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Review

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Review

Immune mechanisms induced by sublingual immunotherapy in allergic respiratory diseases

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Summary

Allergic respiratory diseases (ARDs) are still a major burden on global public health. Sublingual immunotherapy (SLIT) is a mode of allergen immunotherapy (AIT) which involves administration of the allergen under the tongue, and benefits from tolerogenic properties of the oral mucosa. Studies revealed reduced levels of eosinophilia and eosinophil-dominated inflammation in airways of both animals and humans after SLIT. SLIT was also suggested to lower basophil responsiveness and innate lymphoid cell-2 function in blood samples collected from patients with ARD. Moreover, apart from shifting pathogenic type 2 (T_H2) to a type 1 (T_H1) and protective regulatory (Treg) polarization of helper T-cell immune response, antibody isotype switch from IgE to IgG1, IgG2, IgG4 and IgA was also reported in patients with ARD receiving SLIT. Today, the literature on SLIT-mediated activities is still scarce and more studies are required to further enlighten the mechanisms utilized by SLIT for the induction of tolerance. The aim of this review is to summarize the current knowledge about the immune-regulatory mechanisms induced by SLIT against ARDs.

Keywords: SLIT, allergic respiratory diseases, adaptive, innate, immune response

Introduction

Today allergic respiratory diseases (ARDs) continue to be a significant global public health problem [1–3]. However, albeit humans are continuously in contact with allergens, not everyone develops allergic reaction which highlights the importance of both extrinsic as well as intrinsic factors for the inception of the disease. Numerous genetic studies correlated the risk of developing allergies with multiple genes many of which were immunity related [4, 5]. Moreover, according to the *hygiene hypothesis*, the elevated prevalence of allergic diseases is due to increased sterile hygiene practices in the modern world leading to dysregulation of immune system and microbiome dysbiosis because of diminished exposure to immune-stimulating microbial agents in the early childhood [6].

The AIT differs from the other pharmacological approaches for ARDs (e.g. anti-histamines, non-steroidal anti-inflammatory drugs-NSAIDs) as it can both provide protection against the symptoms and improve the natural course of the allergy. The effects are mediated by its distinctive ability to modulate the allergen-specific T-helper type 2 (T_H2) immune responses and induce the generation of tolerance against the causative agent by inducing immunosuppressive cytokine secretion and antigen-specific regulatory T-cell (T_{reg}) activation [7].

The AIT has subtypes that differ in the routes of allergen administration. Among those subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) are the two main forms of AIT that are practiced by the clinicians against ARDs [8, 9]. The SLIT is considered to be a convenient alternative to SCIT which requires frequent injections and periodic visits to medical centers, as it involves self-administration of the allergen under the tongue [10]. The SLIT is also regarded as a safer approach as it is associated with lower risk of severe systemic reactions [10]. This review summarizes the current literature on the mechanisms of action induced by SLIT against ARDs.

Immunopathogenesis of ARDs: an overview

Respiratory epithelium provides an effective barrier against invading foreign particles including microbial agents and allergens, through its physical barrier function (i.e. epithelial tight junctions), innate immune defence function (i.e. foreign particle detection and cytokine release) and mucociliary activities [11]. Various environmental factors including smoking, detergents, ozone, diesel exhaust as well as some allergens with protease enzymatic activity are known to disrupt the epithelial barrier function and tight junctions (TJs) [12]. Entry of foreign substances into the body due to disruption of the epithelial barrier initiates protective innate immune response by activating myeloid and dendritic cells (DCs), which in turn leads to generation of adaptive immune response by antigen presentation to helper T cells and cytokine release [13].

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The DCs play an important role in allergic responses by presenting the internalized allergens/antigens to naïve T cells during ARDs [14]. Following the engulfment of the foreign particles, DCs display CCR7- and CCR8-dependent migration to neighboring lymph nodes in order to induce naive CD4+ T-cell activation. In the presence of cytokines released by DCs (e.g. IL-4 and IL-13), activated T-cells differentiate to T_H2 cells which in turn would promote IgE-mediated immune response [15]. Cytokines such as interleukin (IL)-25 and IL-33 which are released locally to initiate epithelial repair are known to increase T_H2-polarizing capacity of DCs [16].

