

Immune Mechanisms of Sublingual Immunotherapy

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Abstract Sublingual immunotherapy (SLIT) is a well-established allergen-specific immunotherapy and a safe and effective strategy to reorient inappropriate immune responses in allergic patients. SLIT takes advantage of the tolerogenic environment of the oral mucosa to promote tolerance to the allergen. Several clinical studies have investigated the complex interplay of innate and adaptive immune responses that SLIT exploits. The oral immune system is composed of tolerogenic dendritic cells that, following uptake of allergen during SLIT, support the differentiation of T helper cell type 1 (Th1) and the induction of IL-10-producing regulatory T cells. Following SLIT, allergic disease-promoting T helper cell type 2 (Th2) responses shift to a Th1 inflammatory response, and IL-10 and transforming growth factor (TGF)- β production by regulatory T cells and tolerogenic dendritic cells suppress allergen-specific T cell responses. These immune changes occur both in the sublingual mucosa and in the periphery of a patient following SLIT. SLIT also promotes the synthesis of allergen-specific IgG and IgA antibodies that block allergen-IgE complex formation and binding to inflammatory cells, thus encouraging an anti-inflammatory environment. Several of these revealing findings have also paved the way for the identification of biomarkers of the clinical efficacy of SLIT. This review presents the emerging elucidation of the immune mechanisms mediated by SLIT.

Keywords Sublingual immunotherapy · Allergy · Regulatory T cell · Tolerance · Mucosal immunity · Biomarker

Abbreviations

SLIT Sublingual immunotherapy
SCIT Subcutaneous immunotherapy

OIT Oral immunotherapy
mDC Myeloid dendritic cell
pDC Plasmacytoid dendritic cell
oLC Oral Langerhans cell
Treg Regulatory T cell

Introduction

Allergen immunotherapy has been proven to be a clinically safe and effective strategy to reorient inappropriate immune responses in allergic patients. Administration of allergens via the oral mucosal route using sublingual immunotherapy (SLIT) has gained prominence as an effective allergen-specific immunotherapy alternative to subcutaneous injections. The clinical safety of SLIT largely benefits from keeping adverse reactions to the treatment local and transient, helping to avoid interruption or cessation of treatment [1]. In SLIT, allergens are delivered to the oral mucosa via drops or tablets that are held under the tongue. While SLIT has not yet been FDA approved in the USA, it is commonly used in European clinics as an alternative to subcutaneous immunotherapy (SCIT) [2]. Several clinical trials and meta-analyses have emphasized the effectiveness of SLIT for the treatment of allergic rhinitis, asthma and food allergies, though there are apparent differences in clinical efficacy depending on the allergen utilized [3–8].

SLIT takes advantage of oral tolerance, an evolutionary conserved mechanism that promotes tolerance to environmental allergens that come in contact with the oral mucosa. The tolerogenic potential of the oral mucosal tissue is emphasized by the general lack of immune cells located in the oral mucosa following SLIT in allergic individuals [9]. Human oral tissues contain low numbers of mast cells, eosinophils, and basophils, which can release pro-inflammatory mediators in response to allergen-specific immunotherapy and promote adverse reactions, making the oral mucosa an ideal site for the avoidance of inappropriate immune responses.

The underlying immunological mechanisms exploited by SLIT have been the focus of several studies. Analogous to

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mechanisms that have been delineated for other allergen-specific immunotherapies, SLIT is thought to promote a shift from the T helper cell type 2 (Th2)-dominant inflammatory environment promoted by many allergic diseases towards T helper cell type 1 (Th1) immune responses, as well as the induction of regulatory T cells (Treg) and changes in IgG4, IgE, and IgA antibody production. Such immunological changes may help develop long-lasting tolerance or desensitization to the offending allergen [10]. Studies of the complex interplay between adaptive and innate immune responses within the oral mucosa and the periphery following SLIT are critical for the development of efficacious SLIT that induces tolerance. The following review of the immune mechanisms of SLIT will focus on published clinical trial data, primarily centering on modulations of dendritic cells, T cells, and humoral responses following SLIT and how those immune changes promote tolerance induction following efficacious SLIT (Table 1).

