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Basic Science for the Clinician: Mechanisms of sublingual and subcutaneous immunotherapy

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Introduction

Subcutaneous immunotherapy (SCIT) has been in use since 1911 for the treatment of respiratory and venom allergies, and current practice involves administering escalating doses of antigen via one or more subcutaneous injections weekly until a maintenance dose is achieved. Maintenance SCIT is generally continued every 4–8 weeks for a period of 3–5 years and has been shown in multiple studies to provide sustained benefit in reducing clinical symptoms to allergens as well as modulate the immune response to allergens (reviewed in¹). Recently, newer routes of IT involving the mucosal immune system have emerged and gained popularity including sublingual immunotherapy (SLIT) and oral immunotherapy (OIT)². SLIT is administered daily by holding antigen drops or tablets under the tongue, while OIT involves swallowing escalating daily doses of antigen. Characterizing the mechanism of action of IT has been the focus of intense investigation over the past several decades¹ and is important for the practicing clinician to understand as he or she considers the different routes of IT available to administer in clinical practice.

Changes in the allergen-specific T cell response in SCIT and SLIT

Mechanisms of T cell modulation following IT—The conventional theory regarding successful IT, be it SCIT or SLIT, has invoked the induction of regulatory T (Treg) cells, the nomenclature of which we will review briefly here^{3,4}. Several types of Tregs have been described including thymic-derived and peripherally derived Tregs^{5,6}. Thymus-derived Treg (tTreg; also referred to as natural (nTreg)) cells are characterized as CD4⁺CD25⁺ T cells that

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constitutively express the transcription factor Foxp3 and have a unique, hypomethylated CpG rich Foxp3 locus⁷⁻⁹. tTreg cells are produced in the thymus in response to expression of self-antigens and are therefore important in the prevention of autoimmunity. While they constitute 5–10% of peripheral CD4⁺ T cells, they are unlikely to be involved in tolerance to antigens not presented in the thymus such as allergens. An additional Treg subset has been described that develops in the periphery (pTreg; also referred to as inducible (iTreg))^{6, 10}. These pTreg cells can differentiate from preexisting T effector lymphocytes or circulating naïve T cells and may or may not express Foxp3 and CD25 while possessing suppressive activity. These cells are difficult to identify and study based on using CD25 as a surface marker or Foxp3 as an intracellular marker, as both of these markers can be transiently expressed by CD4⁺ and CD8⁺ effector T cells following T cell receptor activation and in response to IL-2 via a positive feedback loop¹¹. A sub-group of pTregs that is primarily gut-derived and generates mucosal tolerance has been referred to as Th3. Reflecting their prominent production of transforming growth factor (TGF)- β , in addition to mediating tolerance, Th3 cells are relevant to inducing secretory IgA production and oral tolerance. Suppression by Tregs occurs through contact inhibition, secretion of IL-10, TGF- β and IL-35 that inhibit cellular activity, surface interactions by negative co-stimulatory molecules such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or by competition for IL-2^{12, 13}.

An alternative mechanism for successful immunotherapy has been proposed that involves a shift in allergen-specific T cells from a T helper 2 (Th2) to a Th1 phenotype¹⁴. The recent development of major histocompatibility class (MHC) class II tetramers and epitope-guided mapping has allowed researchers to monitor the allergen-specific T cell responses to IT. The frequency of Fel d 1-specific T cells in allergic individuals prior to IT ranges from 1 in 7,000 to 1 in 30,000, with similar frequencies found for other allergen-specific T cells¹⁵. Allergen-specific T cells have also been identified in non-allergic subjects, albeit with lower numbers and decreased avidity of the T cell receptor¹⁶.

T cell changes during SCIT—A prominent role for IL-10-producing pTreg cells was first described in studies involving bee venom SCIT³. Induction of peripheral tolerance occurred with the use of either whole bee venom extract or short peptides to phospholipase A₂, the major bee venom allergen. The peripheral T cell response demonstrated that both Th2 (IL-4, IL-5 and IL-13) and Th1 (IFN- γ) cytokine production decreased, while IL-10 levels were increased. These IL-10 producing T cells suppressed allergen-specific T cell proliferation and activation and are now recognized as pTregs¹⁷.

