Expert Opinion

Sublingual immunotherapy of allergic diseases

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The only disease-modifying treatment that is available for allergic patients is allergen-specific immunotherapy. Two competing application forms are used: subcutaneous immunotherapy, which has been used for > 90 years, and a relatively new immunotherapy where the allergen is applied sublingually. Numerous studies have shown efficacy for subcutaneous immunotherapy and have identified possible mechanisms that are responsible for the observed reduction in allergic responses. In contrast, the efficacy of sublingual immunotherapy has not been documented to the same degree and the responsible immunological mechanisms have not yet been clearly defined. This review focuses on the published clinical and experimental data on sublingual immunotherapy and points at possible mechanisms of how sublingual immunotherapy may differ from subcutaneous immunotherapy in its mode of action, and also discusses the potential advantages and pit falls of both therapies.

Keywords: allergy, immune mechanisms, subcutaneous immunotherapy, sublingual immunotherapy

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1. Introduction

Allergic immune responses to common environmental antigens lead to clinical disorders, such as allergic asthma, hay fever, eczema and allergic rhinitis. It is clear that the development of allergies involves both genetic and environmental factors leading to the production of IL-4. Animal studies have revealed that naive T cells are activated by antigen-presenting cells (APCs) and IL-4, through IL-4 receptor/Stat6-mediated signals and the activation of specific downstream transcription factors. These events lead to the development of allergen-specific T helper 2-type $CD4^+$ T cells (T_H2) in the lymph nodes or spleen. Once generated, effector T_H2 cells migrate to the sites of allergen exposure under the guidance of specific chemokines and adhesion molecules, where they produce IL-4, -5, -9 and -13. The secretion of these cytokines induces the production of allergen-specific IgE by B cells, the development of eosinophilia, smooth muscle contraction and mucus secretion. IgE crosslinking induces the degranulation of eosinophils and mast cells, leading to the release of preformed or *de novo* synthesised inflammatory mediators, which are also critical factors in inducing the development of allergic disorders [1].

Although there is relatively much that is known about the immunological mechanisms that are involved in the development of allergic disorders, only very few effective medications are available. The most important ones are steroids in combination with leukotriene modifiers, antihistamines or long-acting β-agonists (for the treatment of asthma). However, these treatment regiments do not show any disease-modifying effects [2,3]. At present, the only well-established disease-modifying treatment that is available for allergy sufferers is the allergen-specific immunotherapy (SIT). This involves the application of increasing doses of different allergens (usually standardised extracts of the allergen) over a period of up to 3 – 5 years. Although this treatment does not improve allergic symptoms in all patients, its general effectiveness in allergic diseases is no longer questioned.

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SIT is not a novel therapy and was already being used a century ago [4]. Even though oral immunotherapy and subcutaneous immunotherapy (SCIT) were developed and used in parallel, oral immunotherapy was stopped in the early 1990s due to the controversies over its effectiveness and the often-observed gastrointestinal side effects [5]. Local bronchial administration also failed to show clear clinical efficiency [5,6] and local nasal immunotherapy is not widely used due to technical reasons [7]. Furthermore, local nasal immunotherapy showed no clinical efficacy after stopping the immunotherapy, suggesting the need for an annual pre-seasonal treatment [8]. For these reasons, SCIT has been the SIT of choice. However, SCIT has some safety and compliance issues that are associated with the injection route (see Section 2). For this reason, other application methods have been tested. In particular, the sublingual delivery of allergens (SLIT) has gained substantial interest in the last two decades. During SLIT, the allergen preparation (drop medication or a soluble tablet) is kept under the tongue for at least 2 min and is then either swallowed (SLIT-swallow) or spat out (SLIT-spit). The latter method is rarely used as it has no proven clinical efficiency. At present, SLIT is well established and accepted in Europe, making up ~ 40% of the European SIT market, whereas it is not widely used in the US or Japan.

2. Sublingual immunotherapy versus subcutaneous immunotherapy: the clinical evidence

SLIT was originally proposed as a safe alternative treatment for SCIT. Reports on deaths associated with SCIT [9,10] raised serious concerns about the safety profile of SCIT, although subsequently it was demonstrated that most life-threatening events were due to human errors [5]. However, these cases have led to strict regulations concerning the subcutaneous application of the allergen, such as observation of the patient for up to 1 h after SCIT [11]. Nevertheless, even if all of the guidelines were followed correctly, a small risk of systemic reactions remained in 3.7% of the patients, including life-threatening episodes of systemic anaphylaxis [12]. In contrast, during SLIT, > 500 million doses have been administered to humans, and, so far, no severe adverse side effects have been reported, although much higher doses per application (up to 500-fold) than in SCIT were used [13-17]. The most frequent side effects that were described were local and self-remitting mild oral or sublingual itching, whereas headache, rhinorhea, nasal obstruction or urticaria were described only sporadically. Gastrointestinal reactions seemed to increase with higher antigen doses [18]. With the knowledge of possible severe side effects, using SCIT in children below five years of age is not advisable [19]. In contrast, SLIT showed safety even in very young children of < 5 years of age [20,21]. However, further prospective studies with high-dose SLIT will have to be conducted before definitive conclusions can be made on its use in very young children. An ultra-rush regimen of SLIT induction is characterised by higher doses earlier during treatment (i.e., a short build-up phase in the range of 1 to a few hours. Even with an ultra-rush regimen, no severe adverse reactions were observed in children and adults [22-24]. As SLIT is safe in adults and children, repeatedly demonstrated in controlled trials and postmarketing surveillance studies [21,25,26], self-medication is feasible and possibly reduces costs. But is SLIT also clinically effective? And is it as effective as SCIT? Much scepticism remains over the reasons of why to replace SCIT with SLIT, particularly if the new treatment may not be as effective as the old one.

SIT is considered to be the only treatment that is able to modify the allergic immune response, altering the natural course of the disease [19,27]. SCIT leads to sustained clinical improvement even after discontinuation, prevents new sensitisations in adults and children [28-32] and prevents the development of asthma in rhinitis patients [19,33]. In patients with established allergic asthma, it reduces the symptom and medication score and improves bronchial hyper-responsiveness [34]. It is a very effective therapy for the treatment of bee- and wasp-venom anaphylaxis, and allergic rhinoconjunctivitis caused by pollen and house dust-mite allergy, as shown in numerous controlled studies [19,33]. The symptom medication score is reduced by 30 – 45% on average. SCIT is quite potent, especially for seasonal hay fever, and has been shown to reduce seasonal asthma symptoms, decrease bronchial hyper-responsiveness and improve the patients' quality of life [35]. Although SCIT was effective in patients with intermittent IgE-mediated allergic asthma, older patients with long-standing and allergen-independent asthma did not profit from this treatment [36]. Only a few studies have been conducted using allergens derived from animals or fungi [37,38].

After the first double-blind, placebo-controlled trial of SLIT had been conducted in 1986 [39], in 1998 the WHO [19] and the EAACI/ESPACI (European Academy of Allergology and Clinical Immunology/European Society of Pediatric Allergy and Clinical Immunology) position paper [40], followed by the Allergic Rhinitis and its Impact on Asthma (ARIA) document [33], as well as the meta-analysis Cochrane review [16,41], stated clinical efficacy of SLIT in allergic rhinitis and on asthma symptoms. Primary outcome measures were the symptom or medication score, and secondary measurements included serum levels of IgE and IgG antibodies, assessments of allergen sensitivity in the eye, nose or skin, as well as adverse event reports. Whereas the WHO position paper suggested SLIT as an alternative approach to SCIT in adults, the ARIA document also suggested the use of SLIT in children. In studies encompassing adults or children, efficacy of SLIT for allergic rhinitis caused by common allergens such as grass, mites, birch and *Parietaria judaica* [42-61] was reported. There are also anecdotal studies on olive [62], cypress [63] and mixed-tree extracts [64]. Moreover, in some studies, beneficial effects were observed on rhinitis and asthma symptoms, such as an increase in the days free of asthma symptoms, reduced intake of β_2-agonists and systemic

steroids [43,45,54,57]. The majority of these studies were included in a critical analysis [65] of 23 placebo-controlled, double-blind studies on SLIT with information on symptom and medication scores. In contrast to the primary literature, the author concluded that only 26% showed unequivocally clinical efficiency, and that 35% were possibly effective, defined by improvement in either medication or symptom score. The remaining studies (39%) failed to prove statistically significant efficacy. For allergic rhinitis due to perennial antigens, such as the house dust mite, it has been more difficult to prove the efficacy of SLIT; positive results for some studies were reported [43,54,55,66,67], but negative for others [48,49]. Some of these studies were also included and analysed in the Cochrane review [16,41], which failed to show statistical significance for perennial antigens for both medication and symptom scores. However, SCIT is also more effective in patients with seasonal allergies compared with patients with a perennial allergy.

Controversy remains over the use of SLIT in children. Several studies in children for seasonal rhinoconjunctivitis with or without asthma due to grass pollen were undertaken with different results: statistical reductions in i) both symptom and medication scores [44,46,59,68]; ii) medication, but not symptom scores [45,69]; or iii) neither medication nor symptom scores were observed between treated and placebo groups [51,53,57]. A non-double-blind, placebo-controlled study reported that co-seasonal SLIT reduced the development of asthma in children with grass pollen-induced allergic rhinoconjunctivitis [70]. Interestingly, a review of paediatric literature [71] revealed that SLIT improved asthmatic symptoms in children who were allergic to house dust mites, without a clear effect on rhinitis and rhinoconjunctivitis. However, as stated in a very recent review on the use of SLIT in children [72], further paediatric studies are needed to confirm that SLIT is at least equally efficient as SCIT.