The alarmins can also promote T_H2-mediated immune response via local innate lymphoid cell (ILC)-mediated activities which are regarded as innate counterparts of helper T cells (T_H cells) and important sources of early innate effector cytokines [5]. Among the ILC subtypes, ILC2s are increasingly recognized as essential in the initiation and orchestration of allergic T_H2 inflammation [17]. In atopic subjects, the resultant T_H2-mediated immune response can result in further disruption of the epithelial barrier function by reducing TJ expression levels which would lead to exacerbation of allergy symptoms by increasing sensitization to the allergen and consequently promoting inflammatory response [18, 19].

Allergic sensitization results in the generation of predominantly T_H2 cells which are responsible for the induction of allergen-specific IgE production by the follicular B-cells in the secondary lymphoid tissues; germinal centre B-cells which are activated by CD40-signaling undergo isotype switching from IgM to IgE in the presence of T_H2 cytokines such as IL-4 and IL-13 [20, 21]. The secreted IgE antibodies set the stage for the effector phase of allergic reactions by occupying the high-affinity IgE receptors (FcεRI) on mast cells and basophils. During the effector phase, the cross-linking of FcεRI receptors upon interaction with IgE-bound allergens leads to release of granular mediators (e.g. histamine), lipid mediators (e.g. leukotrienes) and type 2 cytokines by mast cells and basophils [21, 22]. The released mediators lead to vasodilation, increased vascular permeability, bronchoconstruction, and mucus production during the acute allergic reaction [23].

While the early signs of immediate allergic immune response are IgE-dependent, the late-phase reactions are mainly regulated by infiltrated T_H2-cells which results in further activation of the immune cells in the microenvironment including basophils, MCs, eosinophils, and B cells. This leads to the second wave of type 2 cytokine (e.g. IL-4, IL-5) and mediator (e.g. leukotrienes, prostaglandins, histamine, tryptase, eosinophilic cationic protein, and peroxidases) release that can elevate IgE production and eosinophil infiltration [7]. Among the T_H2-cytokines released in the microenvironment, IL-5 serves as the key mediator for eosinophil proliferation, while IL-4 and IL-13 facilitate the eosinophil recruitment by increasing expression levels of endothelial adhesion molecules (e.g. L-selectin, and very late antigen-4) [24]. Upon degranulation, eosinophils release granule-associated basic proteins, lipid mediators and reactive oxygen species which further exert epithelial cell damage and airway hyperactivity with mucus production. Moreover, eosinophils can also facilitate airway edema and bronchoconstriction indirectly through their modulating activities on other leukocytes including mast cells and T cells [25].

SLIT on immune cell functions during ARDs

Despite the growing number of studies on the contribution of AIT in the management of allergic diseases [26], the relevant literature on the SLIT in the context of ARDs is still scarce. The SLIT aims to induce mucosal immunity against airway allergic reactions through administration of allergen extract as drops or dissolvable tablet under the tongue [27, 28]. While the allergen can arrive in local lymph nodes as unbound via free diffusion, it can also be delivered by oral DCs which can migrate to the proximal lymph nodes for the activation of both allergen-specific T- and B-cells upon engulfment of the foreign particle [29]. Generation of allergen specific blocking antibodies and skewing allergen-specific immune response away from T_H2 to type 1 T cell (T_H1) and Treg cells are essential for the pro-tolerogenic adaptive immune responses [30].