Use of inhalant allergens in SCIT, including house dust mite and birch pollen, have confirmed the importance of IL-10 (and TGF- β) production by CD4⁺CD25⁺ T cells (pTregs) in the suppression of Th2 and Th1 proliferative responses and cytokine production¹⁸. Several studies have also demonstrated new or increased IL-10 production by CD4⁺ cells in the absence of changes in allergen-induced proliferation, but with decreases in Th2 cytokine production including IL-5 within 10 months of treatment^{4, 19-21}. Reasons for differences in T cell responses could include dose of allergen used, different adjuvants added to the allergen and differences related to the allergen itself and the ability to induce an immune response.

What is consistent is that each of these studies has found cells capable of making high levels of IL-10 ($\pm$ TGF- β) consistent with the pTreg cell type. While the appearance of the IL-10 producing pTregs in bee venom and inhalant allergen SCIT is rapid, within 7 days, full tolerance requires 3–5 years of sustained high-dose treatment. The tolerogenic effect of pTreg cells is evident from studies demonstrating that following 4 years of high dose SCIT, subjects remain non-responsive for at least 3 years after SCIT has been stopped, similar to those who had continued SCIT for the full 7 year period.²²

Using the tetramer-guided approach described earlier to monitor the frequency and characteristics of alder pollen specific CD4⁺ T cells during the course of IT, led to the observation that two populations of allergen-specific memory T cells could be separated based on the expression of CD27. A terminally differentiated CD27⁻ population has characteristics of classical Th2 cells with high levels of CRTh2 and CCR4 on the cell surface and high intracellular levels of IL-4, IL-5 and IL-13. In contrast, the CD27⁺ population had high CXCR3, CCR7 and CD7 on the cell surface with high intracellular interferon (IFN)- γ levels, characteristics of Th1 cells, as well as suppressive IL-10²³. It was the ratio of CD27⁻ (Th2) to CD27⁺ (Th1) cells that distinguished allergic from non-atopic individuals and suggested that this could be a biomarker for successful immunotherapy. Successful SCIT was associated with preferential loss of the pathogenic CD27⁻ (Th2) population, while the surviving IL-10 secreting CD27⁺ T cells maintained a suppressed state and limited the development of new allergen-specific Th2 cells²⁴.

T cell changes during SLIT—In an early mechanistic study of SLIT in humans, peripheral blood mononuclear cells (PBMCs) from patients treated with house dust mite (HDM) SLIT for 3 years produced more IL-10 following mitogen stimulation when compared to patients with untreated rhinitis²⁵. Similar to SCIT, this provided indirect evidence for the immunoregulatory role of IL-10 in SLIT. A smaller mechanistic study examining 9 patients undergoing birch SLIT suggested an early and late phase response to therapy²⁶. By 4 weeks of therapy, there was an increase in circulating pTregs accompanied by an increase in IL-10 and Foxp3 mRNA expression levels and non-specific suppression of antigen-driven lymphocyte proliferation. At the end of 52 weeks of therapy, suppression of proliferation was specific to Bet v 1 (birch pollen) and the levels of circulating pTregs as well as IL-10 returned to baseline. Persistent reduction of IL-4 mRNA and an increase in IFN- γ mRNA was maintained. This study suggests that early in therapy, IL-10 producing pTregs act non-specifically to suppress lymphocyte proliferation in response to antigen, but over time this non-specific response is replaced by antigen-specific tolerance on the backdrop of a Th1 skewed cytokine milieu²⁷. Other studies have confirmed increased pTreg numbers, decreased allergen-specific T cell proliferation, and a shift from a Th2-to-Th1 cytokine milieu following SLIT, accompanied by clinical improvement in symptoms^{28, 29}.