From an economical point of view, SIT should maintain clinical improvement and efficiency for at least 3 years after discontinuation. An economic evaluation of 3 years of SCIT, compared with continuous symptomatic treatment, has shown that the break-even point was reached 3 – 5 years after therapy had been completed [73]. Long-lasting clinical improvement [30-32] and prevention of new sensitisations [28,29,74] has so far only been reported for SCIT. A recent study in children with severe grass pollen allergy reported an ongoing clinical benefit and prevention of new sensitisations even 12 years after cessation of SCIT, when compared with seasonal pharmacotherapy alone [75]. It is thus unclear if SLIT will reach similar efficacy levels reported for SCIT. However, a recent study provided evidence that SLIT maintains its clinical efficiency in children for 4 – 5 years after discontinuation [76], but without an appreciable effect on the prevention of new sensitisations.

As for whether SLIT is as effective as SCIT, only three studies have been published comparing SLIT directly with SCIT in terms of clinical efficiency. Only one study, conducted in patients with birch pollen allergy, was designed in a double-blind and placebo- and dummy-controlled fashion [77]. This trial showed equivalent efficacy of both treatments in terms of symptom and medication scores, reducing the disease severity significantly. Two other studies (non-randomised and placebo-controlled) reached the same conclusion [78,79]. An open trial conducted in patients with *Alternaria tenuis* allergy [80] found an even greater clinical improvement in the SLIT- versus SCIT-treated group. However, in two open studies with patients who were allergic to the house dust mite, SCIT was more effective than SLIT [66,81].

In conclusion, there is mounting evidence that SLIT is safe and effective in treating patients with allergic disorders. Nevertheless, more double-blind, placebo- and dummy-controlled studies directly comparing SLIT with SCIT have to be conducted in the future, to establish any efficacy advantage of SLIT or SCIT as the therapy for allergic disorders. Furthermore, it is also possible that any advantage may vary according to the type of allergy treated.

3. Pharmacokinetics and allergen dosing during sublingual immunotherapy

Reports on the efficacy of oral immunotherapy have been controversial, and the allergen seemed to get completely degraded in the duodenum in humans [82]. In contrast to the oral route, with the allergen being immediately swallowed, sublingual delivery leads to mucosal exposure of the allergen. Small synthetic molecules get efficiently absorbed into the blood without passing through the intestine and liver. Albumin, with a molecular weight exceeding that of most allergens, was directly absorbed within 30 min by the nasal mucosa in patients with allergic rhinitis. Interestingly, the absorbed dose was significantly higher in allergic patients, compared with control subjects [83], which is in line with the observed rapid clearance of allergen from the nasal cavity, and little local persistence in subjects with *P. judaica* allergy, as compared with healthy subjects [84]. In animal models, a significant absorption of the antigen by the sublingual and nasal mucosa was detected [85]. Notwithstanding, studies with purified and radiolabelled allergens from *P. judaica* and house dust mite Par j 1 [86] and Der p 2 [87], respectively, failed to detect direct absorption of the allergen into the blood through the oral mucosa, even when the allergen was kept for 30 min in the mouth [86]. Interestingly, the allergen was retained for a long period, from 20 – 48 h at the mucosal level in the mouth or nose [87,88]. In line with these observations, saliva appears to have limited proteolytic activity against allergens as opposed to gastrointestinal fluids [82,89,90]. Plasma radioactivity increased only after swallowing of the antigen, slowly and peaking at 2 h after swallowing of the antigen, although this was due to the presence of free radiolabel or radiolabelled peptides. Only allergoids, i.e., chemically modified allergens, seem to get absorbed as intact molecules [87]. When SLIT-swallow with SLIT-spit was compared, it became apparent that ~ 70% of the allergen was lost with spitting, although it still reached the

gastrointestinal surface by normal swallowing. These data indicate that although the allergen is not directly absorbed by the oral mucosa, contact of the allergen with the oral mucosa is essential for clinical efficiency.

As each supplier uses its own standardisation procedure, it is not possible to directly compare the amounts of allergen present in the extracts of the different manufacturers. It is also unlikely that this will change in the near future, as the production of the allergen extract is often part of the companies' intellectual property. However, there are efforts within the FDA to develop a standard quantification method to make the studies more comparable. The doses that are used in SLIT are referred to the ratio of the cumulative doses administered in SCIT. The ARIA document identified that, in SLIT, doses at least 50- to 100-fold higher than those in SCIT were required to obtain clinical efficiency [33]. A current comparative review of published studies [18] concluded that adverse events were not dose dependent, although the predefinition of the cutoff value between the high and low SLIT/SCIT ratio was questioned [91]. In agreement with another study [52], reporting an association of high antigen doses with gastrointestinal side effects, the questioned analysis indeed shows an increased occurrence of gastrointestinal symptoms at higher antigen doses. In clinical trials, antigen doses as high as a SLIT/SCIT ratio of 500 were used. Only anecdotal studies on the dose dependence of the immunological response to immunotherapy exist. High doses of allergen were superior to low doses with regard to reduced symptom score, seasonal rise of IgE and IgG4 and the production of IL-4 [92,93]. Obviously, the reported dose range in SLIT is wide: high doses seem to outperform low and medium doses [26,94]. However, this issue awaits more dose-ranging studies, preferably with standardised extracts or recombinant allergens. The question of why higher antigen amounts in SLIT are needed is yet unanswered. In SCIT, the antigen in combination with adjuvants, such as calcium phosphate or aluminium hydroxide, is directly injected into the subcutis underlying the epithelium, where professional APCs, such as Langerhans' cells, reside. In SLIT, the antigen first has to be exposed to the oral mucosa before it can be taken up by Langerhans' cells or other APCs. Bearing in mind that the oral mucosa shows limited absorption of allergens, one explanation for the observed dose dependence may be that insufficient amounts of the antigen are available for presentation by APCs, when using the sublingual route at low allergen doses. In addition, the adjuvants could play a role providing slow release of the allergen, preventing rapid degradation or dispersal of the allergen or modulating the allergic immune response in favour of a more T_H1- or T_{reg}-dominated direction.

4. The potential immunological mechanisms of sublingual immunotherapy

It is believed that the underlying immunological mechanisms of SLIT are similar, if not identical, to SCIT [95]. Although both of the treatments exert similar effects on immediate- and late-phase allergen-induced symptoms in allergic inflammation, the tissue of application is different: subcutis in SCIT and oral mucosa in SLIT. It is well documented that the organisation of the oral mucosa is quite unique in terms of APCs, the expression of adhesion molecules and in its immunostimulatory or -tolerating capacity. Dendritic cells in mucosa-associated lymphatic tissues behaved differently from their counterparts in peripheral tissues: if stimulated under identical conditions, dendritic cells from Peyer's patches produced IL-10 and not IL-12 (as did splenic dendritic cells) [96]. The skin, oral mucosa, airways, gut and peripheral blood appear to contain a certain set of dendritic cells [97,98] that differ not only in their phenotype but also in their maturation state, translating into differences in their T-cell stimulatory capacity [99-103]. It seems only plausible that dendritic cells in distinct tissue compartments are adapted to the local environment. Langerhans' cells of the oral mucosa differed profoundly from their counterparts in the subcutis, as they expressed higher levels of MHC I/II, co-stimulatory molecules, the high-affinity receptor for IgE (FcεRI) and other Fcγ receptors [104]. These cells had a higher capacity to stimulate allogenic T cells in comparison with Langerhans' cells from the skin and oral epithelium [105]. These data are supported by two studies, one conducted in humans [105] and the other in rodents [103]. Although all of these studies did not use preparations of highly purified Langerhans' cells for the stimulation of T cells, but rather epidermal and oral mucosa cell suspensions, it is clear from these data that oral mucosa and skin substantially differ in many immunological aspects. As the oral cavity is the first part of the gastrointestinal tract that is exposed to pathogenic (microbes) and non-pathogenic (food) antigens, it is possible that this environment may result in the upregulation of the Langerhans' cells maturation status. A recent comparative analysis reported on differential expression of MHC I/II and co-stimulatory molecules on dendritic cells, even from two mucosal tissues: the oral and nasal mucosa [104].

The lymph nodes draining the site of allergen exposure may also be vital for tolerance induction. It was shown that when lymph nodes from mucosal sites were removed and replaced with non-mucosal lymph nodes, mucosal tolerance was not formed [106]. This suggests that not only the mucosal tissue, but also the draining lymph nodes, provide a specialised and unique microenvironment. Therefore, the authors believe that it is oversimplified to extrapolate findings on immunological mechanisms underlying SCIT to SLIT without conducting further studies. The mechanisms of SIT have recently been reviewed elsewhere [27,95], but this paper only highlights the similarities and differences between SLIT and SCIT.

