustained and clinically relevant alterations in the gut mucosa.

The potential evolutionary rationale for the skin-mucosal surface immune connection uncovered with the use of EPIT is intriguing. There is a strong precedent for skin epithelial barrier dysfunction driving allergic sensitization as illustrated by the atopic march, hallmarked by disease progression from atopic dermatitis to allergic rhinitis, asthma, and food allergies, driven by pathophysiology whereby local inflammatory responses facilitate barrier breakdown in distant organs, including the gastrointestinal and respiratory tracts [133]. In a paralleled fashion that underlies the pattern of immune dysregulation in the atopic march, EPIT appears to harness skin-gut immune mechanisms, namely unique tissue-homing markers, to transduce stimuli at the cutaneous level to establish non-responsiveness in the gut.

In regards to the humoral response to EPIT, clinical trials have demonstrated elevations in allergen-specific IgG4 [86, 95, 96]. However, the significance of the humoral response is uncertain with one study demonstrating that passive sensitization of naïve mice with sera from EPIT-treated mice was ineffective in preventing anaphylaxis, arguing against systemically accessible IgE-blocking IgG antibodies as the sole mechanism of non-responsiveness to oral allergens in EPIT [131]. Consistent with SCIT, EPIT has been shown to induce initial elevations in allergen-specific IgE followed by return to baseline levels in clinical studies [86, 96].

**Intradermal immunotherapy**

The precedent for intradermal immunotherapy (IDIT) was established by Phillips in 1926 who reported symptomatic improvement with IDIT in a group of 29 patients with grass pollen allergy [134]. IDIT has since seen use in both aeroallergens and insect venom therapy (Table 2). An ex vivo study of human skin demonstrated that initial antigen acquisition in IDIT is most efficiently achieved by CD14+ dermal DCs, followed by CD1a+ dermal DCs [135]. Another murine model study identified a significant role of Langerin+ dermal DCs in mediating the immunoregulatory impacts of IDIT [136]. IDIT, as compared to SLIT or EPIT, resulted in high allergen-specific IgG production with reductions in allergen-specific IgE [136]. Notably, upon experimental deletion of Langerin+ dermal DCs, there was a restoration of allergen-specific IgE production, highlighting the likely significance of these APCs in mediating the response to IDIT therapy.

Despite promising initial findings [101], the first RCT evaluating IDIT in grass pollen failed to demonstrate clear clinical improvements [102]. Immunologic biomarkers demonstrated an increase in allergen-specific IgE and cutaneous biopsies revealed increased expression of Th2 surface marker CRTH2 and decreased expression of Th1 marker CXCR3. However, a later RCT using IDIT in grass pollen did demonstrate symptomatic improvements with an accompanying reduction in allergen-specific IgE and no changes in allergen-specific IgG4 [104]. Other studies of IDIT in house dust mite (HDM) allergy were suggestive of clinical efficacy and demonstrated increases in allergen-specific IgG4 and IL-10 [103, 105]. These findings, taken as a whole, suggest that while IDIT may rely on unique APCs for antigen acquisition and presentation, the mechanisms of allergen-specific IgG4 generation and the shift towards regulatory cytokine production in IDIT appear to be shared with SCIT and SLIT.

To circumvent the side effects and risk of IgE-mediated reactions that can accompany administration of whole allergen, peptide AIT was conceptualized. Peptide AIT utilizes either short peptides or long contiguous overlapping peptides, featuring key CD4 T cell epitopes of allergens while lacking the conformation of the intact allergen to prevent IgE recognition [137]. Peptide AIT is often administered subcutaneously or intradermally and has been recently reviewed elsewhere [137, 138].

**Intralymphatic immunotherapy**

In intralymphatic immunotherapy (ILIT), aeroallergens are generally administered to the inguinal LNs to induce a strong T-cell response while requiring smaller amounts of allergen and reduced treatment durations (~2 months) [139, 140]. As compared to SCIT, ILIT appears to cause fewer adverse effects and improves patient compliance [106]. In a murine model study comparing SCIT and ILIT, ILIT generated greater than 10-fold higher allergen-specific IgG2a response despite requiring 100-fold lower allergen doses than SCIT [141]. Biodistribution evaluations confirmed that ILIT had superior antigen delivery and retention in the LNs, whereas SCIT delivered a significant fraction of antigen to the liver. The lower allergen doses required in ILIT correlate with robust safety profiles in multiple meta-analyses of ILIT [139, 142]. Furthermore, a murine model study comparing immune responses to subcutaneous, intradermal, intramuscular, and intralymphatic routes of administration suggested that only the intralymphatic route was able to elicit a robust IgG2a response as well as Th1-skewed response with increased IFN-γ production [143]. In contrast, all routes of administration were able to induce a robust IgG1 response.

In clinical settings, ILIT administered in 4-week intervals as three doses has shown comparable symptomatic improvements to a 3-year course of SCIT with similar decreases in allergen-specific IgE [106]. In a study where ILIT was administered over 2-week intervals as opposed to 4-week intervals, there was no evidence of symptomatic improvement despite increases in allergen-specific IgG4 and a tendency towards higher IL-10 levels [108]. It was suggested that the 4-week interval may be important in the success of ILIT due to improved memory B-cell development and antibody affinity maturation [80]. Notably, under the 4-week intervals, there may have been periods of limited antigen availability in the lymphatic follicles, which could influence competitive selection of high-affinity memory B cells.

