

Mechanisms of Sublingual Immunotherapy

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KEYWORDS

• Sublingual • Immunotherapy • SLIT • Mechanisms • IgG4

The efficacy of sublingual immunotherapy (SLIT) in the treatment of allergic airways disease, particularly seasonal pollen-induced rhinitis, is now well established.^{1,2} Applications of SLIT, at least in clinical trial settings, have been extended to include treatment of food allergy and latex allergy.^{3–5} SLIT is convenient for patients and seems safe,⁶ providing advantages over classic subcutaneous immunotherapy (SCIT), although head-to-head trials are awaited.

The exact mechanisms by which SLIT exerts its effects have yet to be resolved. These mechanisms are likely to overlap considerably with those of SCIT, yet discrepancies exist, particularly concerning the magnitude of systemic immunologic changes. Moreover, these mechanistic effects do not necessarily correlate with clinical efficacy.

SLIT may exploit the naturally protolerogenic milieu of the oral mucosa (a site exposed to numerous innocuous food proteins on a daily basis) but it is not clear to what extent the oral environment is distinct from the rest of the gastrointestinal tract with regards to immune responses. Much progress has been made in elucidating the early interactions between allergen, antigen-presenting cells, and T cells, but the precise cell types involved are unknown.

Strong evidence exists for effects on allergen-specific IgE and IgG levels (similar to those seen with SCIT) but these changes correlate best with allergen dose, rather than clinical efficacy. As for SCIT, researchers have also looked for evidence of reprogramming of T-cell responses, particularly the induction of regulatory T cells, after SLIT.

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However, despite much progress, satisfactory biomarkers of the efficacy of SLIT have still to be established. Recent data^{7,8} have confirmed long-lasting, tolerogenic effects of SLIT, the immunologic correlates of which require further study. The use of adjuvants to enhance the efficacy of SLIT, and potentially reduce the high allergen doses required, has also been an area of interest.^{9–11}

This article considers the possible mechanisms of SLIT, beginning with the processes involved within the oral mucosa. There follows an evaluation of the major studies concerning downstream immunologic events, both locally and systemically. Areas of uncertainty are highlighted.

CURRENT PROPOSED MECHANISM OF SLIT

Fig. 1 illustrates a hypothetical model of the pathway of allergen and downstream effects of SLIT. In this model, allergen is taken up by submucosal dendritic cells (perhaps oral Langerhans cells), which then migrate to local regional lymphoid tissues, including submandibular and cervical lymph nodes. There, dendritic cells present peptide fragments to allergen-specific T cells in a protolerogenic manner, resulting in inhibition of activation and proliferation of Th2 cells and stimulation of Th1 and/or regulatory T cells. Subsequently, these T cells influence B cells to produce protective antibody responses, including secretion of allergen-specific IgG4 and IgA, and, later, inhibition of IgE. Regulatory T cells may have further suppressive effects on other inflammatory cells, including eosinophils, mast cells, and basophils, either by cytokine secretion or direct cell-to-cell contact. Given the aggregation of antigen-presenting

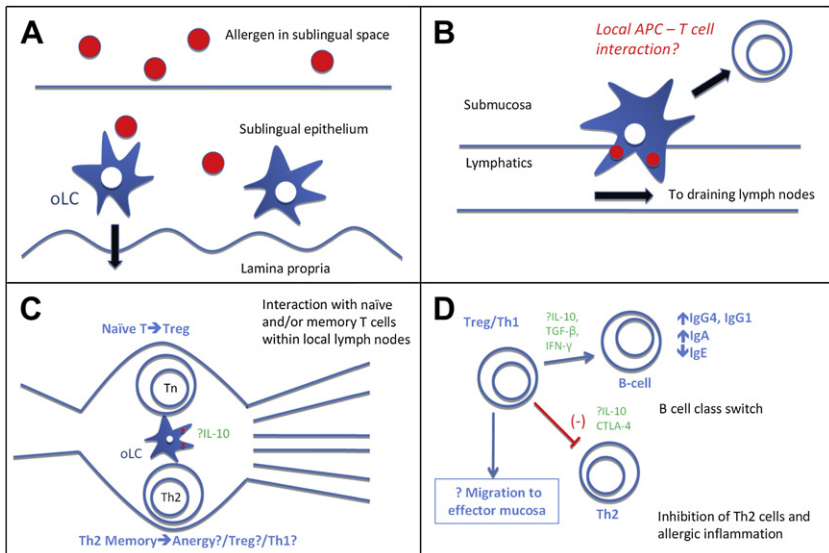


Fig. 1. Postulated mechanisms of sublingual immunotherapy. (A) Allergen up-take by dendritic cells, including oral Langerhans' cells, in the sublingual mucosa. (B) Dendritic cells transport allergen to regional lymph nodes and/or mucosal T-cell rich areas. (C) Within lymph nodes dendritic cells present allergen to T-cells in the context of protolerogenic signals, such as IL-10, leading to the induction of regulatory T cells and Th1 cells, and the inhibition of Th2 cells. (D) Regulatory T-cells and Th1-cells suppress Th2-mediated allergic inflammation and induce B cell class switch to IgG4, IgG1 and IgA. (From Scadding G, Durham S. Mechanisms of sublingual immunotherapy. *J Asthma* 2009;46(4):324; with permission.)

cells and lymphocytes in some areas of the oral mucosa itself,^{12–14} the possibility of local allergen presentation, bypassing regional lymphoid tissues, is considered.

