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Review article

Immunological mechanisms of sublingual immunotherapy

Administration of allergen-specific immunotherapy by the oral route, sublingual immunotherapy (SLIT), has been shown to be effective, with an improved safety profile compared with subcutaneous administration. However, the precise mechanisms underlying the induction of immune tolerance by SLIT remain unclear. Contact of the allergen with the antigen-presenting cells in oral mucosa is likely to be critical. Mucosal Langerhans cells can capture the allergen and transport it to local lymph nodes, which may favour the induction of T lymphocytes that suppress the allergic response. In addition, the production of blocking IgG4 antibodies and the involvement of mucosal B cells appear to play a role. There is a growing evidence to support the role of regulatory T cells in controlling the development of asthma and allergic disease. Nevertheless, there remains a lack of firm evidence that SLIT induces regulatory T cells, although preliminary *in vitro* data suggest that SLIT may increase interleukin-10, which has a clear role in suppressing the allergic immune response. Further studies are required to determine the involvement of regulatory T cells, the role of different dendritic cell subsets, mucosal B cells as well as the potential use of adjuvants during SLIT.

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Specific immunotherapy (SIT) by the subcutaneous route has been shown to be an effective therapeutic tool for the treatment of respiratory allergic disorders. However, in early studies, subcutaneous immunotherapy (SCIT) was associated with uncommon but severe or even fatal systemic reactions (1). Therefore, investigators have proposed alternative routes of allergen administration involving mucosal absorption and suggested that the natural mechanisms underlying the induction of oral tolerance at mucosal surfaces may be an effective therapeutic strategy for suppression of ongoing pathological immune responses in allergic diseases (2). The precise mechanisms by which oral tolerance is induced remain unclear, but it seems likely that the route of allergen processing and presentation is a critical determinant of the subsequent T cell response (3).

Positive results of studies evaluating the effectiveness and safety of SIT by the sublingual route in patients with rhinitis and asthma symptoms because of pollen or the house dust mite (*Dermatophagoides pteronyssinus*) are encouraging (4). Moreover, several studies have shown a long-lasting effect, which documents a preventive effect on the development and natural history of asthma (5, 6).

Hypotheses for the mechanism of action of SLIT

Although most studies of sublingual immunotherapy (SLIT) have examined its safety and efficacy, now investigations are beginning to elucidate the underlying immunological mechanisms. It seems likely that contact of the allergen with the oral mucosa is critical for the success of SLIT (7). To provide the experimental basis for understanding the relationship between the route of administration and the mechanism of action, Allergen labelled with iodine^{123} has been used for pharmacokinetic studies (8). In subjects receiving the radiolabelled allergen orally, plasma radioactivity increased rapidly, peaking at about 30 min. However, when given sublingually, plasma radioactivity was undetectable until swallowing. Moreover, a relevant amount of iodine^{123}-labelled allergen persisted in the sublingual region for up to 40 h after administration.

What then makes the oral mucosa efficient in the immunological processes leading to a reduction of allergic symptoms during SLIT? It is postulated that the Langerhans-like local dendritic cells may play a critical role in this process (9). During SLIT, the allergen is captured within the oral mucosa by Langerhans-like dendritic cells and, subsequently, the dendritic cells mature and migrate to proximal draining lymph nodes. The significance of the role of these local lymph nodes in successful allergen-SIT lies in their preferential production of blocking IgG antibodies and the induction of T lymphocytes with suppressive function (10).

Role of blocking antibodies

One mechanism by which immunotherapy suppresses the allergic response is by an increase in the production of

IgG antibodies, primarily the IgG4 subtype, over IgE. Because the production of IgE against 'harmless' antigens is the hallmark of allergic responses, the production of antigen-specific IgG antibodies, can antagonize and 'block' the allergic inflammation cascade resulting from antigen recognition by IgE. Therefore, the shift in balance between IgE and IgG4 is a phenomenon crucial for successful allergen-specific immunotherapy. In conventional SIT, while specific IgE levels did not change significantly after 70 days of house dust mite immunotherapy, specific IgA, IgG1, and particularly IgG4, significantly increased (11). This increase in specific IgA and IgG4 in serum coincides with increased transforming growth factor (TGF)-β and interleukin (IL)-10 in allergen-specific peripheral blood mononuclear cell cultures. Both TGF-β and IL-10 have roles in suppressing the allergic response, through different mechanisms.