ILIT likely operates through similar humoral and cell-mediated mechanisms as SCIT and SLIT. One of the initial RCTs of ILIT demonstrated induction of allergen-specific IgG4 and IL-10 [107]. ILIT-mediated generation of allergen-specific IgG4 has since been recapitulated in multiple studies [111, 112, 116, 118, 144]. However, the effect of ILIT on allergen-specific IgE levels appeared unclear in a recent meta-analysis [144]. Furthermore, ILIT may induce immunologic changes in target nasopharyngeal tissues with one murine model study of ILIT demonstrating decreased eosinophil counts and reduced mRNA expression of IL-4, IL-6, IL-17A, and IL-33.
in nasal mucosa [145]. Diminished eosinophil recruitment in ILIT appears to be mediated by reduced expression of the chemokine eotaxin-2, while diminished neutrophil recruitment may be related to reduced expression of CXCL1 and CXCL2 [146].

A recent murine model study revealed that ILIT can diminish the production of serum Th2 cytokines including IL-13, IL-25, and IL-33, consistent with the Th2 dampening observed across AIT modalities [143]. However, in contrast to the Th1 skewing observed in many AIT modalities, a number of ILIT studies have found evidence of ILIT-induced downregulation of Th1 and Th17 pathways [145, 146].

In an RCT, ILIT appeared to increase levels of CD4+ CD25+ effector memory Tregs in the peripheral blood, consistent with shifts towards regulatory immunity [112]. Furthermore, ILIT can induce increases in effector memory T cells within the LNs, suggesting that some of the immunoregulatory effects of ILIT can be mirrored in both the blood and LNs [112, 122].

Some studies did not demonstrate that ILIT was able to specifically induce FOXP3+ Tregs [110, 113]. However, a recent murine model study did demonstrate significantly higher levels of FOXP3+ Tregs following ILIT [145]. A recent study gave evidence of the ‘bystander effect’ in ILIT. In this regard, patients sensitized to both birch and grass pollen had received ILIT for either birch or grass and then demonstrated symptomatic improvements not only during the targeted allergen season but also during the non-targeted allergen season [120]. This finding occurred in the setting of increases in IL-10 secretion and upregulation of CD4dimCD25+FoxP3+ Tregs and activated CD3+ CD4+ CD45RA−FoxP3dim Tregs. This transition to a regulatory phenotype was hypothesized to confer a dampening of overall allergic inflammation, occurring independently of the triggering allergen, comparable to the bystander phenotypes described in EPIT and SCIT [128, 147, 148].

Another study demonstrated that ILIT-induced CD3+ CD4+ Foxp3+ Treg cells with increased expression of CCR7, a LN homing marker, suggestive of increased propensity for Treg recirculation in lymphoid tissues [149], which may be expected to suppress both new and recall lymphocyte reactions to allergen. Furthermore, studies have shown decreased basophil FceR1 expression and a trend towards diminished basophil reactivity, highlighting potential modulation at the level of the innate immune system [117, 122]. As a whole, multiple recent systematic reviews have concluded that ILIT appears to be safe in treatment of allergic rhinitis though with widely varying reports of long-term efficacy in setting of significant trial heterogeneity [139, 142, 144].

**Conclusion**

Allergen contact appears to be required for some individuals to develop hypersensitivity to that allergen. Paradoxically, allergen exposure, presumably processed through similar pathways of tissue and immune interactions, is also a path for desensitization. The different outcomes of allergen-mediated sensitization versus desensitization by contact with the same allergen may have at their root differences in allergen dosage, frequency, context, host factors, adjuvant availability, and route through which allergen gains access to the body. In this light, although different routes may have different propensities for context-dependent immune responses, the fact that each of the diverse routes of allergen entry reviewed here can achieve desensitization is consistent with a model that sensitization may also not depend upon a particular entry portal. Even aerosolized allergen delivered through the nasal route, a likely route for pollen sensitization, can lead to allergen-specific desensitization [150–152]. In addition, pollen delivery to the gastrointestinal tract can desensitize to aeroallergens in the case of birch [153, 154] and possibly ragweed [155, 156], consistent with a model of cross-system induction of immunologic non-responsiveness to allergens.

**Immune system analysis in controlled desensitization experiments illuminates a general structure of antigen/allergen processing in different barrier tissues. In general, there is indirect or direct antigen delivery to LNs, followed by upregulation of Tregs to establish peripheral non-responsiveness to allergen. The immunologic differences between AIT routes reside in which APCs facilitate antigen acquisition, their physiologic state/inclination, and aspects of their movement. Other differences include the kinetics of antigen presentation in LNs, the allergen-specific Ig responses generated, and potentially, the type of Treg induced, which may be connected to variation in tissue-specific homing properties.

Regarding this latter point, a connection between skin and gut is illuminated by the efficacy of EPIT in the treatment of peanut allergy, perhaps related mechanistically by the induction of gut-homing LAP+ Tregs as well as long-lived naive CD62L+ Tregs. Meanwhile, in ILIT, the induced Treg population harbors homing markers for LNs, facilitating continued recirculation through secondary lymphoid organs, potentially influencing the anatomic reach and longevity of allergen-specific—and perhaps bystander allergen—non-responsiveness. Important questions that emerge from mechanistic considerations of desensitization is what do these regulatory pathways look like during the allergen sensitization phase, and what are the mechanisms that shift the balance instead towards intolerance. Ongoing work promises to lead to deeper insights into these questions and other long-standing puzzles of allergic disease. In addition, a more complete understanding of the mechanisms that facilitate the persistence of immunologic non-responsiveness to allergen after discontinuation of immunotherapy will be vital in maximizing the clinical impact of AIT.

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Conflicts of interest
Duane Wesemann is an Editorial Board Member of Immunotherapy Advances and as such has been blinded from reviewing or making decisions on the manuscript.

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