4.1 Induction of allergen-specific antibody responses

In patients who are allergic to seasonal allergens and the house dust mite and who underwent SCIT, serum IgE initially rose, but dropped off during the following months [107-112]. Due to

natural exposure to the allergen, the IgE titre of patients allergic to grass pollen normally appears to increase during the season, which was blunted by SCIT [110,112]. As well as this, SCIT could induce allergen-specific IgG responses (mostly IgG1 and IgG4), and, in a few cases, also IgA [113-117]. Sublingual delivery of the allergen also induced a consistent increase of allergen-specific IgG4 [16,43,45,52]. There are two reports that indicate that SLIT affects allergen-specific IgA: high levels of local mucosal IgA in the lungs and nose after SLIT was demonstrated in mice [118]. In patients undergoing SLIT, specific IgA levels, which were initially decreased compared with healthy individuals, no longer differed between the two groups [119]. In both SCIT and SLIT, clinical efficacy of the treatment often, but not always, correlated with a decrease of the IgE/IgG4 ratio [16,45,53,54,61]. IgG4 is considered to be an antibody with low inflammatory potential (i.e., it does not activate the complement system). The induction of allergen-specific IgG or IgA isotypes may contribute to the clinical improvement of allergic symptoms by competing with IgE for binding to allergen, thereby preventing degranulation of effector cells of the immediate reaction, the basophils and mast cells [111,112], or preventing antigen uptake by APCs bearing suitable Fc receptors and subsequent presentation to T cells [111,112,120,121]. Moreover, binding of allergen/IgG complexes by FcγRII-expressing cells, such as B cells, mast cells or basophils, may transduce a negative signal through the receptor's immune receptor tyrosine-based inhibitory motif, preventing activation and release of effector molecules when co-aggregated with stimulatory Fc receptors such as FcεRI [115,116]. As discussed above, clinical improvement did not always correlate with an increase in blocking antibody isotypes. A change in the IgE/IgG ratio may just be a marker of high allergen exposure, shifting the immune response from T_H2 to T_H1, and translating into a dominating IgG response and reduced influx of eosinophils (Section 4.2). Interestingly, it was suggested that the tolerance status is related to distinct IgG specificities rather than to isotypes [122-124]. In just a few hours after onset of SCIT for wasp venom, the set of epitopes recognised by IgG had changed and was linked to early clinical tolerance [125]. Although the exact mechanism of this observation is unknown, it may result from the specific depletion of high-avidity antibodies by large allergen doses.

4.2. Recruitment of pro-inflammatory cells

SCIT reduces the recruitment of pro-inflammatory cells, such as eosinophils, basophils and mast cells, into the skin and mucosal tissue of the nose, eyes and lungs [126-132]. Clinical efficacy could be correlated with a decrease in eotaxin levels. Likewise, in SLIT, infiltration of the site of allergen exposure by eosinophils [56,133-136] and neutrophils [56] was reduced in conjunction with reduced expression of the adhesion molecule ICAM-1, and correlated with reduced medication and symptom scores [56]. Reduced or unchanged levels of eosinophil cationic protein or tryptase after allergen challenge suggested that activation of eosinophils and mast cells was impaired [133,134]. These data indicate that there are no major qualitative differences in terms of reducing the recruitment and activation of pro-inflammatory cells after SCIT or SLIT. The exact mechanism of how SCIT/SLIT leads to the inhibition of the recruitment and activation of inflammatory cells remains unclear, but it may be due to the overall reduction in allergen-specific T_H2 responses.

4.3 Immune deviation from T_H2 to T_H1 and/or T-regulatory responses

T cells control the allergic response by producing cytokines that induce the allergic inflammation. Patients who have allergies usually mount a strong allergen-specific T_H2-response and T_H2 cells are found in mucosal surfaces during late allergen responses. T_H2 cells produce mainly IL-4, -5, -9 and -13, but not IFN-γ, in contrast to T_H1 cells. Due to their central role in the allergic inflammation, a pivotal point of immunotherapy may be the deviation of the T-cell response from an allergic (T_H2-) to a non-allergic (T_H1)-response. The induction of T_H1 cells would further protect from T_H2 cell development by the enhanced secretion of IFN-γ by T_H1 cells. Interestingly, the antigen dose also seems to influence the T_H1/ T_H2 balance. When low antigen amounts were used, the induction of T_H1 responses were enhanced. In contrast, high antigen doses were associated with a T_H2 bias [137]. However, *in vitro* experiments with house dust mite showed that high antigen concentrations enriched the proportion of T_H1 cells with effector phenotype [138].

A further mechanism of how SLIT may protect from allergy is by inducing peripheral tolerance to allergens. Tolerance can be established by clonal or functional deletion of allergen-specific T_H2 cells. Functional deletion (i.e., T-cell anergy) affects proliferation or cytokine production to different extents depending on the tolerating conditions. SCIT can have an impact on the T_H2/T_H1 ratio by downregulating allergen-specific T_H2 responses, both in terms of proliferation and cytokine production [139-152]. This downregulation may be only observed in the maintenance phase, as the T_H2 response can initially rise during the build-up period [139,146]. In some studies, the T_H2/T_H1 balance seemed to be shifted by a systemic decrease in IL-4, rather than an increase in IFN-γ production [140,141,150,151], whereas in others, increased IFN-γ production was observed [145,152,153]. Studies examining changes in the local response obtained comparable results (i.e., decreased infiltration to the site of allergen challenge with $CD4^+$ T cells and eosinophils [140], and an increase in IFN-γ [140,145,154] and IL-12 [145]). A shift in the T_H2/T_H1 balance correlated with clinical improvement, allergen-specific IgE levels and late-phase skin reactions [139,153]. In contrast to SCIT, the question remains as to whether SLIT influences or changes T-cell responses in allergic patients. Studies on house dust-mite and bee-venom allergies reported reduced proliferation [133,155-157], increased IL-10 production [155,156] by peripheral blood mononuclear cells or decreased serum levels of IL-13 [158] in patients who were treated with SLIT. Others did not detect significant changes in terms of cytokine production (IL-4, -5, -12, IFN-γ) and the T_H1/T_H2 balance [53,133,159], despite positive effects on medication or symptom scores.

As well as clonal and functional deletion of allergen-specific T cells, the induction of regulatory T (T_{reg}) cells represent a different means of suppressing inflammatory responses. T_{reg} cells can generally be subdivided into different subsets: naturally occurring $CD4^+CD25^+$ T cells and adaptive T_{reg} cells. The latter are further subdivided into T_H3 cells, which can be induced following oral administration of the antigen, and $T_{reg}1$ cells that can be induced in the presence of IL-10. Other subtypes are also defined and described, but in the authors' opinion, the available data are not clear if these cells are a novel T_{reg} type or a subtype of the already well-defined T_{reg} populations. Natural and adaptive regulatory T cells are antigen specific, but regulate immune responses in a non-specific manner, by cell–cell contact or by the production of IL-10 or TGF-β. T_{reg} cells can exert their suppressive functions at multiple levels: i) cytokine secretion; ii) chemokine receptor expression; iii) inhibition of cytolytic function; or iv) induction of granzyme A/perforin-dependent apoptosis in activated $CD4^+$ and $CD8^+$ T cells. The list of the cell types that are killed by $CD4^+CD25^+$ T_{reg} cells can now be complemented by B cells, as very recently shown [160]. For SCIT, several groups provided firm evidence for a role of T_{reg} cells in the control of the allergic response by immunotherapy. In house dust-mite and birch pollen allergies with whole allergen, the number of IL-10- or TGF-β-producing $CD4^+$ T cells increased. These T cells were predominantly confined to the $CD4^+CD25^+$ population [138,161,162] and associated with decreased proliferation or cytokine production of both T_H1 and T_H2 cells [161,162]. In contrast, evidence for T_{reg} cells in SLIT is missing, with the exception of one report showing increased IL-10 production by peripheral blood mononuclear cells [156]. IL-10 and TGF-β both suppressed T-cell activity [163] and affected isotype switching, thereby skewing the allergen-specific antibody isotype from IgE to an IgG4- or IgA-dominated response. In turn, signalling via the FcγRIIB-receptor (e.g., binding of IgG4-allergen complexes) seemed to be required for the induction of T_{reg} cells and mucosal tolerance [164]. IL-10 also blocked CD28 co-stimulation [165], which was important for cytokine secretion by T_H2 cells, namely IL-5 and IL-13 [166-168].

The influence of the antigen amount on the development and function of T_{reg} cells is unclear. There is evidence from TCR-transgenic mouse models that extrathymic $CD4^+CD25^+$ T_{reg} cells can be generated *de novo* from $CD4^+CD25^-$ T cells in response to agonist ligands [169,170]. This process was most efficient at low antigen doses and dependent on TGF-β and subimmunogenic conditions. Interestingly, in mice, induction of TGF-β and IL-10 was highest when oral tolerance was induced by administering low doses of antigen [171]. This suggests that SLIT, due to the low exposure level with antigen, may favour the development of T_{reg} cells.

5. Conclusion

SIT is the only means to modify the natural course of the disease and to maintain efficiency for several years after cessation. SCIT can prevent insect-venom anaphylaxis and allergic rhinoconjunctivitis to seasonal and perennial allergens. It can also prevent the progression to asthma in rhinitis patients, as well as development of new sensitisations, and shows long-term efficacy. In contrast, SLIT is mostly used as an alternative treatment for allergic rhinitis, and not for perennial antigens and allergic asthma to inhalant allergen. Only limited information exists regarding the long-term efficacy of SLIT, and it is not firmly established whether it prevents new desensitisations. Due to insufficient data, SLIT should not yet be routinely used in small children. Furthermore, it is unclear if SCIT and SLIT are equally efficient. Only few studies exist with regard to allergen dosing and clinical outcome after SLIT [26,94]. The underlying immunological mechanisms in both treatments show similarities, as well as dissimilarities. SCIT and SLIT can both modify the allergen-specific antibody response and reduce the recruitment of pro-inflammatory innate immune cells. It was shown that SCIT can shift the immune response towards a T_H1-dominated response and induce T_{reg} cells. It is not clear if this is also the case for SLIT. However, the authors believe that it is very likely that SLIT also mediates some of the observed suppressive effects on T_H2 cells by inducing T_H1 and/or T_{reg} responses. Figure 1 shows the potential mechanisms of how SLIT may suppress the development of allergen-specific T_H2 responses.