Dendritic Cells

Dendritic cells (DCs) are considered the primary target cells of SLIT, largely because of their close proximity to the mucosal

surface and their ability to take up the allergens and present them to T cells, priming and polarizing effector or regulatory T cell responses. Among DCs in the oral mucosa, there are both myeloid dendritic cell (mDC) and plasmacytoid (pDC) subsets, though the vast majority of DCs in the oral mucosa are mDCs. Oral mucosal Langerhans cells (oLC) represent the predominant mDC population that binds and processes allergens during SLIT [11]. Resting Langerhans cells (LCs) are immature and reside mainly within the vestibular region, but upon maturation, they upregulate CD83 and CCR7 and traffic to lymph nodes for antigen presentation to T cells following allergen uptake [12, 13]. It has been suggested that oLCs remain relatively immature and that this immature state may help them promote tolerance induction to the specific allergen during SLIT [14].

Using an ex vivo model of allergen resorption as a surrogate for human SLIT subjects, a study found that treatment with grass pollen on the sublingual mucosa did not upregulate CCR7 on the oLCs that took up the allergen and thus maintained an immature state—a hallmark of tolerogenic DCs [15]. The lack of CCR7 upregulation by oLCs that have taken up allergen also indicated that oLCs do not migrate rapidly to the lymph node following SLIT, but instead may initially present to T cells directly in the mucosa. This potential defect

Table 1 Immune modulations mediated during the course of SLIT

Parameters	Effects of SLIT	Allergens	Indications
Dendritic cells	• oLCs produce IL-10 and TGF-β1 • oLCs remain immature and do not upregulate CCR7 • oLCs rapidly bind allergen • Peripheral mDCs exhibit regulatory DC markers • Decreased sublingual subepithelium mDCs • Promote Th1 and tolerogenic cytokine production • Decreased basophil activation	Birch pollen Grass pollen House dust mite Cow's milk Peanut	Allergic rhinitis Allergic rhinoconjunctivitis Food allergy
T cells	• Induction of peripheral FoxP3-expressing IL-10-producing Tregs • Induction of sublingual epithelium IL-10-producing Tregs • Decreased Th2 cytokine profile ($\downarrow$ IL-4-producing T cells, IL-5, IL-13) • increased Th1 cytokine profile ($\uparrow$ IFNγ-producing T cells) • Induction of TGF-β-mediated suppression by Tregs • Suppression of T cell proliferation and allergen-specific T cell responses • Reduced methylation of CpG site in <i>Foxp3</i> locus of inducible Tregs	Grass pollen House dust mite Birch pollen Japanese cedar pollen Peanut	Allergic rhinitis Cedar pollinosis Allergic rhinoconjunctivitis Food allergy
Antibody responses	• Decreased allergen-specific serum IgE antibody • Increased allergen-specific serum IgG1 and IgG4 antibody • Increased allergen-specific serum and salivary IgA antibody • Induction of blocking allergen-specific IgG4 and IgA antibody	Grass pollen House dust mite Birch pollen Japanese cedar pollen Peanut Cow's milk Ragweed Peach	Allergic rhinitis Cedar pollinosis Allergic rhinoconjunctivitis Food allergy Asthma

oLC oral Langerhans cells, *Treg* regulatory T cell, *DC* dendritic cell, *mDC* myeloid dendritic cell

in oLC migration to the lymph node following SLIT has been supported by another related oLC localization study [13]. Compellingly, oLCs that had taken up the allergen produced the regulatory cytokines IL-10 and transforming growth factor (TGF)- β 1, which are both known to contribute to a tolerogenic microenvironment [15]. The immature phenotype of oLCs and their regulatory cytokine production strongly implicates their involvement in the development of a tolerogenic microenvironment. Later studies have further supported the postulate that SLIT induces tolerogenic DCs. Children undergoing SLIT to a common house dust mite allergen possessed peripheral mDCs that produced IL-10 upon activation, and DCs from patients undergoing SLIT for grass pollen expressed markers highly associated with regulatory DCs that are known to promote the development of a tolerogenic environment [16, 17]. Thus, tolerogenic DCs may represent a biomarker for the clinical efficacy of SLIT [18].