ALLERGEN UPTAKE AND PRESENTATION IN SLIT

Some years ago, a series of pharmacodynamic studies by Bagnasco and colleagues^{15,16} explored the local and systemic distribution of allergen in vivo after application to the human sublingual region. Radiolabeled *Parietaria* allergen was tracked, by both local scintillography and plasma radioactivity, to identify the distribution of allergen after sublingual application, compared with oral (swallowed) allergen. Not only was allergen retained within the sublingual space for several hours, with little direct absorption into the circulation from the mouth (Fig. 2), but allergen could also be detected within the mucosa for 20 hours after dosing.

Further similar in vivo human research is lacking; however, several groups have used murine SLIT models and ex vivo human antigen-presenting cells (including those purified from the oral mucosa) to investigate local processes in more detail.^{9–11,17–19} Immunostaining of tissue sections from the oral mucosa of mice treated sublingually with labeled ovalbumin has shown the passage of allergen across the epithelium and into the subepithelium over the course of 30 to 60 minutes.¹⁹ At 60 minutes allergen seems to disappear from the submucosa, suggested by the investigators to coincide with uptake by dendritic cells. In a series of experiments, the same group showed allergen uptake, processing, and presentation to T cells in vitro, using dendritic cells purified from the oral mucosa. Furthermore, these dendritic cells appeared to have tolerogenic properties, being capable of inducing T cells secreting interleukin 10 (IL-10) and interferon γ (IFN- γ), and with the ability to suppress T-cell proliferation in vitro.

Previous studies using an ovalbumin-sensitized mouse model of asthma had reported improved efficacy of SLIT using allergen coupled to a mucoadhesive polysaccharide core.⁹ Later, using the same SLIT model, the same investigators extracted

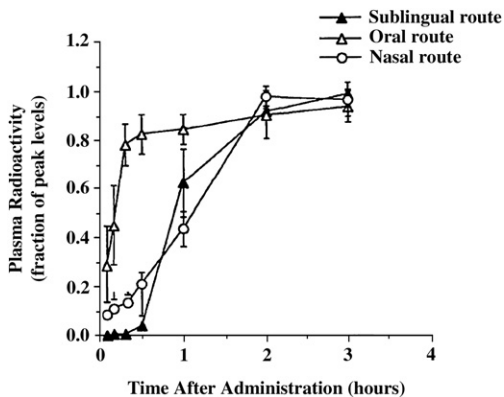


Fig. 2. Average plasma radioactivity curves (mean $\pm$ SEM, normalized to peak levels) observed in three groups of healthy volunteers receiving ^{123}I -Par j 1 (radio-iodine labelled *Parietaria judaica* major allergen) by sublingual, oral, and nasal routes. Sublingual administration results in negligible direct absorption into the blood stream until after swallowing at 30 minutes. (From Bagnasco M, Mariani G, Passalacqua G, et al. Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 1997;100(1):125; with permission.)

allergen-specific T cells with regulatory properties from the cervical lymph nodes of treated mice.¹⁹

Further murine studies have identified CD11b+CD11c— monocytoïd dendritic cells to be the most abundant antigen-presenting cell within the lingual immune system,¹³ existing alongside both effector and suppressor CD4+ T cells. The duration of allergen-mucosal contact (longer being more efficacious) and the frequency of administration (more frequent being more efficacious) have been investigated during sublingual treatment in mice.^{20,21} A study involving a mouse model of grass pollen-induced allergic rhinitis showed reduced airway inflammation after allergen challenge in sublingually treated animals, associated with impaired T-cell proliferative responses in cervical lymph nodes but not spleens, again suggesting the importance of the local immune response.²²

Researchers have studied the use of several adjuvants in mouse SLIT models. High molecular weight, charged chitosan particles coupled to ovalbumin-enhanced allergen uptake and presentation *in vitro*, and had improved efficacy *in vivo*.¹¹ Use of certain strains of lactic acid bacteria concurrently with allergen may enhance Th1 and regulatory T-cell responses.²³ Ovalbumin SLIT along with a combination of dexamethasone and vitamin D₃ was also more effective than ovalbumin treatment alone.¹⁰

Sublingual ovalbumin coupled to cholera toxin subunit B^{24,25} has been shown to enhance B-cell-dependent expansion of regulatory T cells and inhibition of effector T cells. The investigators suggest B lymphocytes may play an important role as antigen-presenting cells during tolerance induction by this route, facilitated by the presence of cholera toxin B subunit, which binds to GM1 ganglioside receptors, allowing efficient nonallergen-specific uptake. Similar results were also achieved using intragastric administration of allergen-cholera toxin B complexes.²⁶

Studying the local pathway of allergen after sublingual application in human patients is more difficult. However, several studies have used human oral mucosal biopsies (either from fresh cadavers or during intraoral surgery) to investigate local immunologic tissues and the events after allergen application.^{12,17,18,27} Oral Langerhans cells (the oral equivalent of the skin Langerhans cell, expressing CD1a and Langerhans cell-specific lectin/CD207) were identified as possible candidate antigen-presenting cells for SLIT, because of their expression of high amounts of major histocompatibility complex (MHC) class I and II, surface IgG receptors, and, most crucially, the high-affinity IgE receptor, FcεRI. Expression of this last receptor was found to be greatest in atopic individuals and to positively correlate with serum total IgE levels.²⁷ Further evidence suggests possible antiinflammatory effects of this receptor, with cross-linking of FcεRI on monocytes, resulting in the release of IL-10 and production of indoleamine 2,3-dioxygenase.^{28,29}

Subsequently, purified oral Langerhans cells have been shown to produce IL-10 *in vitro*, a process augmented by coligation of toll-like receptor 4 with monophosphoryl lipid A.¹⁷ Moreover, these cells could then induce T cells expressing the prototypic regulatory cell transcription factor Foxp3, and secreting IL-10, transforming growth factor β 1 (TGF-β1) and IFN-γ. These studies led to the hypothesis that allergen was taken up via IgE-FcεRI on the surface of oral Langerhans cells during SLIT in atopic individuals.