6. Expert opinion

It appears that SCIT is more effective than SLIT, but SLIT is safer than SCIT. In order to enhance the efficacy of SLIT it may be promising to combine the allergen with adjuvants. So far, studies addressing this question have only been published for SCIT [172,173]. Attempts to improve clinical efficiency of SLIT by using Bacillus Calmette-Guérin in combination with house dust-mite allergen failed [174]. Immunstimulatory sequences, monophosphoryl lipid A or triterpenoid glycosides from *Quillaja saponaria* may not only be potent adjuvants for the generation of T_H1 responses but may also enhance antigen uptake [175], and may be used in combination with allergen during SLIT (provided that they are safe and allergen specific). For example, 1,25-dihydroxyvitamin D3 together with dexamethasone or *Lactobacillus plantarum* may prove useful as immunmodulators in shifting the T-cell response towards a more regulatory path [176]. Once recombinant allergens become more available, they may also improve the efficacy of SLIT, as the currently used allergen extracts may contain unwanted substances or not sufficient amounts of the relevant allergen [176-179]. However, it is not yet proven that recombinant allergens will lead to higher efficacy, as the additional components included in the extract may actually function as adjuvants. Furthermore, for some types of allergies (e.g., ragweed or timothy grass) there is more than one dominant allergen that is responsible for the induction of allergic responses. To treat patients with

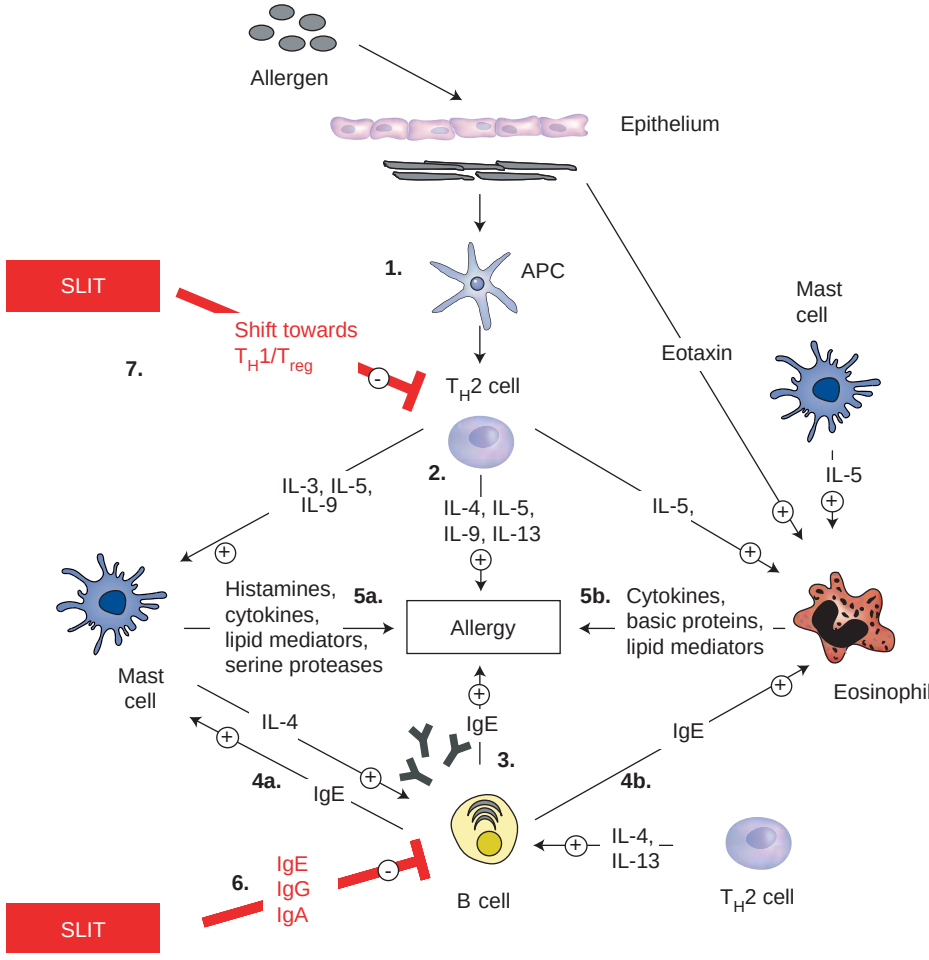

Figure 1. Potential mechanisms of SLIT-reducing allergic responses. Allergen that crosses the epithelial barrier is taken up and presented by APCs to T cells, which differentiate into T_H2 cells (1). The T_H2 cytokines (2), IL-4 and IL-13 affect isotype-switching of allergen-specific B cells towards an IgE-dominated response (3). IL-5 induces the development of eosinophils in the bone marrow and recruits eosinophils to the site of allergic inflammation, whereas IL-9 enhances proliferation and differentiation of mast cells. Recruitment of eosinophils is further mediated by the epithelium, which produces eotaxin (CCL11). IL-4 and IL-5 prime mast cells to release their mediators on activation (e.g., by allergen-mediated crosslinking of surface-bound IgE [4a, b]). Mediators released by eosinophils and mast cells increase vascular permeability, airway hyper-reactivity and mucus production (5a, b). Airway hyper-reactivity and mucus production is also directly enhanced by IL-13. SLIT decreases IgE production and increases allergen-specific IgG and possibly IgA levels (6). IgG and IgA compete with IgE for allergen binding and thereby inhibit the degranulation of mast cells and eosinophils and antigen uptake by APCs bearing suitable Fc receptors and its subsequent presentation to T cells. Binding of allergen/IgG complexes by FcγRII-expressing cells, such as B cells and mast cells, transduce a negative, inhibitory signal preventing degranulation. Furthermore, SLIT may shift the T-cell response from T_H2 towards a more T_H1 type leading to a general reduction in allergic responses (7). It is possible that T_{reg} cells are also induced during SLIT, inhibiting allergic responses by producing TGF-β and/or IL-10, or by directly inhibiting the activation and proliferation of allergen-specific T_H2 cells through a cell–cell contact-mediated mechanism.

APC: Antigen-presenting cell; Ig: Immunoglobulin; IL: Interleukin; SLIT: Sublingual delivery of allergens; TGF: Transforming growth factor; T_H1: T helper cell type 1; T_H2: T helper cell type 2; T_{reg}: Regulatory T cell.

these types of allergies, a combination of all of the major allergens may be needed. In order to achieve the best result, numerous studies may have to be performed, titrating the amount of one recombinant allergen against the others. This is a task that may be even more difficult when patients with multiple sensitisations are treated. A further possibility to improve SLIT is to use recombinant hypoallergenic allergen derivatives [177-179]. They may maintain their immunogenicity while displaying reduced allergenic activity, leading to decreased side effects and increased efficacy.

Although there are options to possibly increase the efficacy of SLIT, it is most important to identify the underlying mechanisms in order to know in which direction to optimise the therapy. Nevertheless, the major question remains. Why