Interestingly, the binding of grass pollen allergen by oLCs happened rapidly within 5 min of exposure, and those oLCs that bound grass pollen allergen began to migrate within 24 h of allergen exposure [15]. A recent study has shown that sublingual ductal epithelial cells help with this rapid transfer of allergen to sublingual DCs [19]. oLCs were able to uptake a high concentration of allergen but had a peak concentration where no more allergen could be bound [15]. These findings help explain the results of early SLIT clinical trials where low allergen doses were ineffective and high allergen doses were effective, but an even higher allergen dosage was associated with marked local adverse reactions [20, 21]. This indicates that the amount of allergen that is rapidly bound by oLCs reaches a saturation point during SLIT where oLCs are no longer able to take up allergen, and instead the *free* allergen is able to react with local inflammatory cells and mediates unwanted inflammatory responses.

Further, while it has been proposed that tolerogenic DCs promote the polarization of Th1 over Th2 responses following SLIT, few studies have attempted to test this hypothesis. One of the few studies that attempted to test this postulate utilized a DC-T cell co-culture approach, where peripheral mDCs from patients who had received one course of birch pollen SLIT were co-cultured with T cells stimulated with birch pollen allergen. After SLIT, significantly increased IL-10 and decreased Th2 cytokine IL-13 as well as increased Th1 cytokines were observed in the supernatants of the co-cultures from treated allergic patients [22]. While an *in vitro* T-DC co-culture clearly does not recapitulate the complex *in vivo* environment, this study shows that mDCs from the periphery, not just the oral mucosa, can mediate the modulations in cytokine production by allergen-specific T cells and promote a tolerogenic environment following SLIT. However, further studies are needed to elucidate the roles that tolerogenic DCs play in directing early T cell differentiation and function following SLIT.

T Cells

SLIT also promotes the development of Tregs and a polarization from an allergic disease-promoting Th2 response to a Th1 response. The lingual immune system possesses both T cells with immunosuppressive functions and Th1, Th2, and Th17 effector T cells [23]. One of the earliest reports on the effects of SLIT on T cells showed significantly increased IL-10 production by peripheral blood mononuclear cells (PBMCs) from patients treated with house dust mite SLIT for 3 years compared to untreated patients [24]. Increased IL-10 was also found in later studies following grass pollen SLIT [25]. These early studies emphasized a correlation between increased IL-10 production and the clinical efficacy of SLIT.

Following a small number of patients undergoing birch pollen SLIT, a thorough study of the immune mechanisms of SLIT revealed that after 4 weeks of SLIT, higher frequencies of Forkhead box P3 (FoxP3)-expressing Tregs with increased IL-10 messenger RNA (mRNA) transcripts were detectable in the periphery as well as the suppression of allergen-specific T cell proliferation [26]. The regulatory family of T cells has been implicated in other clinically efficacious allergen-specific immunotherapies [27, 28]. Tregs are largely characterized by their constitutive expression of the IL-2 receptor alpha chain (CD25) and the transcription factor FoxP3. FoxP3 is responsible for the transcriptional control of regulatory T cell function, though it is not necessary for Treg development. Tregs can be thymus-derived or inducible; in each case, they can suppress effector immune responses directly or indirectly through TGF- β and/or IL-10 production [29]. Inducible Tregs play a key role in suppressing aberrant allergen-specific effector T cell responses following allergen-specific immunotherapy via SCIT and oral immunotherapy (OIT) [9, 30–32]. After 52 weeks of birch pollen SLIT, a late phase of the immune response to SLIT was revealed, where there was significantly decreased IL-4 production but increased IFN γ production, indicating that late during SLIT there is a shift from Th2 to Th1 responses [26]. Thus, over time, it appears that SLIT promotes the development of inducible IL-10-producing Tregs that suppress allergen-specific Th2 cells and promote a Th1-skewed and regulatory inflammatory environment. Additionally, though TGF- β may mediate the suppression of allergen-specific T cell responses at early and late phases during clinically efficacious SLIT, the roles of TGF- β production by Tregs and other immune cells during SLIT need further investigation [33, 34]. Interestingly, IL-10-producing inducible Tregs from SLIT-treated patients exhibited a high T cell repertoire diversity, emphasizing that Treg-mediated suppression is not necessarily allergen-specific, suggesting that SLIT may be able to induce tolerance to

multiple allergens in an allergen-nonspecific manner, possibly via IL-10 production [35]. However, the antigen specificity of Treg-mediated suppression induced following SLIT also needs further investigation, as it may differ depending on the allergen utilized.