Nevertheless, the current paucity of studies to support the role of blocking IgG4 gives rise to some apparently contradictory data. One study reported changes in specific IgG4 levels (10), whereas others reported a lack of change in specific IgG4 (4) and IgE levels (12). Furthermore, in some SLIT studies, investigators observed a decrease in the IgE/IgG4 ratio (13). A recent study of house dust mite-SLIT treatment of asthmatic children reported the successful down-regulation of specific IgE in serum in combination with slight upregulation specific IgA, but with no effect on IgG4 and IgG1 (13). Further studies are needed to clarify the role of blocking antibodies in SLIT. It has to be noted here that IgG4 and IgA are noninflammatory and noncomplement activating antibodies. By contrast, IgG1, IgG2 and IgG3 are activating the complement system and may play a role on antibody-dependent cellular cytotoxicity.

Role of T lymphocytes

Understanding the healthy immune response to allergens may have implications for successful allergen-SIT (14). In healthy individuals, T regulatory cells represent the dominant allergen-specific subset. By contrast, allergic individuals characteristically display a high frequency of T helper (Th) 2 cells specific for common environmental allergens (Fig. 1).

T regulatory cells comprise two major types: a constitutively expressed subtype of $CD4^+CD25^+$ cells, which are characterized by the expression of the transcription factor FoxP3, and an inducible subtype (Tr1), which is characterized by the secretion of IL-10 and TGF-β. In humans, circumstantial evidence suggests that both types of T regulatory cells play a major role in the inhibition of allergic disorders (15). Furthermore, another type of regulatory cell, T helper type 3 (Th3) produces IL-4, TGF-β and IL-10, and is induced following oral administration of the antigen (10).

Regulatory T cells control an established allergic response through distinct mechanisms, including T cell tolerance, whereby T cells are rendered nonresponsive to potential antigens or the body's own tissues. T cell tolerance can be directly initiated by the autocrine actions of IL-10 and TGF-β (11, 16). Interleukin-10 is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production (16). Meanwhile, TGF-β induces IgA production (17). This may account for the role of IgA and TGF-β, as well as IgG4 and IL-10, in peripheral mucosal immune responses to allergens in healthy individuals. As noted previously, the balance between IgE, IgG and IgA is critical to the successful outcome of SIT (11).

Animal experiments suggest that mucosal vaccination with antigens is able to induce mucosal tolerance. In a murine model of birch (*Betula* sp.) pollen allergy, intranasal application of Bet v 1, the major allergen of birch pollen, suppressed IgE-dependent basophil degranulation, specific IgG1 and IgG2a titres, and IL-5 production *in vitro*. In addition, analyses of splenocytes with reverse transcriptase-polymerase chain reaction revealed that tolerance induction was associated with enhanced expression of TGF-β, IL-10 and Foxp3 mRNA in $CD4^+$ T cells (18).

As of today, there remains a scarcity of evidence to support the ability of SLIT to induce regulatory T cells in clinical practice. Nevertheless, a recent preliminary study showed that, compared with untreated controls, SLIT increased IL-10 production in peripheral blood mononuclear cells from patients with house dust mite allergy following *in vitro* stimulation with *Dermatophagoides* antigens, as well as recall antigens, the common antigens to which most patients should react, such as *Candida albicans* or phytohaemaglutinin (10). In another study in childhood asthma with house dust mite allergy, 6 and 12 months of SLIT down-regulated the specific IgE response, while slightly increasing specific IgA (13). This suggests that further investigations are warranted to study the role of T regulatory cells, IL-10 and TGF-β in allergic subjects treated with SLIT.

Role of oral mucosal dendritic cells

Accumulating clinical trial data have contributed to the understanding of SLIT as an efficient approach to treat respiratory allergic diseases, with an excellent safety profile (4). One explanation for the success of SLIT is the profound difference between oral Langerhans cells and their skin counterparts. Oral Langerhans cells exhibit constitutive high expression of the Fc portion of IgE (Fc-epsilon receptor-type I, or FcεRI+), major histocompatibility complex (MHC) class I and II, as well as co-stimulatory molecules (CD40, CD80/B7.1, CD86/B7.2), which may suggest the specific function of these cells within the regional immune system of the oral mucosa (19).

In a recent study, nasal and oral mucosal $CD1a^+$ myeloid dendritic cells of atopic and nonatopic individuals