favour SLIT over SCIT if SLIT is not as effective and the side effects of SCIT seem to be tolerable (when performed under medical supervision)? More studies are needed to directly compare the efficacy of SLIT versus SCIT to reach a conclusion as to which one is better and for which indication. Therefore, quantification of allergen preparations (in order to directly compare the amount of the major allergens present in different extracts) and dose-finding studies need to be performed and used side by side in clinical studies, directly comparing SCIT with SLIT. In this context, recombinant allergens clearly have an advantage over extracts. Even though SLIT may never reach the same level of effectiveness as SCIT, it may have the advantage of being used in very young children, as preschool age is considered to be the limit for SCIT, due to the possible side effects. Furthermore, compliance may also be higher in this group due to the painless administration of sublingual vaccines. These may be very important points, as SIT appears to be more effective in younger children [180]. Taken together, it is clear that SIT is effective and that there are currently two competing applications: SCIT, with proven efficacy but possible severe side effects (anaphylaxis) and the need to be performed under medical supervision; and SLIT, also with proven efficacy (albeit lower than SCIT), few side effects and the possibility of self medication. In this regard, SLIT may substitute for SCIT in the long run, under the condition that the efficacy can be improved without increasing the side effect profile. It may also be possible that SLIT may be more suited than SCIT for the treatment of a certain atopic phenotype and *vice versa*.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. HAWRYLOWICZ CM, O'GARRA A: Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat. Rev. Immunol.* (2005) 5:271-283.
2. BARNES PJ: New drugs for asthma. *Nat. Rev. Drug Discov.* (2004) 3:831-844.
3. BRYAN SA, LECKIE MJ, HANSEL TT, BARNES PJ: Novel therapy for asthma. *Expert. Opin. Investig. Drugs* (2000) 9(1):25-42.
4. NOON L: Prophylactic inoculation against hay fever. *Lancet* (1911) 1:1572-1573.
5. CANONICA GW, PASSALACQUA G: Noninjection routes for immunotherapy. *J. Allergy Clin. Immunol.* (2003) 111:437-448.
6. CRIMI E, VOLTOLINI S, TROISE C *et al.*: Local immunotherapy with *Dermatophagoides* extract in asthma. *J. Allergy Clin. Immunol.* (1991) 87:721-728.
7. BJORKSTEN B: Local immunotherapy is not documented for clinical use. *Allergy* (1994) 49:299-301.
8. PASSALACQUA G, ALBANO M, RUFFONI S *et al.*: Nasal immunotherapy to *Parietaria*: evidence of reduction of local allergic inflammation. *Am. J. Respir. Crit. Care Med.* (1995) 152:461-466.
9. DUBUSKE LM: Appropriate and inappropriate use of immunotherapy. *Ann. Allergy Asthma Immunol.* (2001) 87:56-67.
10. FINEGOLD I: Analyzing meta-analyses of specific immunotherapy in the treatment of asthma. *Ann. Allergy Asthma Immunol.* (2001) 87:33-37.
11. WILLIAMS AP, KRISHNA MT, FREW AJ: The safety of immunotherapy. *Clin. Exp. Allergy* (2004) 34:513-514.
12. MORENO C, CUESTA-HERRANZ J, FERNANDEZ-TAVORA L, ALVAREZ-CUESTA E: Immunotherapy safety: a prospective multi-centric monitoring study of biologically standardized therapeutic vaccines for allergic diseases. *Clin. Exp. Allergy* (2004) 34:527-531.
13. AGOSTINIS F, TELLARINI L, CANONICA GW, FALAGIANI P, PASSALACQUA G: Safety of sublingual immunotherapy with a monomeric allergoid in very young children. *Allergy* (2005) 60:133.
14. DAHL R, STENDER A, RAK S: Specific immunotherapy with SQ standardized grass allergen tablets in asthmatics with rhinoconjunctivitis. *Allergy* (2006) 61:185-190.
15. FREW AJ, SMITH HE: Sublingual immunotherapy. *J. Allergy Clin. Immunol.* (2001) 107:441-444.
16. WILSON DR, LIMA MT, DURHAM SR: Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* (2005) 60:4-12.
•• Comprehensive analysis of published clinical trials for SLIT in allergic rhinitis.
17. GROSCLAUDE M, BOUILLOT P, ALT R *et al.*: Safety of various dosage regimens during induction of sublingual immunotherapy. A preliminary study. *Int. Arch. Allergy Immunol.* (2002) 129:248-253.
18. GIDARO GB, MARCUCCI F, SENSI L, INCORVAIA C, FRATI F, CIPRANDI G: The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin. Exp. Allergy* (2005) 35:565-571.
19. BOUSQUET J, LOCKEY R, MALLING HJ: Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J. Allergy Clin. Immunol.* (1998) 102:558-562.
20. FIOCCHI A, PAJNO G, LA GRUTTA S *et al.*: Safety of sublingual-swallow immunotherapy in children aged 3 to 7 years. *Ann. Allergy Asthma Immunol.* (2005) 95:254-258.
21. RIENZO VD, MINELLI M, MUSARRA A *et al.*: Post-marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. *Clin. Exp. Allergy* (2005) 35:560-564.
22. GAMMERI E, ARENA A, D'ANNEO R, LA GRUTTA S: Safety and tolerability of ultra-Rush (20 minutes) sublingual immunotherapy in patients with allergic rhinitis and/or asthma. *Allergol. Immunopathol.* (2005) 33:142-144.
23. ROSSI RE, MONASTEROLO G: A pilot study of feasibility of ultra-rush (20-25 minutes) sublingual-swallow immunotherapy in 679 patients (699 sessions) with allergic rhinitis and/or asthma. *Int. J. Immunopathol. Pharmacol.* (2005) 18:277-285.

24. TRIPODI S, DI RIENZO BA, BENINCORI N, SCALA G, PINGITORE G: Safety and tolerability of ultra-rush induction, less than one hour, of sublingual immunotherapy in children. *Int. Arch. Allergy Immunol.* (2006) 139:149-152.

25. PASSALACQUA G, GUERRA L, PASQUALI M, LOMBARDI C, CANONICA GW: Efficacy and safety of sublingual immunotherapy. *Ann. Allergy Asthma Immunol.* (2004) 93:3-12.

26. ANDRE C, VATRINET C, GALVAIN S, CARAT F, SICARD H: Safety of sublingual-swallow immunotherapy in children and adults. *Int. Arch. Allergy Immunol.* (2000) 121:229-234.

27. TILL SJ, FRANCIS JN, NOURI-ARIA K, DURHAM SR: Mechanisms of immunotherapy. *J. Allergy Clin. Immunol.* (2004) 113:1025-1034.

28. PAJNO GB, BARBERIO G, DE LUCA F, MORABITO L, PARMIANI S: Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin. Exp. Allergy* (2001) 31:1392-1397.

29. DES ROCHES A, PARADIS L, MENARDO JL, BOUGES S, DAURES JP, BOUSQUET J: Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J. Allergy Clin. Immunol.* (1997) 99:450-453.

30. HEDLIN G, HEILBORN H, LILJA G *et al.*: Long-term follow-up of patients treated with a three-year course of cat or dog immunotherapy. *J. Allergy Clin. Immunol.* (1995) 96:879-885.

31. ARIANO R, KROON AM, AUGERI G, CANONICA GW, PASSALACQUA G: Long-term treatment with allergoid immunotherapy with *Parietaria*. Clinical and immunologic effects in a randomized, controlled trial. *Allergy* (1999) 54:313-319.

32. DURHAM SR, WALKER SM, VARGA EM *et al.*: Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.* (1999) 341:468-475.

33. BOUSQUET J, VAN CAUWENBERGE P, KHALTAEV N: Allergic rhinitis and its impact on asthma. *J. Allergy Clin. Immunol.* (2001) 108:S147-S334.

34. ABRAMSON MJ, PUY RM, WEINER JM: Allergen immunotherapy for asthma. *Cochrane Database Syst. Rev.* (2003):CD001186.

35. VARNEY VA, GAGA M, FREW AJ, ABER VR, KAY AB, DURHAM SR: Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. *Br. Med. J.* (1991) 302:265-269.

36. BOUSQUET J: Specific immunotherapy in asthma. *Allergy* (1999) 54(Suppl. 56):37-38.

37. BUCUR J, DREBORG S, EINARSSON R, LJUNGSTEDT-PAHLMAN I, NILSSON JE, PERSSON G: Immunotherapy with dog and cat allergen preparations in dog-sensitive and cat-sensitive asthmatics. *Ann. Allergy* (1989) 62:355-361.

38. HORST M, HEJJAOUI A, HORST V, MICHEL FB, BOUSQUET J: Double-blind, placebo-controlled rush immunotherapy with a standardized *Alternaria* extract. *J. Allergy Clin. Immunol.* (1990) 85:460-472.

39. SCADDING GK, BROSTOFF J: Low dose sublingual therapy in patients with allergic rhinitis due to house dust mite. *Clin. Allergy* (1986) 16:483-491.

40. MALLING HJ, ABREU-NOGUEIRA J, ALVAREZ-CUESTA E *et al.*: Local immunotherapy. *Allergy* (1998) 53:933-944.

41. WILSON DR, TORRES LI, DURHAM SR: Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst. Rev.* (2003):CD002893.

42. BAHCECILER NN, ISIK U, BARLAN IB, BASARAN MM: Efficacy of sublingual immunotherapy in children with asthma and rhinitis: a double-blind, placebo-controlled study. *Pediatr. Pulmonol.* (2001) 32:49-55.

43. BOUSQUET J, SCHEINMANN P, GUINNEPAIN MT *et al.*: Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy* (1999) 54:249-260.

44. CAFFARELLI C, SENSI LG, MARCUCCI F, CAVAGNI G: Preseasonal local allergoid immunotherapy to grass pollen in children: a double-blind, placebo-controlled, randomized trial. *Allergy* (2000) 55:1142-1147.

45. CLAVEL R, BOUSQUET J, ANDRE C: Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. *Allergy* (1998) 53:493-498.

46. FELIZIANI V, LATTUADA G, PARMIANI S, DALL'AGLIO PP: Safety and efficacy of sublingual rush immunotherapy with grass allergen extracts. A double blind study. *Allergol. Immunopathol.* (1995) 23:224-230.

47. GIOVANE AL, BARDARE M, PASSALACQUA G *et al.*: A three-year double-blind placebo-controlled study with specific oral immunotherapy to *Dermatophagoides*: evidence of safety and efficacy in paediatric patients. *Clin. Exp. Allergy* (1994) 24:53-59.

48. GUEZ S, VATRINET C, FADEL R, ANDRE C: House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis: a double-blind, placebo-controlled study. *Allergy* (2000) 55:369-375.

49. HIRSCH T, SAHN M, LEUPOLD W: Double-blind placebo-controlled study of sublingual immunotherapy with house dust mite extract (D.pt.) in children. *Pediatr. Allergy Immunol.* (1997) 8:21-27.

50. HORAK F, STUBNER P, BERGER UE, MARKS B, TOTH J, JAGER S: Immunotherapy with sublingual birch pollen extract. A short-term double-blind placebo study. *J. Investig. Allergol. Clin. Immunol.* (1998) 8:165-171.

51. HORDIJK GJ, ANTVELINK JB, LUWEMA RA: Sublingual immunotherapy with a standardised grass pollen extract; a double-blind placebo-controlled study. *Allergol. Immunopathol.* (1998) 26:234-240.

52. LA ROSA M, RANNO C, ANDRE C, CARAT F, TOSCA MA, CANONICA GW: Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J. Allergy Clin. Immunol.* (1999) 104:425-432.

53. LIMA MT, WILSON D, PITKIN L *et al.*: Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. *Clin. Exp. Allergy* (2002) 32:507-514.