Recent studies continue to confirm that the clinical efficacy of SLIT is associated with an increase in inducible Tregs, but concomitant changes in cytokine expression and suppression of T cell proliferation are not consistently observed. While the studies above assessed the systemic effects of SLIT on changing the peripheral induction of IL-10-producing Tregs, a clinical trial studying the impact of SLIT on local mucosal immune responses looked at changes in Tregs and cytokine production in sublingual biopsies from patients who had undergone 12 to 18 months of grass pollen SLIT. This study found that FoxP3-expressing Tregs were increased in the sublingual epithelium following grass pollen SLIT [36]. Interestingly, while both IL-10 and TGF- β mRNA transcripts were detectable in the sublingual mucosa, there were surprisingly no changes in their expression following SLIT. Further, while a decrease in subepithelial mDCs was detected following SLIT, in accordance with previous SLIT clinical studies, there was also an observed significant increase in sublingual epithelial T cells, indicating some local T cell proliferation following SLIT [36]. The differences in IL-10 production observed between Tregs from the periphery and Tregs from the local oral mucosa, as well as observed differences in T cell proliferation, may represent unique changes in the sublingual immune environment that do not necessarily reflect the systemic changes induced by SLIT.

Studying only allergen-specific T cells, a recent study of PBMCs from patients following 2 to 4 months of grass pollen SLIT observed increased FoxP3-expressing Tregs, both decreased Th1 and Th2 cells, decreased IL-4 production, and increased IL-10 production. Remarkably, these changes were also largely observed in the placebo group, with the exception of decreased IL-4 and increased IL-10 following SLIT, and did not correlate with observed early improvements in clinical symptoms [37]. These findings raise the possibility that improved clinical symptoms following SLIT, while often observed within a month, may require more than a year to be reflected in a shift from peripheral Th2 to Th1 cytokine production and the induction of IL-10-producing Tregs. In contradiction, a recent study of patients during 2 years of grass pollen SLIT correlated decreased IL-4-producing T cells with the early clinical efficacy of SLIT, as well as increased Th1 cells and IL-10-producing Treg frequencies after more than a year of SLIT, which persisted to the end of the study [38]. The differences between the findings of these two studies may have much to do with the important, but narrow focus of studying only antigen-specific T cells following SLIT. The immunomodulatory effects that SLIT mediates may be better

observed systemically among a heterogeneous population of T cells, rather than for any one particular antigen-specific T cell.

The changes in T cells induced by SLIT are not relegated to modulation of cytokine production and T cell differentiation. DNA analysis of Tregs reveals that they have an unmethylated CpG-rich *Foxp3* locus, while the *Foxp3* locus from other T cells is heavily methylated [39, 40]. This suggests that epigenetic regulation of the *Foxp3* locus, signified by its methylation status, is important for stable and suppressive Tregs. Epigenetics is the heritable changes in gene expression that occur without changing the DNA sequence, and methylation of CpG residues is important for the epigenetic regulation of countless genes. The stability of *Foxp3* as a result of its methylation status is an important measure of Treg stability and function, as decreased methylation of *Foxp3* is significantly related to increased FoxP3 expression, improved Treg stability, and suppressive capacity [41]. In a clinical trial assessing the clinical efficacy and safety of dual SLIT, that being SLIT for two allergens simultaneously, it was found that after 1 year of SLIT, allergen-specific Tregs developed that suppressed allergen-specific effector T cell responses [42]. The induced Tregs had a memory T cell phenotype and exhibited markedly decreased methylation at CpG sites within the *Foxp3* locus, which in turn correlated with their increased *Foxp3* mRNA transcript expression and stability [42]. Thus, Tregs with unmethylated CpGs and increased suppressive function may be indicative of clinically efficacious SLIT. The methylation status of Tregs following SLIT appears to be a predictive marker of subjects who have developed tolerance, as has also been observed following clinically efficacious OIT [43].