Innate immune cells

In mice, sublingual antigens were able to pass through the epithelial cells to reach ductal APCs [31]. The DCs were crucial in the induction of tolerogenic T cells against allergic airway responses following SLIT [32]. Facilitating antigen uptake by DCs had a positive contribution to enhance the SLIT efficiency against ARDs [33, 34]. Moreover, inclusion of adjuvants such as Pam3CSK4; 1,25-dihydroxyvitamin D3 plus dexamethasone; and *Lactobacillus plantarum* which would skew the immune response away from T_H2, increased SLIT-associated protective activities mediated by murine DCs against airway hyperesponsiveness [35, 36]. Nevertheless, there has not yet been any study on the role played by distinct DC subtypes including myeloid and Langerhans types (LCs) latter of which was shown to be at levels higher in oral mucosa of allergic subjects compared with healthy controls [37]. Moreover, oral LCs were previously reported to differ from skin counterparts in terms of constitutive expression of IgE receptor, and elevated expression levels of major histocompatibility complex class I and II molecules as well as co-stimulatory molecules [38] that suggest tissue-specific phenotype in the oral microenvironment which may influence SLIT-induced mechanisms [39]. On the other hand, oral macrophage-like cells are also thought to migrate to cervical lymph nodes and support regulatory CD4+ T-cell function in asthmatic mice following SLIT [40].

Antigen-specific IgE production is central in pathogenesis of atopic diseases, and due to the expression of high-affinity IgE receptors eosinophils, MCs and basophils are considered as essential cells in the allergic reactions responsible for ARDs. Of those, while MCs are tissue resident cells, basophils and eosinophils home to areas of allergic inflammation [41]. In a previous study by Kaminuma *et al.*, treatment of mice sublingually with ovalbumin (OVA) diminished eosinophil infiltration to bronchoelavolar and nasal cavities after intratracheally or intranasally challenge with OVA [42]. Following SLIT with birch pollen (BP) extract, BP-sensitized mice displayed reduced lung eosinophilia [43]. Moreover, HDM-based SLIT exhibited both prophylactic and therapeutic effects in an experimental mouse model of HDM-induced eosinophil-dominated airway inflammation [44].

Similar results were also observed in human studies since significantly reduced nasal eosinophilia was reported in patients with allergic rhinitis and/or asthma following SLIT by two independent randomized controlled studies [45, 46]. Additionally, reduced fractional exhaled nitric oxide (an

index of eosinophilic airway inflammation) and nasal eosinophil cationic protein levels were reported in patients with ARD upon SLIT [47, 48]. On the other hand, while basophil responsiveness also displayed reduction following SLIT in patients with respiratory allergies to house dust mite and timothy grass [49, 50], there has not yet any report on the effect of SLIT on the allergen-specific MC level and activity in the context of ARDs.

The ILC2s represent another tissue-resident immune cell population distributed in mucosal tissues with important roles in type 2 inflammation and allergic diseases. Peripheral blood samples collected from subjects with allergic rhinitis (AR) displayed elevated levels of ILC2 which were in positive correlation with IL-13 levels and symptom scores [51, 52]. While a previous study by Shamji *et al*. reported SLIT-induced elevated levels of IL-35-inducible regulatory T cells which have inhibitory activity against ILC2-mediated type 2 immune responses [52], there has not been any data demonstrating the airway ILC2 frequency and/or function in patients with ARD after SLIT.

Adaptive immune cells

The SLIT was able to prevent the development of allergic reaction in sensitized animals by normalizing imbalance between T_H1 and T_H2 responses by exerting inhibitory and promoting activities on T_H2 (e.g. IL-4) and T_H1 (e.g. interferon, IFN-γ) cytokine secretion, respectively [43, 44, 53–55]. Moreover, modification of SLIT with inclusion of adjuvants or adenylate cyclase vector system to target oral DCs were shown to act through skewing CD4+ T-cell polarization away from T_H2 cells to Treg and T_H1 cells in asthmatic mice model [35, 40, 56]. Elevated blood Treg cell levels and T_H1 cytokine concentration were associated with clinical improvement in patients with ARD after SLIT [57, 58]. Peripheral blood mononuclear cells (PBMCs) isolated after a year of SLIT demonstrated reduced level of allergen-induced proliferation relative to the baseline levels obtained from PBMCs collected before SLIT [59]. In a study by Swamy *et* al, epigenetic modifications within the Foxp3 locus was shown to have a crucial role in the development of SLIT-mediated T-cell tolerance against allergic reactions [50]. On the other hand, besides of Treg cells, IL-10/IFN-γ double positive Tr1-like cells were also involved in the induction of tolerance in patients with ARD following SLIT [60].