54. PAJNO GB, MORABITO L, BARBERIO G, PARMIANI S: Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. *Allergy* (2000) 55:842-849.

55. PASSALACQUA G, ALBANO M, FREGONESE L *et al.*: Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* (1998) 351:629-632.

56. PASSALACQUA G, ALBANO M, RICCIO A *et al.*: Clinical and immunologic effects of a rush sublingual immunotherapy to *Parietaria* species: a double-blind, placebo-controlled trial. *J. Allergy Clin. Immunol.* (1999) 104:964-968.

57. PRADALIER A, BASSET D, CLAUDEL A *et al.*: Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. *Allergy* (1999) 54:819-828.

58. PURELLO-D'AMBROSIO F, GANGEMI S, ISOLA S *et al.*: Sublingual immunotherapy: a double-blind, placebo-controlled trial with *Parietaria judaica* extract standardized in mass units in patients with rhinoconjunctivitis, asthma, or both. *Allergy* (1999) 54:968-973.

59. SABBAH A, HASSOUN S, LE SELLIN J, ANDRE C, SICARD H: A double-blind, placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. *Allergy* (1994) 49:309-313.

60. TARI MG, MANCINO M, MONTI G: Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double-blind study. *Allergol. Immunopathol.* (1990) 18:277-284.

61. TROISE C, VOLTOLINI S, CANESSA A, PECORA S, NEGRINI AC: Sublingual immunotherapy in *Parietaria* pollen-induced rhinitis: a double-blind study. *J. Investig. Allergol. Clin. Immunol.* (1995) 5:25-30.

62. VOURDAS D, SYRIGOU E, POTAMIANOU P *et al.*: Double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. *Allergy* (1998) 53:662-672.

63. ARIANO R, PANZANI RC, MISTRELLO G: Efficacy of sublingual coseasonal immunotherapy with a monomeric allergoid in Cupressaceae pollen allergy-preliminary data. *Allerg. Immunol.* (2005) 37:103-108.

64. VOLTOLINI S, MODENA P, MINALE P *et al.*: Sublingual immunotherapy in tree pollen allergy. Double-blind, placebo-controlled study with a biologically standardised extract of three pollens (alder, birch and hazel) administered by a rush schedule. *Allergol. Immunopathol.* (2001) 29:103-110.

65. MALLING HJ: Is sublingual immunotherapy clinically effective? *Curr. Opin. Allergy Clin. Immunol.* (2002) 2:523-531.

66. MUNGAN D, MISIRLIGIL Z, GURBUZ L: Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma-a placebo controlled study. *Ann. Allergy Asthma Immunol.* (1999) 82:485-490.

67. TONNEL AB, SCHERPEREEL A, DOUAY B *et al.*: Allergic rhinitis due to house dust mites: evaluation of the efficacy of specific sublingual immunotherapy. *Allergy* (2004) 59:491-497.

68. MARCUCCI F, SENSI LG, CAFFARELLI C *et al.*: Low-dose local nasal immunotherapy in children with perennial allergic rhinitis due to *Dermatophagoides*. *Allergy* (2002) 57:23-28.

69. ROLINCK-WERNINGHAUS C, WOLF H, LIEBKE C *et al.*: A prospective, randomized, double-blind, placebo-controlled multi-centre study on the efficacy and safety of sublingual immunotherapy (SLIT) in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Allergy* (2004) 59:1285-1293.

70. NOVEMBRE E, GALLI E, LANDI F *et al.*: Coseasonal sublingual immunotherapy reduces the development of asthma in children with allergic rhinoconjunctivitis. *J. Allergy Clin. Immunol.* (2004) 114:851-857.

71. SOPO SM, MACCHIAIOLO M, ZORZI G, TRIPODI S: Sublingual immunotherapy in asthma and rhinoconjunctivitis; systematic review of paediatric literature. *Arch. Dis. Child* (2004) 89:620-624.

72. PHAM-THI N, DE BLIC J, SCHEINMANN P: Sublingual immunotherapy in the treatment of children. *Allergy* (2006) 61(Suppl. 81):7-10.

73. SCHADLICH PK, BRECHT JG: Economic evaluation of specific immunotherapy versus symptomatic treatment of allergic rhinitis in Germany. *Pharmacoeconomics* (2000) 17:37-52.

74. MOLLER C, DREBORG S, FERDOUSI HA *et al.*: Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J. Allergy Clin. Immunol.* (2002) 109:251-256.

75. ENG PA, BORER-REINHOLD M, HEIJNEN IA, GNEHM HP: Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. *Allergy* (2006) 61:198-201.

76. DI RIENZO V, MARCUCCI F, PUCCINELLI P *et al.*: Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study. *Clin. Exp. Allergy* (2003) 33:206-210.

•• This study shows that SLIT maintains its clinical efficiency in children for 4 – 5 years after discontinuation.

77. KHINCHI MS, OULSEN LK, CARAT F, ANDRE C, HANSEN AB, MALLING HJ: Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* (2004) 59:45-53.

•• This study directly compares SLIT with SCIT and demonstrates that no significant differences were observed between both routes with regard to clinical effectiveness.

78. QUIRINO T, IEMOLI E, SICILIANI E, PARMIANI S, MILAZZO F: Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study. *Clin. Exp. Allergy* (1996) 26:1253-1261.

79. MALLING HJ: Comparison of the clinical efficacy and safety of subcutaneous and sublingual immunotherapy: methodological approaches and experimental results. *Curr. Opin. Allergy Clin. Immunol.* (2004) 4:539-542.

80. BERNARDIS P, AGNOLETTO M, PUCCINELLI P, PARMIANI S, POZZAN M: Injective versus sublingual immunotherapy in *Alternaria tenuis* allergic patients. *J. Investig. Allergol. Clin. Immunol.* (1996) 6:55-62.

81. PIAZZA I, BIZZARO N: Humoral response to subcutaneous, oral, and nasal immunotherapy for allergic rhinitis due to *Dermatophagoides pteronyssinus*. *Ann. Allergy* (1993) 71:461-469.

82. EINARSSON R, RENCK B, TAUDORF E: *In vitro* studies of degradation of birch and timothy pollen allergen preparations by human duodenal juice. *Allergy* (1988) 43:469-472.

83. BUCKLE FG, COHEN AB: Nasal mucosal hyperpermeability to macromolecules in atopic rhinitis and extrinsic asthma. *J. Allergy Clin. Immunol.* (1975) 55:213-221.

84. PASSALACQUA G, ALTRINETTI V, MARIANI G *et al.*: Pharmacokinetics of radiolabelled Par j 1 administered intranasally to allergic and healthy subjects. *Clin. Exp. Allergy* (2005) 35:880-883.

85. MISTRELLO G, RAPISARDA G, FALAGIANI P: Detection of IgE-binding activity in serum after intranasal treatment of normal rabbits with *P. judaica* extract. *Allergy* (1991) 46:52-58.

86. BAGNASCO M, PASSALACQUA G, VILLA G *et al.*: Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin. Exp. Allergy* (2001) 31:54-60.

87. BAGNASCO M, ALTRINETTI V, PESCE G *et al.*: Pharmacokinetics of Der p 2 allergen and derived monomeric allergoid in allergic volunteers. *Int. Arch. Allergy Immunol.* (2005) 138:197-202.

88. 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) 100:122-129.

89. WALKER GF, LANGOTH N, BERNKOP-SCHNURCH A: Peptidase activity on the surface of the porcine buccal mucosa. *Int. J. Pharm.* (2002) 233:141-147.

90. IGEA JM, CUEVAS M, LAZARO M, QUIRCE S, CUESTA J: Susceptibility of a grass-pollen oral immunotherapy extract to the saliva and gastric fluid digestive process. *Allergol. Immunopathol.* (1994) 22:55-59.

91. BURASTERO S: Regarding Gidaro GB, Marcucci F, Sensi L, Incorvaia C, Frati F, Ciprandi G. The safety of sublingual-swallow immunotherapy: an analysis of published studies. Clin Exp Allergy 2005; 35:565-71. *Clin. Exp. Allergy* (2005) 35:1407-1408.

92. EWBANK PA, MURRAY J, SANDERS K, CURRAN-EVERETT D, DRESKIN S, NELSON HS: A double-blind, placebo-controlled immunotherapy dose-response study with standardized cat extract. *J. Allergy Clin. Immunol.* (2003) 111:155-161.

93. WERFEL T, BREUER K, RUEFF F *et al.*: Usefulness of specific immunotherapy in patients with atopic dermatitis and allergic sensitization to house dust mites: a multi-centre, randomized, dose-response study. *Allergy* (2006) 61:202-205.

94. DIDIER A, MELAC M, COMBEBIAS A, ANDRE C: Efficacy and safety of sublingual immunotherapy [SLIT] tablets in grass pollen rhinoconjunctivitis [abstract]. *J. Allergy Clin. Immunol.* (2006) 117:721.

95. MOINGEON P, BATARD T, FADEL R, FRATI F, SIEBER J, OVERTVELT L: Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* (2006) 61:151-165.

96. WILLIAMSON E, O'MALLEY JM, VINEY JL: Visualizing the T-cell response elicited by oral administration of soluble protein antigen. *Immunology* (1999) 97:565-572.

97. ALLAM JP, NIEDERHAGEN B, BUCHELER M *et al.*: Comparative analysis of nasal and oral mucosa dendritic cells. *Allergy* (2006) 61:166-172.

