

How does sublingual immunotherapy work?

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There is now a growing consensus that specific sublingual immunotherapy (SLIT) does actually work. Initial skepticism about the results of uncontrolled or open studies has been removed after the positive outcomes of a series of large, double-blind, placebo-controlled trials. A recent meta-analysis found 21 trials of SLIT that were of sufficient quality to merit analysis of their efficacy against allergic symptoms.¹ Although there are some differences in dosing, patient selection, and outcome measures, the overall effect of SLIT was found to be beneficial, both on symptoms and on rescue medication requirements. This meta-analysis did not include data from the latest series of large-scale studies to be published, namely those assessing the grass pollen tablet that has recently been granted a product license and marketed in Europe under the trade name Grazax.²⁻⁴ However, it is interesting to note that the Grazax studies show a degree of efficacy almost identical to that found in the meta-analysis, and therefore when these newer studies are eventually included in a future meta-analysis, the overall effect size will remain much the same.

If one accepts that SLIT works, it is then relevant to ask how it works. This has an important bearing on our understanding of how to improve current forms of SLIT and to predict those who are going to respond but might also shed some light on the mechanisms of conventional subcutaneous injection immunotherapy (SCIT).

Within the allergy community, we sometimes forget that we do not really know how conventional immunotherapy works. After all, the regimens that we currently use were invented between 1890 and 1930, long before the discovery of IgE or the cytokines that underpin the development of allergy and its clinical expression. Back in the 1930s, it was observed that patients undergoing SCIT had antibody responses to the injected allergen. These were described by Cooke et al⁵ as blocking antibodies on the presumption that they might in some way block the access of the allergenic proteins to their targets. Subsequently, we have learned that many of these

Abbreviations used

SCIT: Subcutaneous injection immunotherapy

SLIT: Sublingual immunotherapy

antibodies are of the IgG4 subclass⁶ and that their production is favored by IL-10.⁷ However, the time course of antibody production is different from that of clinical benefit.⁸ Moreover, there is little correlation between antibody levels and protection when this is assessed at the single-patient level.⁹ Arguably, the IgG response could be an epiphenomenon, simply reflecting the stimulation of an IgG response after repeated injection of foreign protein, quite independent of the delivery of specific clinical benefit.

At the clinical level, it is clear that SCIT does not abolish immediate allergic responses but uncouples these from the usual downstream consequences. This is exemplified by the ability of SCIT to attenuate the late-phase allergic skin reaction.¹⁰ It is generally accepted that the late-phase response is a more relevant indicator of allergic inflammation than the immediate response, not least because glucocorticosteroids have profound effects on the late-phase response but little or no effect on immediate response to allergen. Knowing this, attention turned to the effects of SCIT on the cellular and cytokine responses to allergen. Initial thoughts that SCIT might induce a T_H1 response¹¹ have proved naive. Although some features of a T_H1 response were observed, the general picture was of a reduction in cellular inflammation, with some subjects showing increased recruitment of IFN- γ -producing T cells but others really not demonstrating any measurable T_H1 response, despite good clinical efficacy.^{12,13} In patients with hymenoptera allergy, SCIT induced increased numbers of CD25⁺ IL-10-producing cells by using the FoxP3 transcription factor and fitting the T-regulatory cell phenotype.¹⁴⁻¹⁶ Most recent reviews have emphasized the importance of inducing T-regulatory cells as the likely mechanism of action of SCIT, although some caution is needed before concluding that this is the only active mechanism or indeed the most important one.

Against this background, what do we know of the mechanisms of successful SLIT? Several possibilities exist. There is a long story about oral tolerance, which is mainly based on rodent experiments. If animals that are easily sensitized through the parenteral route are fed allergenic material by mouth early in life, they become resistant to subsequent parenteral sensitization. This suggests that allergens given by mouth are handled differently from the same material given parenterally but does not really help to explain how sublingual administration of

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allergen could cure an existing allergy. Studies of absorption of radiolabelled allergens have shown that the proteins take longer to be broken down if given sublingually compared with swallowing them without keeping them under the tongue for 2 minutes.¹⁷ Radioactivity is retained in the buccal mucosa and can be shown migrating to the regional lymph nodes. This suggests that the allergens are taken up by dendritic cells in the buccal mucosa and then presented centrally to the immune system. Dendritic cells from the oral mucosa of atopic and nonatopic individuals constitutively express FcεRI, and compared with skin dendritic cells (Langerhans cells), there is greater expression of CD40, MHC class I, and MGC class II and also increased expression of the CD80 and CD86 costimulatory molecules.¹⁸ The precise functional relevance of this is not proved, but at the very least, it indicates that buccal dendritic cells are active and very capable of interacting with lymphocytes.

