

REVIEW ARTICLE

Immunological mechanisms of sublingual allergen-specific immunotherapy

N. Novak, T. Bieber & J.-P. Allam

Department of Dermatology and Allergy, University of Bonn, Bonn, Germany

To cite this article: Novak N, Bieber T, Allam J-P. Immunological mechanisms of sublingual allergen-specific immunotherapy. *Allergy* 2011; DOI: 10.1111/j.1398-9995.2010.02535.x

Keywords

adjuvants; dendritic cells; oral mucosa; sublingual allergen-specific immunotherapy; tolerance.

Correspondence

Natalija Novak, MD, Department of Dermatology and Allergy, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany.

Tel.: +49 228 287 15370

Fax: +49 228 287 14333

E-mail: Natalija.Novak@ukb.uni-bonn.de

Accepted for publication 6 December 2010

DOI:10.1111/j.1398-9995.2010.02535.x

Edited by: Thomas Bieber

Abstract

Within the last 100 years of allergen-specific immunotherapy, many clinical and scientific efforts have been made to establish alternative noninvasive allergen application strategies. Thus, intra-oral allergen delivery to the sublingual mucosa has been proven to be safe and effective. As a consequence, to date, sublingual immunotherapy (SLIT) is widely accepted by most allergists as an alternative to conventional subcutaneous immunotherapy. Although immunological mechanisms remain to be elucidated in detail, several studies in mice and humans within recent years provided deeper insights into local as well as systemic immunological features in response to SLIT. First of all, it was shown that the target organ, the oral mucosa, harbours a sophisticated immunological network as an important prerequisite for SLIT, which contains among other cells, local antigen-presenting cells (APC), such as dendritic cells (DCs), with a constitutive disposition to enforce tolerogenic mechanisms. Further on, basic research on local DCs within the oral mucosa gave rise to possible alternative strategies to deliver the allergens to other mucosal regions than sublingual tissue, such as the vestibulum oris. Moreover, characterization of oral DCs led to the identification of target structures for both allergens as well as adjuvants, which could be applied during SLIT. Altogether, SLIT came a long way since its very beginning in the last century and some, but not all questions about SLIT could be answered so far. However, recent research efforts as well as clinical approaches paved the way for another exciting 100 years of SLIT.

Since the introduction of allergen-specific immunotherapy (AIT) 100 years ago, several alternative noninvasive allergen application strategies have been investigated, using the oral mucosal, intranasal, bronchial or oral route (1). However, only the administration of allergens via the oral mucosal route during the so-called sublingual allergen-specific immunotherapy (SLIT) gained increasing significance as an alternative application strategy to classical subcutaneous injections. Easy painless access to the target organ oral mucosa with rare occurrence of mild adverse events accounted to this trend in AIT, so that maintenance therapy of SLIT can be

ideally continued at home by patients themselves. Beyond the clinical value of SLIT, more and more evidence is gained in favour of the hypothesis that the oral mucosa represents an immune-privileged site, where severe acute allergic and inflammatory reactions occur relatively rarely and where tolerance to commensal bacterial species and food proteins prevails, because of the constitutive anti-inflammatory properties of cells located in this microenvironment (2). Consequently, these protolerogenic properties might explain not only the clinical effect but also the good safety profile of SLIT.

In SLIT, allergens are administrated to the oral mucosa by drops, sprays and in recent years also tablets. Controlled clinical studies have been initiated to evaluate the clinical effect together with strengths and weaknesses of SLIT. First results of meta-analysis on SLIT were impaired by the heterogeneity of the studies, such as the type of allergens used, the inclusion criteria, definitions of primary and secondary endpoints, the study collectives, duration of the studies and numerous other co-factors impacting on the outcome.

Abbreviations

APCs, antigen-presenting cells; DCs, dendritic cells; FcεRI, high-affinity receptor for IgE; Foxp3, forkhead box transcription factor protein 3; IFN-γ, interferon-γ; Ig, immunoglobulin; IL, interleukin; oLCs, oral Langerhans cells; PD-L, programmed cell death ligand; SLIT, sublingual allergen-specific immunotherapy; TGF-β, transforming growth factor-β; TLR, toll-like receptor.