98. ANJUERE F, MARTIN P, FERRERO I *et al.*: Definition of dendritic cell subpopulations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse. *Blood* (1999) 93:590-598.

99. HEUFLER C, KOCH F, SCHULER G: Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J. Exp. Med.* (1988) 167:700-705.

100. HOLT PG, OLIVER J, BILYK N *et al.*: Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells *in vivo* by resident alveolar macrophages. *J. Exp. Med.* (1993) 177:397-407.

101. LIU LM, MACPHERSON GG: Antigen processing: cultured lymph-borne dendritic cells can process and present native protein antigens. *Immunology* (1995) 84:241-246.

102. O'DOHERTY U, STEINMAN RM, PENG M *et al.*: Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte-conditioned medium. *J. Exp. Med.* (1993) 178:1067-1076.

103. HASSEUS B, JONTELL M, BERGENHOLTZ G, EKLUND C, DAHLGREN UI: Langerhans cells from oral epithelium are more effective in stimulating allogeneic T-cells *in vitro* than Langerhans cells from skin epithelium. *J. Dent. Res.* (1999) 78:751-758.

104. ALLAM JP, NOVAK N, FUCHS C *et al.*: Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcepsilonRI expression. *J. Allergy Clin. Immunol.* (2003) 112:141-148.

105. HASSEUS B, JONTELL M, BERGENHOLTZ G, DAHLGREN UI: Langerhans cells from human oral epithelium are more effective at stimulating allogeneic T cells *in vitro* than Langerhans cells from skin. *Clin. Exp. Immunol.* (2004) 136:483-489.

106. WOLVERS DA, COENEN-DE ROO CJ, MEBIUS RE *et al.*: Intranasally induced immunological tolerance is determined by characteristics of the draining lymph nodes: studies with OVA and human cartilage gp-39. *J. Immunol.* (1999) 162:1994-1998.

107. LICHTENSTEIN LM, ISHIZAKA K, NORMAN PS, SOBOTKA AK, HILL BM: IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest* (1973) 52:472-482.

108. PIERSON-MULLANY LK, JACKOLA D, BLUMENTHAL M, ROSENBERG A: Altered allergen binding capacities of Amb a 1-specific IgE and IgG4 from ragweed-sensitive patients receiving immunotherapy. *Ann. Allergy Asthma Immunol.* (2000) 84:241-243.

109. DJURUP R, MALLING HJ: High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens. *Clin. Allergy* (1987) 17:459-468.

110. HAKANSSON L, HEINRICH C, RAK S, VENGE P: Activation of B-lymphocytes during pollen season. Effect of immunotherapy. *Clin. Exp. Allergy* (1998) 28:791-798.

111. GARCIA BE, SANZ ML, GATO JJ, FERNANDEZ J, OEHLING A: IgG4 blocking effect on the release of antigen-specific histamine. *J. Investig. Allergol. Clin. Immunol.* (1993) 3:26-33.

112. MOTHES N, HEINZKILL M, DRACHENBERG KJ *et al.*: Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin. Exp. Allergy* (2003) 33:1198-1208.

113. SVENSON M, JACOBI HH, BODTGER U, POULSEN LK, RIENECK K, BENDTZEN K: Vaccination for birch pollen allergy. Induction of affinity-matured or blocking IgG antibodies does not account for the reduced binding of IgE to Bet v 1. *Mol. Immunol.* (2003) 39:603-612.

114. GEHLHAR K, SCHLAAK M, BECKER W, BUFE A: Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen-specific IgG4 to IgG1 correlates with clinical outcome. *Clin. Exp. Allergy* (1999) 29:497-506.

115. WACHHOLZ PA, DURHAM SR: Induction of 'blocking' IgG antibodies during immunotherapy. *Clin. Exp. Allergy* (2003) 33:1171-1174.

116. FLICKER S, VALENTA R: Renaissance of the blocking antibody concept in type I allergy. *Int. Arch. Allergy Immunol.* (2003) 132:13-24.

117. JAKOBSEN CG, BODTGER U, POULSEN LK, ROGGEN EL: Vaccination for birch pollen allergy: comparison of the affinities of specific immunoglobulins E, G1 and G4 measured by surface plasmon resonance. *Clin. Exp. Allergy* (2005) 35:193-198.

118. KILDSGAARD J, BRIMNES J, JACOBI H, LUND K: Sublingual immunotherapy in sensitised mice. Abstracts of the World Allergy Congress Munich 2005. *Allergy Clin. Immunol. Int. J. World Allergy Org. Suppl. 1* (2005):500.

- Study in mice implicating a role of IgA for the clinical efficiency of SLIT.

119. BAHCECILER NN, ARIKAN C, TAYLOR A *et al.*: Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int. Arch. Allergy Immunol.* (2005) 136:287-294.

- This study indicates that IgA may be increased in response to SLIT.

120. VAN NEERVEN RJ, WIKBORG T, LUND G *et al.*: Blocking antibodies induced by specific allergy vaccination prevent the activation of $CD4^+$ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol.* (1999) 163:2944-2952.

121. VAN NEERVEN RJ, ARVIDSSON M, IPSEN H, SPARHOLT SH, RAK S, WURTZEN PA: A double-blind, placebo-controlled birch allergy vaccination study: inhibition of CD23-mediated serum-immunoglobulin E-facilitated allergen presentation. *Clin. Exp. Allergy* (2004) 34:420-428.

122. DUCHATEAU J, MICHILS A, MICHEL O, BARAS L: Mite allergy is associated with a specific profile of IgG epitopes recognized on antigen p1 of *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy* (1997) 27:296-305.

123. MICHILS A, VERVIER I, CHOUFANI G, GOSSART B, DUCHATEAU J: Relationship between allergic status and specificity of IgG antibody to inhaled allergens: the grass pollen model. *Clin. Exp. Allergy* (1999) 29:832-839.

- This report shows that the dominant IgG specificities change within the first 12 h of ultra-rush immunotherapy, which suggests that the tolerant status is linked to selective depletion of high-avidity antibodies by high doses of allergen.

124. DUCHATEAU J, MICHILS A, LAMBERT J, GOSSART B, CASIMIR G: Anti-β lactoglobulin IgG antibodies bind to a specific profile of epitopes when patients are allergic to cow's milk proteins. *Clin. Exp. Allergy* (1998) 28:824-833.

125. MICHILS A, BALDASSARRE S, LEDENT C, MAIRESSE M, GOSSART B, DUCHATEAU J: Early effect of ultrarush venom immunotherapy on the IgG antibody response. *Allergy* (2000) 55:455-462.

126. WILSON DR, IRANI AM, WALKER SM *et al.*: Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium. *Clin. Exp. Allergy* (2001) 31:1705-1713.

127. WILSON DR, NOURI-ARIA KT, WALKER SM *et al.*: Grass pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J. Allergy Clin. Immunol.* (2001) 107:971-976.

128. POLOSA R, LI GF, MANGANO G *et al.*: Effect of immunotherapy on asthma progression, BHR and sputum eosinophils in allergic rhinitis. *Allergy* (2004) 59:1224-1228.

129. POLOSA R, LI GF, MANGANO G, MASTRUZZO C, PISTORIO MP, CRIMI N: Monitoring of seasonal variability in bronchial hyper-responsiveness and sputum cell counts in non-asthmatic subjects with rhinitis and effect of specific immunotherapy. *Clin. Exp. Allergy* (2003) 33:873-881.

130. DURHAM SR, VARNEY VA, GAGA M *et al.*: Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin. Exp. Allergy* (1999) 29:1490-1496.

131. MONTESEIRIN J, BONILLA I, CAMACHO J, CONDE J, SOBRINO F: Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: the effect of immunotherapy. *J. Allergy Clin. Immunol.* (2001) 107:623-626.

132. FURIN MJ, NORMAN PS, CRETICOS PS *et al.*: Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J. Allergy Clin. Immunol.* (1991) 88:27-32.

133. FANTA C, BOHLE B, HIRT W *et al.*: Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int. Arch. Allergy Immunol.* (1999) 120:218-224.

134. MARCUCCI F, SENSI L, FRATI F *et al.*: Sublingual tryptase and ECP in children treated with grass pollen sublingual immunotherapy (SLIT): safety and immunologic implications. *Allergy* (2001) 56:1091-1095.

135. MARCUCCI F, SENSI L, FRATI F *et al.*: Effects on inflammation parameters of a double-blind, placebo controlled one-year course of SLIT in children monosensitized to mites. *Allergy* (2003) 58:657-662.

136. SILVESTRI M, SPALLAROSSA D, BATTISTINI E *et al.*: Changes in inflammatory and clinical parameters and in bronchial hyperreactivity asthmatic children sensitized to house dust mites following sublingual immunotherapy. *J. Investig. Allergol. Clin. Immunol.* (2002) 12:52-59.

137. HOSKEN NA, SHIBUYA K, HEATH AW, MURPHY KM, O'GARRA A: The effect of antigen dose on $CD4^+$ T helper cell phenotype development in a T cell receptor-alpha beta-transgenic model. *J. Exp. Med.* (1995) 182:1579-1584.

138. GARDNER LM, THIEN FC, DOUGLASS JA, ROLLAND JM, O'HEHIR RE: Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in $CD4^+$ $CD25^+$ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin. Exp. Allergy* (2004) 34:1209-1219.

139. BENJAPONPITAK S, ORO A, MAGUIRE P, MARINKOVICH V, DEKRUYFF RH, UMETSU DT: The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J. Allergy Clin. Immunol.* (1999) 103:468-475.