However, more recently the same investigators used biopsy material from the vestibular region of the mouth from a larger pool of patients undergoing oral surgery classified according to their atopic and grass pollen allergic status.¹⁸ Using CD1a+ cells as a marker for oral Langerhans cells, these investigators studied allergen uptake and cell migration in an *ex vivo* model, using fluorescently labeled major grass pollen allergen, Phl p5. They were able to show both dose-dependent and time-dependent

saturation kinetics of allergen uptake, as well as IL-10 and TGF- β release from both CD1a+ dendritic cells and cocultured T cells. Such dose-dependent allergen binding fits well with the observed allergen dose-dependency for efficacy in clinical SLIT studies (see later discussion). Moreover, these findings suggest a receptor-mediated allergen uptake process. However, there were no differences between tissues from atopic and nonatopic patients, casting some doubt over the relevance of IgE-Fc ϵ R1-mediated uptake. The investigators suggest possible alternative receptor-mediated uptake pathways, such as via IgG-Fc γ receptors or C-type lectins, or by micropinocytosis or macropinocytosis, the last supported by a previous *in vitro* study of dendritic cell uptake of the grass and birch allergens Phl p1 and Bet v1.³⁰

The use of vestibular rather than sublingual mucosal tissue in the study outlined earlier was informed by a previous biopsy study of different regions within the oral cavity, with dendritic cell and mast cell distribution compared within the different regions and with skin tissue.¹² Immunostaining showed a greater concentration of CD1a+ dendritic cells in the oral vestibulum compared with the sublingual region (**Fig. 3**). Immune cells from distinct intraoral mucosal regions and the skin have since been compared in greater detail.³¹ CD3+ cells were most abundant in the vestibulo-buccal region. This area, and to a lesser extent the sublingual area, were found to represent potentially protolerogenic environments, as shown by mRNA expression of TGF- β 1, IL-10, and Foxp3, plus Th1 gene expression including IFN- γ and Tbet mRNA. These regions also expressed mRNA transcripts of several Th17-type cytokines, not seen in the skin. The relevance of this last finding is unclear.

Investigators have speculated that the oral mucosa has a degree of immune privilege.³² As well as potentially tolerogenic antigen-presenting cells and T cells, the environment is further protected from inflammatory responses by high levels of secretory IgA, antimicrobial peptides in saliva, and the presence of commensal bacteria. All these factors may be important in facilitating tolerogenic responses to SLIT.

Significant evidence points towards a dominant role for the local mucosa and lymphoid tissues during SLIT. Oral antigen-presenting cells do seem to have natural protolerogenic properties, perhaps further enhanced by various adjuvants. The principal allergen-presenting cell may be the oral Langerhans cell, but other cell types are likely to be involved in addition. The mechanisms of allergen uptake (be they receptor-mediated, allergen-specific, or otherwise) require further confirmation.

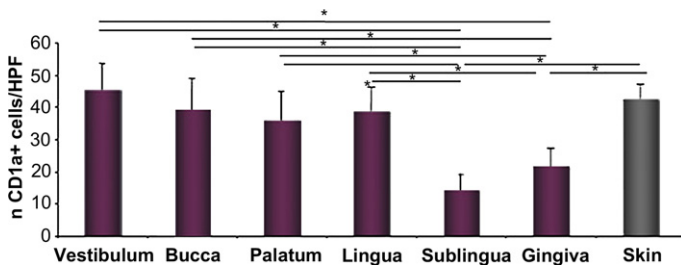


Fig. 3. Higher numbers of oral Langerhans' Cells (oLC) within the vestibulum, bucca, palatum, and lingua compared to sublingual and gingival regions of the oral mucosa. Oral mucosal tissues from different anatomical sites were stained with anti-CD1a antibody for oLC detection. n CD1a+ cells/HPF; mean number of CD1a-positive cells per high powered microscope field. *, $P < .05$. (From Allam JP, Stojanovski G, Friedrichs N, et al. Distribution of Langerhans' cells and mast cells within the human oral mucosa: new application sites of allergens in sublingual immunotherapy? *Allergy* 2008;63(6):725; with permission.)

Evidence does point toward a role for regional lymph glands, although this is largely derived from animal models for obvious reasons. Other areas of uncertainty remain, such as the importance of the tonsils, which have been shown to be rich in regulatory T cells,¹⁴ and may potentially play an important role in SLIT. Moreover, it is perhaps premature to completely rule out a role for other, distal components of the gut immune system. Investigation of allergen uptake and dendritic cell function needs to be extended to the study of human sublingual/vestibular mucosal tissue obtained before and after SLIT in order to determine their relevance to tolerance induction *in vivo* in man.

DOWNSTREAM IMMUNOLOGIC EFFECTS OF SLIT

In considering the immunologic effects of effective SLIT, it seems prudent to first briefly consider the better-established effects of SCIT.^{33,34} SCIT has been shown to have rapid desensitizing effects on mast cells and basophils, providing some protection from anaphylactic responses in days to weeks. Within weeks there is evidence of T-cell tolerance and the induction of regulatory T cells, including cells expressing IL-10 and TGF- β , as well as the prototypic regulatory cell transcription factor Foxp3. B-cell/antibody responses to SCIT appear later, with increases in specific IgG, and in particular IgG4, preceding a decrease in specific IgE that is evident only after 3 to 4 years of treatment³⁵ (itself usually seen to increase during early stages of treatment). Changes are seen at effector mucosae including reductions in mast cell and eosinophil numbers. In the next section, the evidence for analogous processes in SLIT is considered.