Unique local immunomodulatory effects have also been noted with SLIT. In patients treated with grass pollen SLIT for 12–18 months, the number of Foxp3⁺ cells in the sublingual epithelium significantly increased in the treatment group compared to the placebo group and non-atopic controls³⁰. One caveat is that these cells may have been recently activated effector cells rather than true regulatory T cells as the number of IL-10 and TGF- β mRNA positive cells did not change following SLIT. In contrast, another study of grass pollen SLIT

was able to demonstrate an increased number of IL-10 and TGF- β -producing Treg cells following therapy³¹.

Bonvalet et al. demonstrated a significant (41.8%) improvement in symptom scores after grass pollen challenge in subjects given grass SLIT for 4 months³². However, they were unable to detect a significant decrease in grass allergen (Phl p 1 and Phl p 5)-specific CD4⁺ T cells numbers during treatment or 4 months after SLIT was stopped. There was a slight decrease in the number of CD27⁻ or “pro-allergic” Th2 cells³². This raises the possibility that while clinical improvement may be seen as soon as 1 month, parallel immunologic changes involving the shift from a Th2 signature and upregulation of pTreg cells require longer treatment protocols (up to 12 months). Another explanation for the potential failure of immunotherapy is that the epigenetic changes noted in pTregs have been shown to be unstable, i.e. the FoxP3 locus is only transiently hypomethylated^{33, 34}.

Humoral Immunity in SCIT and SLIT

Changes in the Humoral Response During IT—During the course of IT, allergen-specific IgE measured by ImmunoCAP initially rises above baseline and then gradually decreases over months to years of treatment^{35, 36}. However, the normal seasonal increase in IgE due to natural exposure to the allergen is blunted following IT³⁷. In contrast to ImmunoCAP measurements, when Immuno Solid-phase Allergy Chip (ISAC) is used, there is a decrease in grass allergen component-specific IgE seen during the build-up phase of IT, suggesting that ISAC may offer advantages for monitoring the early effects of IT³⁸.

Driven by IL-10 production, there is an accompanying rise in allergen-specific IgG antibodies, particularly IgG4 antibodies, which are proposed to have a “blocking” effect by competing for allergen and thus inhibiting allergen binding to membrane-bound IgE. This would prevent IgE crosslinking and mast cell and basophil degranulation; however, since mast cells are located on mucosal surfaces that directly contact allergen, it is unclear how an IgG antibody could access the allergen prior to its binding to the mast cell³⁹. Another potential effect of IgG4 is to limit activation of T cells via inhibition of IgE-facilitated antigen presentation (FAP) by Fc ϵ RI⁺ and CD23⁺ antigen-presenting cells (APC). Lastly, IgG4 may bind to the low affinity inhibitory Fc γ RIIB, suppressing release of pro-inflammatory mediators⁴⁰. The primary source of IL-10 is not only Tregs, but also so-called regulatory B cells (Bregs). In a classic paper using beekeepers with frequent venom exposure as a model of bee venom IT, the number of Bregs increased over time with higher IL-10 and IgG4 production by the Bregs. Additionally, IL-10⁺ Bregs were found at higher frequency in non-allergic beekeepers⁴¹.

An increase in TGF- β driven allergen-specific IgA and IgG1 production has been reported in some but not all studies of IT^{42–45}. IgA is believed to play a protective role in mucosal immunity, so this mechanism would be more relevant in OIT and SLIT rather than SCIT. As a whole, studies have consistently failed to demonstrate a correlation between the timing or magnitude of the decrease in specific IgE levels and the rise in IgG4 and IgA levels with clinical benefit. Thus, the changes in the humoral compartment are best interpreted as a marker of changes in the overall immune response in systems described elsewhere in this review.