Characteristic inflammation pattern in the patients with allergic diseases involves the IgE production by B-cells under the influence of type 2 cytokines [61]. Data obtained from numerous studies investigating the efficiency of AIT also provided insights on the IgE production dynamics; similar to SCIT which causes a transient increase followed by a decrease in serum IgE production [27], SLIT was able to elevate the allergen-specific IgE levels within the initial few months [62–66], while unaltered or reduced IgE levels were detected after at least a year of treatment in relative to the basal values [49, 50, 67–77]. However, elevated IgE levels after long-time treatment were also demonstrated by some studies which could be due to differences in the treatment protocol (e.g. allergen concentration) used [59, 78].

Table 1: comparison of head to head SLIT versus SCIT studies in terms of clinical and immunological outcome

Author	Study design	No of patients	Allergen	Tx duration (month)	Clinical results	Immunologic results
Schulten [60]	RC	40	Grass	15	NE	IL-5 decreased with SCIT IL-10 increased with SCIT/SLIT
Xian [58]	RC	67	Mite	12	TRS, TRMS, VAS, TAS decreased with SCIT/SLIT	SpIgG4 increased with SCIT/SLIT IFN-δ increased with SCIT CD4+CD25+Fox p3+ T regs increased with SLIT
Keles [85]	RC	60	Mite	18	SS, MS, ICS dose, asthma attack frequency decreased with SCIT/SLIT	IL-10, TGF- β, IFN-δ increased with SCIT/SLIT Sp.IgG4 increased with SCIT
Antunez [67]	RC	23	Mite	24	NE	Sp.IgE, CD8+CD25+ cells decreased, Sp.IgG4 and Sp.IgE/IgG4 increased with SCIT
Karakoc-Aydiner [84]	RC	48	Mite	36	VAS, TRS, TAS, TSS, TMS decreased with SCIT/SLIT	Sp.IgG4 increased with SCIT
Yukselen [83]	RC	30	Mite	24	TRS, TAS, TRMS, TAS decreased NPT threshold increased with SCIT/SLIT	IL-10 and Sp.IgG4 inreased with SLIT/SCIT
Shamji [92]	RC	84	Grass	24	At year 1: lower TNSS was detected in the SCIT-treated group. At year 2 years: TNSS was lower in both SCIT and SLIT groups	Higher nasal Sp.IgA and IgE levels in SLIT group subjects. Serum Sp.IgG, and IgG4 levels were higher in patiens with SCIT

Abbreviations: Tx: treatment; RC: randomized controlled; NE: not evaluated; TRS: total rhinitis score; RMS: total rhinitis medication score; VAS: visual analogue score; TAS: total asthma score; SS: symptom score; MS: medication score; ICS: inhaled corticosteroid; TSS: total symptom score; TMS: total medication score; TNSS; total nasal symptom score; NP: nasal provocation test.