ANTIBODY EFFECTS OF SLIT

Allowing for a break with the proposed chronology of events, as outlined earlier for SCIT, most clinical studies have investigated the effect of SLIT on allergen-specific antibody levels. The observed changes do seem to reflect those of SCIT, albeit at a lower magnitude.

In large, randomized, placebo-controlled trials of grass pollen SLIT significant increases in IgG,³⁶ IgG4,^{14,37} or IgE-blocking antibody³⁸ have been recorded, becoming significant versus placebo from about 8 weeks of treatment. Moreover, 2 of these studies have shown both dose-dependent increases in IgG/IgG4 and dose-dependent clinical efficacy, although not a direct correlation between antibody titers and clinical response (Fig. 4).^{36,37}

Levels of specific IgG show a gradual, progressive increase during prolonged treatment with SLIT (Fig. 5),⁷ whereas specific IgE levels initially increase during treatment, becoming significantly greater than placebo by 2 months, before decreasing to near baseline by about 20 months of treatment (Fig. 6).^{38,39}

However, similar findings have not been universally reported by investigators. Several studies of house dust mite SLIT in particular, as well as other studies of SLIT with seasonal aeroallergens, have yielded few or no significant changes in specific antibody levels.^{40–45} In some cases this finding has been concordant with an absence of significant clinical benefit as per primary outcome measures,^{41,44} but in others there is discordance with clinical benefit in the absence of antibody effects.^{42,43} A recent study of high-dose house dust mite SLIT⁴⁶ produced significant clinical benefit as well as notable T-cell and cytokine responses (discussed later), but produced little in the way of antibody responses, with the only significant finding being an increase specific IgG4 to Der p2 at 2 years of treatment.

These observations raise several questions: foremost, are antibody changes a prerequisite, functional component of successful SLIT or simply a bystander effect,

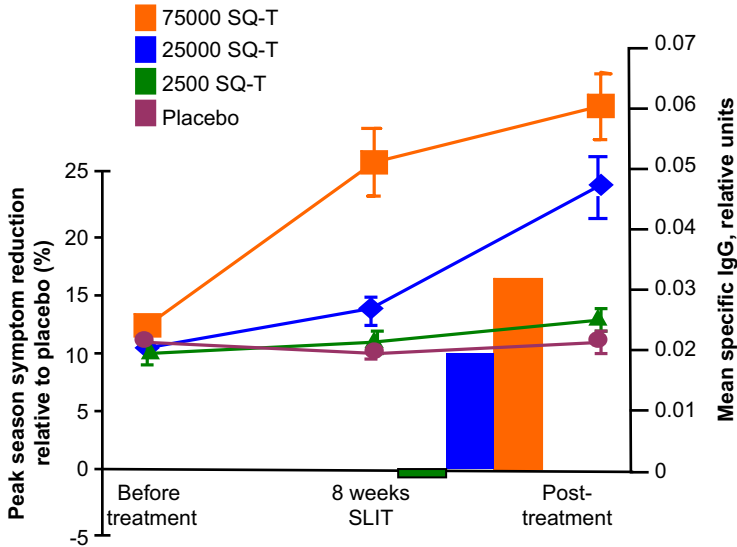


Fig. 4. Serum allergen-specific IgG and peak season symptom reduction during grass pollen SLIT are both dose-dependent. Vertical axis, left; mean peak season symptom reduction relative to placebo in low (2,500 SQ-T/day), medium (25,000 SQ-T/day), and high (75,000 SQ-T/day) dose grass pollen SLIT (histograms). Vertical axis, right; mean allergen-specific IgG in placebo, low, medium and high dose SLIT before, at 8 weeks, and after one year of treatment. (Data from Durham SR, Yang WH, Pederson MR, et al. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006;117(4):806, 807.)

reflecting allergen dose only? Second, are there intrinsic differences between SLIT for house dust mite, a perennial allergen, and for seasonal aeroallergens such as grass pollen? Third, how sensitive are currently used tools such as symptom scores and diaries at measuring the clinical effects of SLIT, particularly in small studies in which allergen exposure between and within individuals is likely to be so variable?

Regarding the first question, there is increasing evidence for a functional role of the induced IgG antibodies. Using an *in vitro* correlate of B-cell allergen uptake and presentation (facilitated allergen binding [FAB] assay, discussed by Shamji and Durham in detail elsewhere in this issue), it was shown that serum from SLIT-treated patients could inhibit B-cell allergen binding via IgE-Fc ϵ R1I.¹⁴ The onset of this inhibition paralleled the increase in IgG4, which, in studies of SCIT,⁴⁷ has been shown to be the serum factor responsible for this inhibition. However, further work is needed, particularly concerning the possible inhibitory effect of these antibodies on mast cells and basophil activation, such as by assays of allergen-induced basophil activation/histamine release.