Differences in humoral immunity induced by SCIT versus SLIT—Studies directly comparing SLIT versus SCIT that include measurements not just of clinical efficacy but also of well-defined immunologic markers, are lacking in the literature. A recent randomized placebo-controlled trial comparing SCIT to SLIT for grass pollen allergy found that the initial increase in grass specific IgE was quicker (1 month compared to 1–2 months) and threefold higher in the SLIT group compared with the SCIT group³⁷. SCIT-treated patients demonstrated a higher level of IgE blocking activity than those treated with SLIT (50% versus 27%) as well as increased inhibition of FAP (22.5% versus 17.2%). This effect was also more rapid with SCIT (3 months versus 15 months)³⁷. Dramatic increases in specific IgG4 antibody levels have been reported in multiple studies of patients receiving maintenance therapy with SCIT (30–40 fold) compared to SLIT (3–4 fold)^{37, 46–48}.

One possible explanation for the increased humoral immune response noted in SCIT versus SLIT pertains to the level of allergen exposure⁴⁹. While the total cumulative dose of allergen given sublingually in SLIT is significantly higher than that given subcutaneously in SCIT (anywhere from 5- to 100-fold lower according to various estimates^{37, 50}, the effective dose that is absorbed by the oral mucosa and available for the immune response is postulated to be much lower in SLIT due to the neutralizing effects of saliva. In addition, SCIT protocols often rely upon use of an adjuvant such as aluminum hydroxide (alum) or calcium phosphate, while such adjuvants are not used in sublingual preparations. The use of adjuvants boosts the immune response, a feature frequently taken advantage of in vaccine development.

Antigen Processing and Presentation: Dendritic Cells in SCIT and SLIT

Antigen Processing and Presentation in IT—In large part, the phenotype of the immune response generated following IT administration depends upon which APC is responsible for processing and presenting the allergen. As the most important “professional” APC, it is the dendritic cell (DC) that has the most important role in driving this component of the subsequent immune response and amongst the DC, it is which class of DC, its degree of maturation, and its inherent state of Th1/Th2/Th17 immune deviating “bias” that determines the response. Dendritic cells include myeloid, plasmacytoid, conventional, Langerhans and many others: each having different anatomical locations and unique inherent tendencies to drive distinct effector and tolerogenic immune responses that are likely to be differently engaged in SCIT and SLIT (reviewed in detail elsewhere⁵¹).

Antigen Processing and Presentation in SCIT—The location of the immune response ensuing after a maintenance allergy injection, whether it is a primary (spleen), secondary (axillary lymph node) or tertiary (local) lymphoid organ, is certain to influence which APC is responsible for initiating the immune response, the nature of the subsequently engaged T effector cells and, ultimately, the phenotype of the ensuing immune response. Surprisingly little is known regarding the natural history of allergen after it is injected, typically in the subcutaneous tissue in the deltoid region. Some of the allergen drains via local lymphatics into secondary lymphoid organs (e.g., axillary lymph nodes). It is estimated, however, that only a very small fraction of the injected allergen reaches these secondary lymphatics^{52, 53}. Subcutaneously injected allergen is also clearly capable of

accessing the circulation either directly through local vasculature or indirectly via the lymphatics and thoracic duct as evinced by the potential for systemic anaphylaxis and the ability to measure allergen in circulation after a subcutaneous injection^{50, 54}. This systemic access suggests that subsequent immune responses could also develop in virtually any lymphoid organ including primary lymphoid organs such as the spleen. Lastly, given the presence of local reactions, including what are often severe, painful reactions that can persist for many days or even weeks after an injection, it is also plausible that T effector immune responses are evolving in tertiary lymphoid organs or developing *de novo* in the subcutaneous tissue itself. Indeed, it is this surprising lack of understanding of the events occurring following subcutaneous injection of allergen that has led to a desire to develop newer techniques with more predictable responses. These include SLIT, but also the consideration of direct intralymphatic injection of allergen, a mechanism that produces much more predictable tolerogenic processes than SCIT and, shares with SLIT the ability to greatly reduce the risk for anaphylaxis⁵⁵.