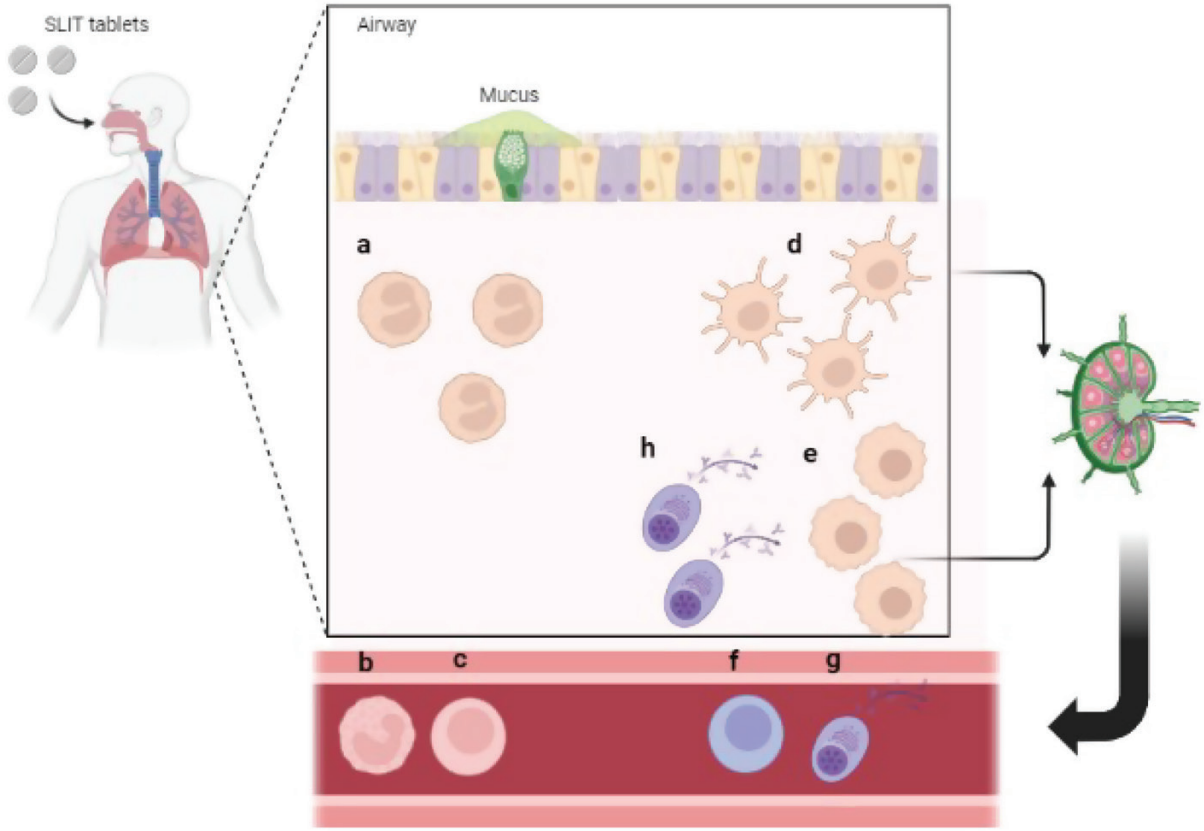

Figure 1: immune-regulatory mechanisms demonstrated for SLIT against ARDs. Both animal and human studies reported reduced level of respiratory eosinophilia and eosinophil-dominated inflammation (a). The SLIT was also suggested to exert inhibitory activities on blood basophil responsiveness (b) and ILC2 function (c) in patients with ARD. Animal studies suggested the positive contribution of facilitating antigen uptake by DCs to increase SLIT activity against ARDs (d). Treg differentiation in response to SLIT is supported by oral macrophage-like cells migrating to cervical lymph nodes (e). The SLIT induced an adaptive immune response toward a T_H1 and Treg, phenotype away from the allergic T_H2 phenotype, in both animal and human patient studies using blood samples (f). In correlation with the skewed T-cell-mediated immune response, the SLIT was also associated with serum antibody isotype switch from IgE to IgG1, IgG2, and IgG4 in patients with ARD (g). The lack of change in serum IgA levels were suggested to be due to enhanced transportation of IgA into the mucosal secretions (h). The figure was generated using BioRender software.