Grass pollen SLIT has also recently been shown to significantly increase serum allergen-specific IgA levels.^{14,48} Similar changes have been seen after SCIT, and, furthermore, the IgA2 produced was able to induce monocyte IL-10 release *in vitro*, and positively correlated with nasal TGF- β expression, perhaps reflecting mucosal origin of these antibodies.⁴⁹ It is tempting to speculate that the IgA induced during SLIT may also have similar functional properties, although this has yet to be proved. Furthermore, one might hypothesize that changes in IgA may be at least as relevant in SLIT as in SCIT, given the mucosal administration of the former. If so, this finding may in part explain the

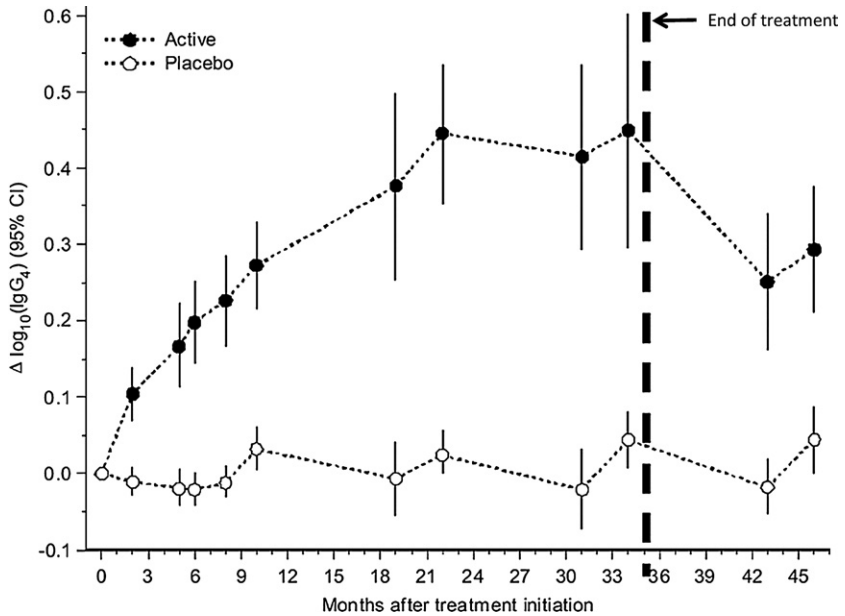


Fig. 5. Change in mean allergen-specific IgG4 level during, and for one season after discontinuation of, 3 years grass pollen SLIT in active and placebo-treated patients. (From Durham SR, Emminger W, Kapp A, et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 2010;125(1):136; with permission.)

comparable clinical efficacy of the 2 treatment approaches despite the fact that SCIT induces approximately 5 times greater increases in specific IgG than SLIT.^{38,47}

T-CELL AND CYTOKINE RESPONSES DURING SLIT

Data concerning the effects of SLIT on T-cell proliferation, cytokine secretion, and on the induction of putative regulatory T cells have been varied and at times conflicting. As with data concerning antibodies, this situation may be the result of different allergens, doses, and treatment regimens, but in addition, may also reflect the increased complexity and variability of the assays used by different researchers.

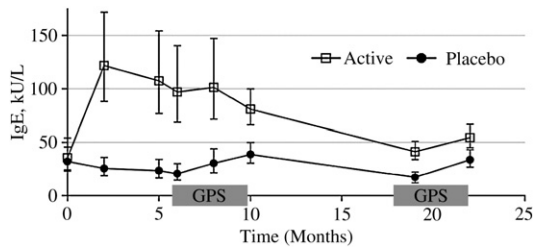


Fig. 6. Allergen-specific IgE levels during 2 years of grass pollen SLIT in active and placebo groups. GPS, grass pollen season. (From Dahl R, Kapp A, Colombo G, et al. Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol* 2008;121(2):516; with permission.)

T-cell Proliferation

A detailed account of immune responses during a year-long treatment with birch pollen SLIT was reported several years ago, but remains one of the most informative studies.⁵⁰ In vitro peripheral blood mononuclear cell (PBMC) proliferation to major birch pollen allergen Bet v1 was decreased at both 4 and 52 weeks of treatment. Inhibition at 4 weeks was reversible either by neutralizing IL-10 using a monoclonal antibody, or by depleting CD25+ cells. Neither approach had any effect at 52 weeks. These findings were accompanied by an increase in allergen-stimulated gene expression (mRNA level) of IL-10 at 4 weeks, and IFN- γ at 52 weeks.

A recent study of house dust mite immunotherapy reported a progressive inhibition of allergen-induced CD4+ cell proliferation over the course of 2 years.⁴⁶ In this case, blocking TGF- β in vitro reversed this inhibition at 6 months, but not significantly at other time points. Several other studies have looked at T-cell proliferative responses, both with^{51,52} and without significant positive findings.^{41,44}

Cytokine Secretion Profiles

A placebo-controlled trial of tree pollen immunotherapy in a pediatric cohort reported increased allergen-induced PBMC IL-10 mRNA in both high-dose and low-dose SLIT-treated patients at 1 and 2 years, although levels peaked at 1 rather than 2 years of treatment.⁵³ This finding was accompanied by suppression of IL-5 mRNA in the high-dose group at the same time points, and IL-5 levels were found to correlate with higher symptom scores (Fig. 7). The same group later published the finding of raised IL-18 mRNA (a Th1 cytokine) in the actively treated patients.⁵⁴

The effects of 6 months of house dust mite SLIT on PBMC allergen-induced cytokine profiles differed according to the concentration of allergen used in vitro: 10 μ g Der p1/mL induced significant IFN- γ release in SLIT-treated but not placebo-treated