IgG responses have been studied in several SLIT trials, but the results have been rather variable. For example, no change of specific IgG or IgE titer was found in a clinical trial of *Dermatophagoides pteronyssinus* SLIT in children with asthma, which showed good efficacy, as judged by a 60% reduction in asthma episodes and nocturnal symptoms.¹⁹ What should we draw from this? If we believe antibodies are important, then either this study shows SLIT can be effective without any change in antibody levels, or else the treatment did not work, and the 60% reduction in symptoms was a chance finding or perhaps caused by unblinding. Either view is uncomfortable if one wants to sustain a belief in the importance of an antibody response. Conversely, another study, this time with a grass pollen extract at the highest dose used to date in clinical trials, did find an increase in allergen-specific IgG levels, although with a relatively small clinical benefit.²⁰ Another key study showed a good clinical improvement after 1 year of SLIT with a modest increase in IgG4 levels, whereas after 2 years, the average specific IgG4 level increased by 2.5-fold; however, the degree of clinical benefit was the same in both years.⁴ We now know that production of IgG4 is controlled by the cytokine IL-10, which downregulates T-cell function and B-cell switching to IgE.²¹ *In vitro* PBMCs from patients treated with SLIT show a clear increase in IL-10 mRNA production when stimulated with allergen.²² This is supported by evidence that SLIT normalizes the cytokine profile of PBMCs. Whereas PBMCs from healthy subjects make a good IL-10 response to allergen, cells from untreated atopic subjects fail to produce IL-10, but cells from patients who have received SLIT respond similarly to those from normal nonatopic subjects.²³ Similar data have been reported in other studies with other allergens and allergoids.²⁴

T-cell responses are also altered after SLIT. Proliferation of PBMCs to timothy grass has been shown to be decreased by the time patients reach the maintenance phase of therapy, and this unresponsiveness persists through the end of the first year of treatment.²⁵ This has also been shown with birch pollen²⁶ and house dust mite preparations.²⁷

Further support for the immunomodulatory effect of SLIT comes from an open but randomized controlled trial in which a relatively modest dose of birch pollen extract (about 12 times the usual SCIT dose) was given to 39 patients with birch pollen allergy.²⁸ Twenty-nine completed the course, whereas 23 of the 40 patients assigned to the control group were followed up to the end of the study. It emerged that there was reduced salbutamol use in the actively treated group during the second and third years of treatment, and this was accompanied by a reduction in nasal eosinophil counts. Although the effects on salbutamol could be distorted by the open study design, this is less likely to alter nasal inflammation and suggests that SLIT does indeed have a measurable effect on allergic inflammation, at least in the nose. This view is further supported by another randomized trial of SLIT in 86 children allergic to house dust mite.²⁹ After 6 months, the active group showed clear reductions in serum eosinophil cationic protein levels and also in cytokine IL-13 levels, which has been associated with several components of airways remodeling.³⁰

Therefore it appears that there are several possible mechanisms at work that might explain the efficacy of SLIT. Oral tolerance certainly exists, but the system described in neonatal rodents is probably not relevant to SLIT. The antibody response to SLIT is variable but differs in some key respects to that seen after SCIT, and there is some evidence of suppression of T-cell responses. It is clear that further work in the area is needed.

In this month's issue of the Journal, Bohle et al³¹ have provided some interesting data that shed some light on these processes. Briefly, their work suggests that there are at least 2 processes operating in parallel to underpin the clinical efficacy of SLIT. Over the first month of treatment, an increased number of CD4⁺CD25⁺ IL-10-producing cells were detected in peripheral blood. These were associated with a nonspecific suppression of antigen-driven T-cell proliferation in that responses were reduced to the inducing allergen (birch pollen) and also to the related apple allergen Mal d 1 and the unrelated antigen tetanus toxoid. When reassessed after 1 year, only the T-cell response to birch pollen was suppressed. Interestingly, after 4 weeks of treatment, the suppression of proliferation was reversible by means of depletion of CD25⁺ cells or by addition of anti-IL-10 antibodies, but neither maneuver could reverse the suppression observed after 1 year. The work itself was well performed and seems sound, and therefore the logical implication is that there is an initial induction of T-regulatory cells that act through IL-10 to suppress specific and nonspecific T-cell responses. However, over time, this phenomenon fades and is replaced by a more specific and durable form of T-cell tolerance.

The authors conclude that SLIT induces immune mechanisms that are comparable with those found after injection SIT, but in fact, their data show more than that. Although at first glance it might appear encouraging that similar mechanisms operate in SLIT and SCIT, there is no

fundamental reason why the 2 forms of immunotherapy should work the same way.

First, some of the immunologic events after allergen injection might simply be responses to injection of large amounts of foreign protein, rather than having any relevance to the clinical benefit. Second, the basic principles of host defense mean that the mucosal immune system is set up to ignore most foreign material and to be tolerant of almost everything else; otherwise, we would spend our days reacting against every piece of food we eat. Only those agents capable of invading the mucosa need to be responded against, so that on future encounter they will be neutralized outside the body, preferably without creating a host-damaging inflammatory response. In contrast, the internal immune system is set up to mount a vigorous response to any agent that is encountered within the tissues on the basis that anything foreign deep inside the body must be dangerous, otherwise it would not have got there in the first place. The cytokine evidence presented by Bohle et al³¹ suggests that TGF- β is not a part of the response, and therefore one would not expect an IgA response to SLIT. This contrasts with studies of SCIT, which have shown both a TGF- β response and a modest increase in allergen-specific IgA levels.³² Does this matter at the clinical level? Well, arguably it might be better not to respond at all to pollen and other allergenic material rather than to create antigen-antibody complexes on the mucosal surface. Once we have agreed what constitutes a comparable dosing regimen for SLIT versus SCIT, it would be instructive to pursue this aspect.

Some caution is needed: the study by Bohle et al³¹ is based on a small number of subjects, and it was not possible to obtain blood samples from all subjects at all time points. However, the data do point toward a phased and progressive response to SLIT and provide a plausible intellectual framework to explain some of the conflicting data that have been reported hitherto. It might also help explain some of the inconsistencies in the literature on the immunologic effects of SCIT, and it will be instructive to see whether applying these techniques and time-course experiments to SCIT yield consistent or discordant results.

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