The isotype-switched antibodies have the potential to inhibit IgE-mediated responses by targeting the IgE epitopes on the allergic antigen and blocking allergen-IgE immune-complexes from binding to IgE receptors [79]. IgG4 is regarded as an efficient blocker of IgE-dependent inflammatory reactions since it has low avidity to allergen and high affinity to inhibitory FcγRIIb receptors [79]. Its induction was suggested as a general maker of successful AIT [80, 81], and in accord, elevated IgG4 levels and decreased IgE/IgG4 ratio were reported after long-term SLIT [49, 50, 59, 67, 75, 76, 78, 82, 83]. However, the relevant literature is not consistent due to studies showing no change in specific IgE, IgG, and IgG4 levels between SLIT-active and SLIT-placebo groups despite the significant decrease in asthmatic symptoms and medication use in the former group [77, 84, 85]. This can be at least partially explained by a mechanism that would generate tolerance against ARD through induction of other specific IgG subclasses including IgG1 and IgG2 [49, 50, 53–55, 57, 59, 62, 63, 65, 67, 75, 76, 78, 82, 86, 87], both of which were also suggested to be with therapeutic importance following AIT [88, 89]. On the other hand, long-term SLIT was also associated with reduced or unchanged serum IgA levels [49, 68] which could be due to enhanced transportation of IgA into the mucosal secretions [75].

When compared with SCIT, SLIT displayed some differences in the efficacy and immune responses that are triggered against ARDs [90, 91] (Table 1). In a study by Schulten *et al*., patients with ARD exhibited reduced IL-5 levels only following SCIT, but not SLIT [60]. In another report, total rhinitis score was inversely correlated with Treg and IFN-γ levels in SLIT and SCIT, respectively [58]. The SCIT was better in elevating IFN-γ/IL-4 ratio as well as the levels of CD4+CD25+ T-cells and allergen specific IgE and IgG4 antibodies [58, 67, 83–85]. Furthermore, quite recently, both AIT forms were suggested to differ in the predominant antibody class for the induction of tolerance (IgG4 and IgA in SCIT and SLIT, respectively) in patients with ARD [92]. Similar differences were also observed in a mouse model for allergic asthma; SLIT was able to suppress allergen-induced AHR and clinically relevant lung function parameters, but was not able to exert any significant effect on type 2 allergic inflammation which was effectively suppressed by SCIT [86]. In the same study, higher IgG1 and IgG2a levels were reported following SCIT and SLIT, respectively [86]. While these can be suggestive of the possible divergence between the two approaches in the mechanism(s) of action, they can also be due to the other factors such as the higher cumulative dose of allergen and the use of adjuvants in SCIT [93], which are needed to be addressed by future studies for more confirmatory conclusions.

Conclusions

Avoidance to limit the allergen exposure and medications including anti-inflammatory drugs and anti-histamines are among the typical treatment approaches which are in use today against ARD. The AIT provides a unique alternative strategy to both improve the symptom scores and reduce the need for medication [94]. Of the two main forms of AIT, SLIT is regarded as a more practical and safer approach than SCIT since it is a self-administered therapy and exploits the tolerogenic properties of the oral mucosa for the induction of tolerance against allergens.

Previous studies demonstrated the skewing of allergen-specific T-cell differentiation away from the allergic T_H2 phenotype to a T_H1 and Treg phenotype; and antibody isotype switch from IgE to IgG and IgA in patients with ARD receiving SLIT. The SLIT was also suggested to exert inhibitory activities on innate immune cells including eosinophils, basophils and ICL2 (Figure 1). Nevertheless, the immune-regulatory mechanisms triggered by SLIT still requires more attention in literature to investigate their possible divergence from those triggered by SCIT against ARDs. The clinical application of SLIT can also benefit from the modifications such as using multi-allergens [95], and combination with SCIT [85], and identification of biomarkers associated with SLIT efficacy which would not only help to distinguish non-responders in clinic but also shorten the duration of the treatment.

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Conflict of interest

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Author contributions

Concept - UG, NNB; Design – UG; Supervision – NNB; Writing - UG; Critical Reviews - NNB.

Data Availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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