140. DURHAM SR, YING S, VARNEY VA *et al.*: Grass pollen immunotherapy inhibits allergen-induced infiltration of $CD4^+$ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-γ. *J. Allergy Clin. Immunol.* (1996) 97:1356-1365.

141. EBNER C, SIEMANN U, BOHLE B *et al.*: Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from T_H2 to T_H1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin. Exp. Allergy* (1997) 27:1007-1015.

142. FAITH A, RICHARDS DF, VERHOEF A, LAMB JR, LEE TH, HAWRYLOWICZ CM: Impaired secretion of interleukin-4 and interleukin-13 by allergen-specific T cells correlates with defective nuclear expression of NF-AT2 and jun B: relevance to immunotherapy. *Clin. Exp. Allergy* (2003) 33:1209-1215.

143. GABRIELSSON S, SODERLUNDA, PAULIE S, VAN DER POUW KRAAN TC, TROYE-BLOMBERG M, RAK S: Specific immunotherapy prevents increased levels of allergen-specific IL-4- and IL-13-producing cells during pollen season. *Allergy* (2001) 56:293-300.

144. GUERRA F, CARRACEDO J, SOLANA-LARA R, SANCHEZ-GUIJO P, RAMIREZ R: T_H2 lymphocytes from atopic patients treated with immunotherapy undergo rapid apoptosis after culture with specific allergens. *J. Allergy Clin. Immunol.* (2001) 107:647-653.

145. HAMID QA, SCHOTMAN E, JACOBSON MR, WALKER SM, DURHAM SR: Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J. Allergy Clin. Immunol.* (1997) 99:254-260.

146. MAJORI M, CAMINATI A, CORRADI M, BRIANTI E, SCARPA S, PESCI A: T-cell cytokine pattern at three time points during specific immunotherapy for mite-sensitive asthma. *Clin. Exp. Allergy* (2000) 30:341-347.

147. MEISSNER N, KOCHS S, COUTELLE J *et al.*: Modified T-cell activation pattern during specific immunotherapy (SIT) in cat-allergic patients. *Clin. Exp. Allergy* (1999) 29:618-625.

148. O'BRIEN RM, BYRON KA, VARIGOS GA, THOMAS WR: House dust mite immunotherapy results in a decrease in Der p 2-specific IFN-γ and IL-4 expression by circulating T lymphocytes. *Clin. Exp. Allergy* (1997) 27:46-51.

149. OLDFIELD WL, LARCHE M, KAY AB: Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* (2002) 360:47-53.

150. SECRIST H, CHELEN CJ, WEN Y, MARSHALL JD, UMETSU DT: Allergen immunotherapy decreases interleukin 4 production in $CD4^+$ T cells from allergic individuals. *J. Exp. Med.* (1993) 178:2123-2130.

151. VAN BEVER HP, VEREECKE IF, BRIDTS CH, DE CLERCK LS, STEVENS WJ: Comparison between the *in vitro* cytokine production of mononuclear cells of young asthmatics with and without immunotherapy (IT). *Clin. Exp. Allergy* (1998) 28:943-949.

152. VARNEY VA, HAMID QA, GAGA M *et al.*: Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J. Clin. Invest.* (1993) 92:644-651.

153. LAAKSONEN K, JUNIKKA M, LAHESMAA R, TERHO EO, SAVOLAINEN J: *In vitro* allergen-induced mRNA expression of signaling lymphocytic activation molecule by PBMC of patients with allergic rhinitis is increased during specific pollen immunotherapy. *J. Allergy Clin. Immunol.* (2003) 112:1171-1177.

154. WACHHOLZ PA, NOURI-ARIA KT, WILSON DR *et al.*: Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral T_H1:T_H2 cytokine ratios. *Immunology* (2002) 105:56-62.

155. BELLINGHAUSEN I, METZ G, ENK AH, CHRISTMANN S, KNOP J, SALOGA J: Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. *Eur. J. Immunol.* (1997) 27:1131-1139.

156. CIPRANDI G, FENOGLIO D, CIRILLO I *et al.*: Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann. Allergy Asthma Immunol.* (2005) 95:38-44.

- In this paper, increased IL-10 production by peripheral blood mononuclear cells isolated from patients who were allergic to house dust mite after successful SLIT was shown.

157. FENOGLIO D, PUPPO F, CIRILLO I *et al.*: Sublingual specific immunotherapy reduces PBMC proliferations. *Allerg. Immunol.* (2005) 37:147-151.

158. IPPOLITI F, DE SANTIS W, VOLTERRANI A *et al.*: Immunomodulation during sublingual therapy in allergic children. *Pediatr. Allergy Immunol.* (2003) 14:216-221.

159. ROLINCK-WERNINGHAUS C, KOPP M, LIEBKE C, LANGE J, WAHN U, NIGGEMANN B: Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int. Arch. Allergy Immunol.* (2005) 136:134-141.

160. ZHAO DM, THORNTON AM, DIPAOLO RJ, SHEVACH EM: Activated $CD4^+CD25^+$ T cells selectively kill B lymphocytes. *Blood* (2006) 107:3925-32.

161. FRANCIS JN, TILL SJ, DURHAM SR: Induction of IL-10+$CD4^+CD25^+$ T cells by grass pollen immunotherapy. *J. Allergy Clin. Immunol.* (2003) 111:1255-1261.

162. JUTEL M, AKDIS M, BUDAK F *et al.*: IL-10 and TGF-β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur. J. Immunol.* (2003) 33:1205-1214.

163. SCHMIDT-WEBER CB, BLASER K: T-cell tolerance in allergic response. *Allergy* (2002) 57:762-768.

164. SAMSOM JN, VAN BERKEL LA, VAN HELVOORT JM *et al.*: Fc γ RIIB regulates nasal and oral tolerance: a role for dendritic cells. *J. Immunol.* (2005) 174:5279-5287.

165. AKDIS CA, JOSS A, AKDIS M, FAITH A, BLASER K: A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *FASEB J.* (2000) 14:1666-1668.

166. JAFFAR ZH, STANCIU L, PANDIT A, LORDAN J, HOLGATE ST, ROBERTS K: Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced T_H2 cytokine production from asthmatic bronchial tissue: role for alpha-beta, but not gamma-delta, T cells. *J. Immunol.* (1999) 163:6283-6291.

167. LARCHE M, TILL SJ, HASELDEN BM *et al.*: Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J. Immunol.* (1998) 161:6375-6382.

168. VAN NEERVEN RJ, VAN DE POL MM, VAN DER ZEE JS, STIEKEMA FE, DE BOER M, KAPSENBERG ML: Requirement of CD28-CD86 costimulation for allergen-specific T cell proliferation and cytokine expression. *Clin. Exp. Allergy* (1998) 28:808-816.

169. KRETSCHMER K, APOSTOLOU I, HAWIGER D, KHAZAIE K, NUSSENZWEIG MC, VON BOEHMER H: Inducing and expanding regulatory T cell populations by foreign antigen. *Nat. Immunol.* (2005) 6:1219-1227.

170. APOSTOLOU I, VON BOEHMER H: *In vivo* instruction of suppressor commitment in naive T cells. *J. Exp. Med.* (2004) 199:1401-1408.

171. FARIA AM, MARON R, FICKER SM, SLAVIN AJ, SPAHN T, WEINER HL: Oral tolerance induced by continuous feeding: enhanced up-regulation of transforming growth factor-β/ interleukin-10 and suppression of experimental autoimmune encephalomyelitis. *J. Autoimmun.* (2003) 20:135-145.

172. MARSHALL JD, ABTAHI S, EIDEN JJ *et al.*: Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes T(H)1 cytokine expression while downregulating T(H)2 cytokine expression in PBMCs from human patients with ragweed allergy. *J. Allergy Clin. Immunol.* (2001) 108:191-197.

173. SIMONS FE, SHIKISHIMA Y, VAN NEST G, EIDEN JJ, HAYGLASS KT: Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA. *J. Allergy Clin. Immunol.* (2004) 113:1144-1151.

174. ARIKAN C, BAHCECILER NN, DENIZ G *et al.*: Bacillus Calmette-Guerin-induced interleukin-12 did not additionally improve clinical and immunologic parameters in asthmatic children treated with sublingual immunotherapy. *Clin. Exp. Allergy* (2004) 34:398-405.

175. VAJDY M, SINGH M: The role of adjuvants in the development of mucosal vaccines. *Expert. Opin. Biol. Ther.* (2005) 5(7):953-965.

176. MOINGEON P: Sublingual immunotherapy: from biological extracts to recombinant allergens. *Allergy* (2006) 61(Suppl. 81):15-19.

177. GAFVELIN G, THUNBERG S, KRONQVIST M *et al.*: Cytokine and antibody responses in birch-pollen-allergic patients treated with genetically modified derivatives of the major birch pollen allergen Bet v 1. *Int. Arch. Allergy Immunol* (2005) 138:59-66.

178. LINHART B, HARTL A, JAHN-SCHMID B *et al.*: A hybrid molecule resembling the epitope spectrum of grass pollen for allergy vaccination. *J. Allergy Clin. Immunol.* (2005) 115:1010-1016.

179. REISINGER J, HORAK F, PAULI G *et al.*: Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. *J. Allergy Clin. Immunol* (2005) 116:347-354.

- The authors of this paper demonstrate that vaccination with recombinant allergens induces nasal IgG, suggesting that recombinant allergens may also be used for SLIT.

180. PAJNO GB: Allergen immunotherapy in early childhood: between Scylla and Charybdis! *Clin. Exp. Allergy* (2005) 35:551-553.

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