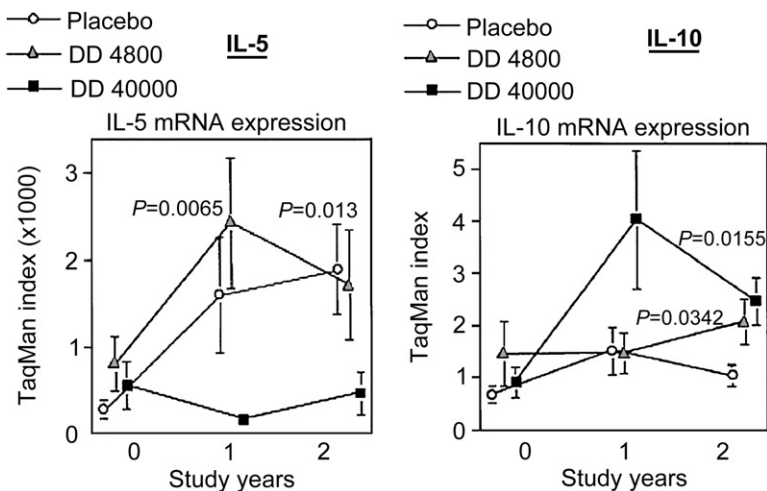


Fig. 7. In vitro allergen-induced IL-5 and IL-10 mRNA expression (real time PCR by TaqMan index) in peripheral blood mononuclear cells from children undergoing a 2 year trial of tree pollen SLIT including placebo, low dose (daily dose 4,800 SQ-U major allergen), and high dose (daily dose 40,000 SQ-U major allergen) treatment. (Adapted from Savolainen J, Jacobsen L, Valovirta E. Sublingual immunotherapy in children modulates allergen-induced in vitro expression of cytokine mRNA in PBMC. *Allergy* 2006; 61(10):1186–97; with permission.)

patients, whereas 0.4 µg Der p1/mL resulted in increased PBMC IL-10 release in the actively treated group.⁵⁵ O'Hehir and colleagues⁴⁶ reported a decrease in mite allergen-induced PBMC IL-5 secretion at 6 months, with a further decrease by 12 months. Levels at 1 year were less than a quarter of those in placebo-treated patients. Increased allergen-induced IL-10 release in SLIT-treated patients was also seen, but not until after 2 years of treatment.

Bohle and colleagues⁵⁰ reported an increase in IL-10 mRNA in allergen-stimulated T cells after only 4 weeks of birch pollen SLIT. However, by 1 year levels had returned to baseline. Conversely, allergen-stimulated IFN-γ gene expression was significantly increased at 1 year, by which point blocking IL-10 or removing CD25+ cells had no effect on allergen-driven T cell proliferation.

Regulatory T cells

Researchers have sought evidence for the generation of regulatory T cells during SLIT. As in SCIT, regulatory T cells may be an important source of antiinflammatory cytokine production, mediators of B-cell class switching, and inhibitors of Th2 cell activation and proliferation. Although in several animal models, particularly those using additional adjuvants, both Foxp3+ and Foxp3– putative regulatory T cells, with demonstrable *in vitro* effects, have been described,^{10,11,19,25} such evidence has proved more elusive in human studies.

O'Hehir and colleagues⁴⁶ were able to isolate nondividing, CD25+ CD4+ Foxp3+ cells and show that these cells could inhibit proliferation of CD25– T cells *in vitro*. However, levels of these cells increased in both SLIT-treated and placebo-treated patients during the trial, without significant difference between the two. However, Bohle and colleagues⁵⁰ found no significant changes in T-cell Foxp3 gene expression, but did see trends towards an increase at 4 weeks and a decrease at 52 weeks.

In a recent Finnish study of tree pollen SLIT in children, allergen-induced Foxp3 mRNA levels in PBMC were increased and correlated positively with IL-10 mRNA at 1 and 2 years and with TGF-β mRNA at 1 year.⁵⁶ Although symptom scores were improved there was no correlation between Foxp3 gene expression and clinical outcomes.

Making firm conclusions on the effects of SLIT on T-cell responses from even the limited number of studies discussed earlier is not possible. An early IL-10-dependent, regulatory T-cell response, later progressing to a Th1-dominant, IFN-γ-secreting response, is suggested by some studies,^{50,55} whereas a more gradual onset regulatory response, with either TGF-β-mediated Th2 suppression (as shown by decreased IL-5) and/or IL-10-mediated inhibition is supported elsewhere.^{46,53,56} In addition, there are several other relevant publications in this field. Whereas some have revealed positive findings concerning cytokines such as increased IL-10,^{57,58} increased IFN-γ⁵⁹ and decreased IL-13,⁶⁰ or inhibition of T-cell proliferation,^{51,52,57} others have found no significant effects on cytokines and/or T-cell proliferation.^{41,44} Discrepancies in laboratory techniques, allergen type and doses, study size, and presence/absence of control groups are all potential confounding factors here. This area warrants further study in larger, controlled trials.

LOCAL MUCOSAL EFFECTS OF SLIT

SCIT has been shown to reduce allergic inflammation at mucosal sites such as the nose and lung, including reductions in mast cell and eosinophil numbers.^{61,62} More recently, phenotypic regulatory T cells have been identified in the nasal mucosa of SCIT-treated patients during natural seasonal pollen exposure.⁶³ These regulatory

cells likely have direct antiinflammatory or tolerogenic effects within the target organ. Somewhat less is known regarding local mucosal effects of SLIT.