Humoral Responses

Several studies have observed changes in antibody responses following SLIT. In the context of an allergic disease, Th2 responses induce class switching of B cells to the ϵ immunoglobulin heavy chain and the production of allergen-specific IgE antibodies. These allergen-specific antibodies bind to the high affinity IgE receptor Fc ϵ RI on the surface of mast cells, basophils, eosinophils, and DCs and induce the release of potent inflammatory mediators and increase allergen uptake [9].

One of the mechanisms through which allergen-specific immunotherapy is proposed to function is through decreasing allergen-specific IgE production and promoting the synthesis of allergen-specific IgG4 antibodies, which block allergen-IgE interaction [10]. IL-10 inhibits the production of IgE and enhances allergen-specific IgG4 production. Allergen-specific IgG responses capable of blocking allergen-specific IgE, which have been found to develop following other allergen-

specific immunotherapies, are proposed to be a strong indicator of clinical tolerance following treatment [44]. Decreased serum IgE and allergen-specific IgE, and increased IgG1 and IgG4, have also been proven to be hallmarks of efficacious SLIT. Following grass pollen SLIT, IgG4 synthesis occurred early in therapy, and the production of blocking allergen-specific IgG4 correlated with a downregulation of Th2 responses [38]. The clinical efficacy of SLIT using several other allergens for the treatment of asthma and allergic rhinitis, including SLIT for house dust mites, cockroaches, and ragweed, has also been closely associated with increased allergen-specific IgG1 and/or IgG4, indicating that changes in allergen-specific IgG are important general markers of clinical tolerance following SLIT, regardless of allergen or indication (Table 1) [42, 45–49]. It is important to note that increased allergen-specific IgG and functional blocking antibody responses following SLIT are more consistent for some allergens, such as grass pollen, compared with other allergens like cockroach, where trending increases in IgG4 are inconsistently observed and do not associate with functional blocking antibody responses [50, 51]. Further, increased allergen-specific IgG has also been observed following SLIT for food allergies, indicating that increased allergen-specific IgG following SLIT is not a phenomenon that only applies to SLIT for aeroallergens [32, 52, 53]. However, some studies have shown that the development of blocking IgG responses following SLIT may only contribute to clinical tolerance in a fraction of patients or none at all, and that additional immune mechanisms may be more necessary and relevant for tolerance induction [54, 55].

SLIT may also induce the production of allergen-specific IgA that could block the binding of allergen-IgE complexes at mucosal surfaces. IgA deficiency has been associated with the pathogenesis of multiple allergic diseases, and the presence of IgA has been associated with healthy patients compared to allergic patients [56]. Because SLIT is administered at the mucosal surface, it is likely that IgA could play multiple roles in immune modulations that associate with clinical tolerance. IgA is well-known for its ability to neutralize toxins and pathogens at mucosal surfaces and shifting the allergen-specific antibody response to IgA from IgE may promote the neutralization of allergen at the mucosal surface following SLIT [57]. Multiple studies of SLIT for food and aeroallergens have shown that an increase in serum and salivary IgA is associated with clinical efficacy, generally after a year or more of treatment [36, 45, 58–60]. Therefore, allergen-specific IgA may be another biomarker for monitoring the clinical efficacy of SLIT. While the mechanisms of IgA class switching following SLIT have yet to be elucidated, TGF- β and IL-10 production by inducible Tregs and tolerogenic mDCs, as well as CD40-CD40 ligand ligation, may help drive the observed allergen-specific IgA response following SLIT [61].

Conclusion

It is clear that sublingual immunotherapy can induce allergen-specific tolerance to the offending allergen in allergic patients. SLIT can exploit the unique tolerogenic properties of the oral mucosa, through regulatory mDCs (mostly Langerhans cells); polarization of Th1 immune responses; induction of IL-10-producing regulatory T cells; promotion of increased allergen-specific IgG1, IgG4, and IgA responses; and decreased IgE production (Table 1). The safety and efficacy of SLIT for treating patients with allergic diseases, including allergic rhinitis, asthma, and food allergies, have been well-documented by several clinical studies. Further clarification of the specific mechanisms of tolerance induced by SLIT will continue to drive the additional applications of SLIT for the treatment of multiple allergic diseases and potentially also for autoimmune diseases.

Compliance with Ethics Guidelines

Conflict of Interest David C. Jay and Kari C. Nadeau declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the authors.

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