Antigen Processing and Presentation in SLIT—In contrast to SCIT, somewhat more (or rather, “something”) is known regarding the role of antigen processing pathways in SLIT. The administration of a SL tablet and its being held in place for ~5 minutes before being swallowed largely focuses the allergen to the sublingual tissue. Not being injected and, as such, being less able to directly access vascular structures, sublingually administered allergen is less capable of being measured in the circulation^{50, 54}. This largely eliminates the risk for anaphylaxis, but also focuses the arena of investigation of the immune response to the sublingual mucosa. The DCs involved in SLIT processing are primarily myeloid (mDC) and Langerhans cells (LCs)^{51, 56, 57}. The capacity of these DCs to rapidly capture sublingually administered antigen can be demonstrated by the take-up of biotinylated ovalbumin^{40, 58}. Both sublingual LCs and mDCs are characterized by inherent production of IL-10, IL-12, and TGF- β . As such, the tendency of both of these DC classes is to promote Th1 (via IL-12) and Th3 immunity (via TGF- β and IL-10), but not Th2 responses, along with also promoting IgA-mediated humoral immune responses (via TGF- β)³¹. These DCs are also characterized by expression of high affinity IgE receptors (Fc ϵ RI)^{58–60}. This expression of Fc ϵ RI promotes beneficial immune responses via IgE-mediated FAP.

While reducing the potential for anaphylaxis and arguably focusing the potential phenotypes of developing immune responses to those that can be mediated by mDCs and LCs (i.e. a Th1/Treg response) there is one potential downside to the sublingual administration of allergen. While the systemic availability of SCIT raises the risk for anaphylaxis, it also makes available the entire immune organ as an arena for antigen processing and presentation. In contrast, the immune response to SLIT is limited to the fixed number of DCs present in the sublingual tongue mucosa. One of the surprising observations of SLIT is the absence of compelling dose responses. That is, in numerous studies once an effective dose is achieved no further benefit is observed with higher doses^{61–63}. And in at least one study, the addition of numerous other allergens to a fixed dose of timothy grass appeared to reduce its beneficial effects for all allergens in the mixture⁶⁴. Neither of these limitations, if confirmed, is generally associated with SCIT and may reflect the unlimited capacity for antigen processing and presentation after subcutaneous administration. In contrast, the

sublingual surface provides only a relatively fixed number of DCs to process and present allergen, suggesting the possibility of saturation and competitive inhibition for available DCs.

Innate Lymphoid Type 2 cells in SCIT and SLIT

A novel subset of immune cells termed the innate lymphoid type 2 cells (ILC2) has been described and recognized as having central importance in pathogenesis of allergic diseases secondary to their capacity to rapidly produce large amounts of IL-5 and IL-13 in response to “danger” and “alarm” signals (reviewed in ^{65–66}). The impact of IT on ILC2 function has only been minimally investigated. However, a recent study demonstrated that ILC2 numbers increased in seasonal allergic rhinitis during the pollen season and that that increase was attenuated among subjects⁶⁷. The increased numbers of ILC2s were characterized by IL-13 expression ⁶⁷.

Conclusion

Both SCIT and SLIT are clinically effective in meta-analyses and both induce similar, but not identical changes in the immune response (summarized Table I). What remains to be seen is if the subtle differences that have been noted have relevance to factors such as the persistence of the protective immune response following discontinuation of treatment, or the ideal allergens targeted by each route. It is clear that for the clinician, the last decade represents a true sea change in the field of allergy with the emergence of SLIT and OIT as viable alternatives to SCIT, and that keeping up to date with these changes will be critical to the practice of allergy in the future.

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Table I**Immune Mechanisms of Immunotherapy**

	Immune Mechanism	Demonstrated for SCIT	Demonstrated for SLIT
Cellular Immunity	Induction of Allergen-Specific pTregs	X	X
	Th2-to-Th1 immune deviation	X	X
	Selective apoptosis of CD27 ⁻ allergen-specific T cells	X	
	Induction of IL-10, TGF- β	X	X
	Induction of TGF- β ⁺ allergen-specific Th3 cells		X
Humoral Immunity	Reduction of ILC2s	X	
	Transient rise and long-term inhibition of allergen-specific IgE	X	X
	Rapid decrease in allergen component IgE	X	
	Increase in allergen-specific IgA and IgG4	X	X
	Inhibition of facilitated antigen presentation (FAP)	X	