A placebo-controlled study of house dust mite SLIT used conjunctival allergen provocation tests as an outcome measure.⁶⁴ After 1 year of treatment, actively treated patients had fewer neutrophils and eosinophils in conjunctival fluid after allergen challenge compared with placebo-treated patients. The SLIT group also had lower serum eosinophil cationic protein levels at 1 year, as well as lower rhinoconjunctivitis symptom scores. Nasal allergen provocation tests after SLIT for *Parietaria* pollen-induced rhinitis also produced fewer inflammatory cells in nasal secretions of active versus placebo groups.⁶⁵ In an open study of birch pollen SLIT, the actively treated group had lower numbers of in-season nasal smear eosinophils as well as higher bronchial metacholine PD20 levels than matched patients treated with pharmacotherapy only.⁶⁶ Conversely, a study of house dust mite SLIT failed to detect differences in nasal smear eosinophils between baseline, 6-month, and 12-month recordings.⁴⁵

Only a handful of studies have looked at the effect of SLIT on the oral mucosa during clinical treatment. Two small studies^{67,68} investigated biopsy tissue from the sublingual region before and after treatment with grass pollen SLIT, including an individual with persistent local reactions to the allergen.⁶⁸ Low numbers of mast cells and eosinophils were found in the tissue, both before and after SLIT. However, the nature and time course of local adverse reactions to SLIT within the mouth is suggestive of mast cell degranulation and allergic inflammation. More recently, 2 larger studies have identified significant numbers of mast cells in sublingual and other regions of the oral mucosa,^{12,14} likely in sufficient numbers to be responsible for these commonly seen clinical symptoms. These mild adverse events usually resolve within days to a couple of weeks of initiating treatment; whether this represents early desensitization of effector cells, as reported for SCIT, or simply the exhaustion of local inflammatory cells, is unclear.

Local sublingual Foxp3-expressing cells have been found to be significantly increased in grass pollen SLIT-treated patients compared with placebo.¹⁴ Alongside other findings including antigen-presenting cell abundance, T-cell presence and anti-inflammatory cytokine gene expression,^{12-14,31} this finding suggests that local mechanisms, with perhaps local interaction between antigen-presenting cells and T cells, may be relevant. It is uncertain whether regulatory T cells are induced locally within the mucosa or migrate there from regional lymphoid tissue. Similarly, unlike for SCIT, there is no firm evidence for the presence of regulatory T cells at other mucosal sites, such as the nose or lung, after SLIT.

TIME COURSE AND LONG-TERM EFFECTS OF SLIT

The clinical time course of onset of specific immunotherapy can be difficult to measure, especially when aeroallergen exposure can differ from season to season, day to day, and between individuals. A recent study made use of an artificial allergen exposure chamber to expose both grass pollen SLIT and placebo-treated patients to standardized levels of pollen before and then at regular intervals during the course of treatment.⁶⁹ Moreover, because the study was entirely conducted outside the pollen season, all other antiallergic medication could reasonably be discontinued to prevent interference.

SLIT-treated patients had significantly lower symptom scores during pollen exposure than placebo-treated patients from 4 weeks onwards; moreover, there was a trend towards a difference at just 1 week (Fig. 8).⁶⁹ Symptom scores showed progressive improvement to 2 months, with a further slight improvement at 4 months.

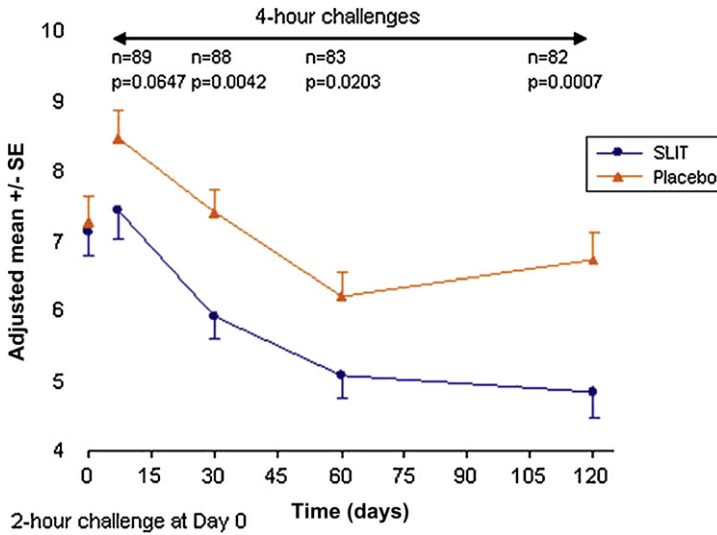


Fig. 8. Time course of effect of grass pollen SLIT versus placebo on symptom scores during exposure to grass pollen allergen within a challenge chamber. Participants were exposed to allergen within the challenge chamber for 2 hours at baseline, and then for 4 hours after one week, 4 weeks, 2 months and 4 months of SLIT or placebo. Average rhinoconjunctivitis total symptom scores (ARTSS, vertical axis) were calculated during the exposure periods. p-values represent difference between SLIT and placebo at each time point. (From Horak F, Ziegelmayer P, Ziegelmayer R, et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J Allergy Clin Immunol* 2009;124(3):474; with permission.)

This latter finding is consistent with previous analyses, suggesting that preseasonal treatment of up to 16 weeks was optimal.^{36,70}

There was also a progressive improvement in placebo-treated patients until 2 months, mirroring the large placebo effect seen in seasonal immunotherapy trials, or, alternatively, suggesting a degree of natural desensitization on recurrent exposure.

Regarding mechanisms, specific IgE and IgG levels increased during the 4-month treatment period in the SLIT group only, but no changes were seen in T-cell proliferation or basophil activation studies. Within the actively treated group, those with the greatest relative improvement in symptom scores from baseline to 4 months had greater increases in specific IgG compared with less responsive individuals.