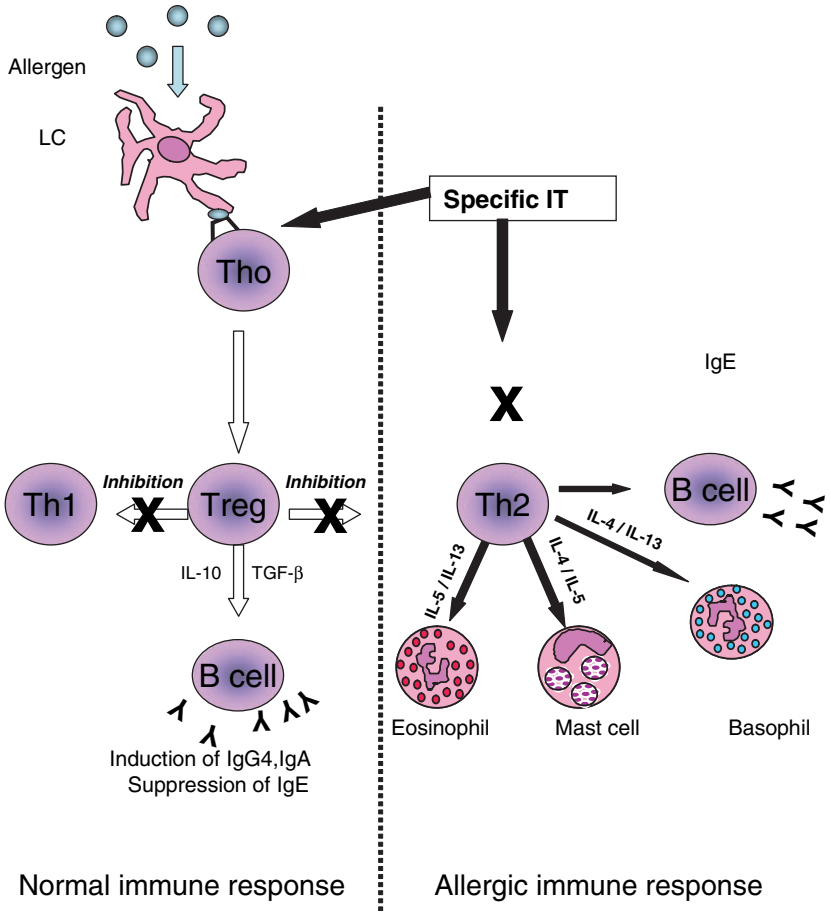

Figure 1. Peripheral tolerance mechanisms in allergen-specific immunotherapy (SIT) and healthy individuals. Immune deviation towards T regulatory cell response is an essential step in allergen-SIT and natural allergen exposure of nonallergic individuals. T regulatory cells secrete interleukin (IL)-10 and transforming growth factor (TGF)-β, which induce IgG4 and IgA production in B cells. These two cytokines directly or indirectly suppress the effector cells of allergic inflammation, such as mast cells, basophils and eosinophils. In addition, T regulatory cells may down-regulate cytokine production in T helper (Th) 2 cells, thus suppressing IgE production.

were studied and compared. Both nasal and oral dendritic cell types shared the feature of FcεRI expression, but differed in (i) surface density of FcεRI, (ii) lineage specificity, (iii) myeloid marker and (iv) costimulatory and MHC class expression. Furthermore, this study found that the lipopolysaccharide receptor CD14, present on both nasal and oral myeloid dendritic cells, was at higher density in oral dendritic cells (9).

Allergen delivery through the sublingual-swallow route appears to be more efficient than either route alone, which suggests an inevitable absorption of allergen through the gastrointestinal tract (8) that potentiates the induction of oral mucosal tolerance.

Studies indicate that short-term, co-seasonal SLIT with grass pollen was able to reduce the development of asthma in children with allergic rhinoconjuctivitis. Compared with SLIT-treated children, development of asthma after 3 years of immunotherapy was 3.8 times more frequent in the control group (5). Moreover, the long-lasting efficacy 5 years after discontinuation of house dust mite-SLIT demonstrated the capacity of this mode of immunotherapy to modify the natural history of paediatric asthma (6).

It is tempting to speculate that the use of allergen in conjunction with an adjuvant within the oral cavity may further potentiate the immunomodulatory effect of SLIT towards protection from allergy, represented by Th0 type immune response with low amounts of production of Th1 and Th2 cytokines or immune tolerance. A previous study tested whether administration of Bacillus Calmette-Guerin (BCG) vaccine as an adjuvant to allergen-specific SLIT has any additive effect on cytokine profiles in children with asthma because of house dust mite. Those treated with both SLIT and SLIT + BCG demonstrated *in vitro* a higher production of *Dermatophagoides pteronyssinus* (Der p) 1-stimulated IFN-γ from peripheral blood mononuclear cells, which correlated with the observed improvement in clinical parameters. Meanwhile, *in vitro*

production of Der p 1-stimulated IL-12 was found to be higher in the group receiving BCG (20).

Conclusion

Despite the significant advances of the past few years, the exact immunological mechanisms by which mucosal vaccines induce tolerance against allergens in humans remain unclear. The use of immunomodulatory and antigen-directing adjuvants together with mucosal SIT routes are being intensely investigated, and fruitful results are reported. Current understanding of the immunological mechanisms underlying allergen-SIT, particularly the role of T regulatory cells in allergen-specific peripheral tolerance, may enable novel treatment strategies. Application of recent advances in understanding of this area may result in more rational and safer approaches that, in the future, could result in the prevention and cure of allergic diseases.

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