Achieving long-lasting tolerance (clinically shown by symptomatic improvement even after discontinuation of treatment) is a major advantage of allergen immunotherapy compared with pharmacologic treatments. Tolerance is well established for SCIT for several different allergens.^{71–73} Two randomized, placebo-controlled studies have confirmed a carry-over effect of grass pollen SLIT for at least 1 year after discontinuation, in both cases after either 3 years or 3 seasons of treatment.^{7,8} In the study by Ott and colleagues⁸ clinical improvement persisted during a fourth season despite specific IgG4 levels decreasing to baseline values. Specific IgE levels increased sharply in years 1 and 2 before gradually decreasing thereafter, but had not completely returned to baseline levels by year 4.⁸ In the study of Durham and colleagues⁷ changes in IgG4 were closely paralleled by serum IgE-blocking activity, and were still significantly greater than placebo a year after treatment discontinuation. One important difference between these 2 studies is the use of perennial treatment⁷ versus

coseasonal treatment only.⁸ Although IgG4 levels seem to correlate with treatment and have *in vitro* blocking activity, an absolute increased level does not seem essential for continued efficacy.

Another study of interest here is a community-based trial of preseasonal and coseasonal grass pollen SLIT carried out in the United Kingdom.⁷³ After a baseline season, volunteers received either 2 years' placebo, 2 years' SLIT, or 1 year's SLIT followed by a second year of placebo. Both treatment groups showed increases in specific IgE and IgG4 at 1 year; however, a significant symptomatic improvement was seen only in the second year of the group receiving 2 years' SLIT. Specific IgG4 levels in the group receiving only 1 year of SLIT fell in the second year, but did not return completely to baseline levels.

An open study, including follow-up of SLIT-treated patients for up to 15 years, suggested the beneficial effect of house dust mite SLIT on clinical symptoms could persist for up to 7 years after a 3-year treatment.⁷⁴ This finding was accompanied by suppression of nasal eosinophils and bronchial hyperreactivity during the same period. In addition, SLIT-treated patients were relatively protected from developing new allergen sensitizations. This finding has been reported previously for SLIT,⁷⁵ along with the prevention of asthma development.⁷⁶ Such findings in open studies require confirmation in double-blind, placebo-controlled trials.

It remains to be conclusively shown for how long specific IgG changes persist after discontinuation of treatment, and whether this or any other biomarker is useful in reflecting the long-term clinical response. The exact relationship between allergen-specific IgG and IgE levels and clinical efficacy is likely to be more complex than simple quantitative values. For example, relative ratios of specific IgE/IgG may be more important, and, furthermore, the antibody affinity or avidity, rather than the absolute level, is likely to be relevant. Moreover, the persistence of a low titer of specific IgG with high allergen affinity may maintain tolerance even when total specific IgG levels decline. Studies looking at functional antibody assays for several years after treatment discontinuation are needed to assess these matters further.

AREAS OF UNCERTAINTY AND FUTURE STUDY

Although the oral Langerhans cell is an attractive candidate for the role of key antigen-presenting cell in SLIT, it is clear, at least from murine studies, that alternative antigen-presenting cells are also likely involved, including other monocyte-derived dendritic cells, macrophages, and potentially B cells.^{19,25} Furthermore, the mechanism of allergen uptake by these cells is as yet uncertain. Further clinical studies using adjuvants are needed, particularly in view of their apparent success in murine models.^{9-11,23-25} Grass pollen SLIT along with monophosphoryl lipid A, a toll-like receptor 4 agonist, has been shown to be safe in a proof-of-concept study,⁷⁷ but clinical and immunologic effects were modest. A suitable adjuvant would either increase the efficacy of current treatments, or allow maintained efficacy despite lower or less frequent allergen dosing.

The optimum site for allergen application is another area for future study. Trials are required to compare sublingual versus vestibular allergen application given the relative merits of these regions reported in investigational studies.^{12,13} Tablet SLIT perhaps has benefits over drops by providing sustained contact in higher concentrations with the mucosa for longer. It remains to be determined if other mucoadhesive formulations can provide further benefit in clinical practice.

Whether the rest of the gastrointestinal tract and mucosal-associated lymphoid tissue plays any part in the mechanism of SLIT is unclear. Although SLIT is considered

to exploit an immune mechanism distinct from classic oral tolerance induction there may be considerable overlap. Recent studies of oral tolerance induction to peanut^{78,79} and the use of SLIT for hazelnut and the peach lipid transferase protein allergy^{3,4} have shown efficacy and may possibly share common mechanisms. It remains to be seen whether either can provide true tolerance (ie, persistence of benefit after treatment discontinuation).

Use of modified recombinant allergens or peptide fragments for SLIT may in future provide safer treatments. Other possibilities to improve efficacy include combining SLIT with anti-IgE or anticytokine drugs, although cost currently makes this approach prohibitive for routine clinical use. The importance of tonsil and adenoidal tissue for sublingual tolerance also warrants further study, as does the role of allergen-specific IgA.

SUMMARY

On a systemic level, current evidence suggests SLIT shares many mechanistic properties in common with SCIT. Evidence points to similar, although less pronounced, effects on specific IgG, IgE, and IgA. Furthermore, IgG antibodies at least seem to be functional. Effects on the T-cell compartment are less certain, but a picture of regulatory and Th1 cell induction and Th2 inhibition is emerging.

Understanding of the local mucosal mechanisms is rapidly progressing, with evidence accumulating for a process involving local antigen-presenting cells as the crucial early mediators of SLIT. Further clarification here may allow for the development of better-targeted vaccines that might simplify, improve, and reduce the costs of treatment. Further such studies will determine whether or not SLIT may replace conventional SCIT as the mainstay of allergen-desensitization treatment.

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