However, with increasing quality and number of studies on SLIT during recent years, allover data on efficiency improved significantly (3, 4). A meta-analysis of 22 randomized clinical studies including a total of 979 adult patients (4) as well as a meta-analysis covering 10 studies conducted in a total of 577 children (5) revealed that SLIT represents an effective treatment alternative to the subcutaneous variant in clinical practice. Aeroallergens such as birch or grass pollen have been used in most of the studies. In contrast, only few approaches on other types of allergens used during SLIT exist. Data obtained with the help of standardized exposition of patients under SLIT using an allergen challenge chamber revealed significant treatment effect already after the first month of therapy (6). Beyond, selected studies conducted for a relatively long time period including follow-up over 1–2 years provide good evidence for a maintained disease-modifying effect of SLIT (7).

Nevertheless, without any doubt weak points of SLIT remain, such as requirement of a good compliance of patients during the whole treatment period combined with the reduced chance for the doctor to control both efficacy as well as putative side-effects of the treatment during a close meshed period of time. However, much scientific effort within the last decades was forced on another important question, namely the key question how SLIT works.

Characteristics of the 'immunological privilege' of the oral mucosa

To understand how SLIT works, detailed knowledge about the immunological characteristics of the oral mucosa is indispensable. The oral mucosa is from various points of view regarded an immune-privileged organ: numerous antigens derived from food proteins as well as from different commensal and pathogenic microbes get in touch with the oral mucosal immune system (8). However, acute allergic and inflammatory reactions occur relatively rarely at this site so that the immune homeostasis is successfully maintained by local protolerogenic mechanisms counteracting allergic reactions and inflammation most of the time. This requires a very sophisticated network of different components, which work hand in hand together (2).

The physical barrier as one of the first parts of this network is represented by the squamous epithelium, which lines the mucosa (9). Furthermore, some oral mucosal regions such as the sublingual compartment, vestibulum oris or buccal region are covered by lining mucosa, while others like the palatum durum and gingiva is formed by masticatory mucosa. Several immunological elements are integrated into this barrier and allow the uptake of required antigens on the one hand, but prevent entry of harmful pathogens on the other hand. This selectivity is indispensable in regard to the relatively high vascularization and permeability of this organ. Soluble factors such as α -amylase or lingual lipase represent a soluble barrier, which proteins have to pass in the oral mucosa. Moreover, although the oral mucosa represents the entry to the gastrointestinal tract (GIT), it differs from other GIT mucosal surfaces especially by lacking mucosa-

associated lymphoid tissue (MALT) (2). MALT together with regional draining lymph node represents inductive sites, while the lamina propria mucosae, exocrine glands and surface epithelia – which are also present within the oral mucosa – are considered as effector sites. Thus, inductive sites have not been clearly defined in this organ. Some authors suppose that dendritic cells (DCs), which have taken up antigens in the epithelium of the oral mucosa mature only partially and migrate to the basal lamina, to present the processed antigens in the so-called oral lymphoid foci located directly within the oral mucosa to T cells to induce an effective immune response (10). However, whether oral lymphoid foci are also detectable and of relevance for the induction of allergen-specific tolerance after allergen uptake via the oral mucosal route during SLIT needs to be further elucidated.

Oral mucosal DCs are concomitantly stimulated by microbial antigens present under physiological conditions in high numbers in the oral mucosa via pattern recognition receptors such as toll-like receptor (TLR)2 or TLR4, which are highly expressed by oral mucosal DCs in contrast to epidermal DCs (11). Stimulation of oral DCs with TLR ligands *in vitro* induces protolerogenic mechanisms, such as upregulation of their co-inhibitory molecule expression (B7H1 and B7H3) or release of IL-10 and enhanced priming of forkhead box transcription factor protein 3 (Foxp3)-expressing T cells and IL-10 and transforming growth factor- β (TGF- β)-producing T cells (12), which *in vivo* might contribute to the maintenance of the immune homeostasis despite frequent stimulation DCs by microbial antigens (12).

Comparing the microenvironment and presence of protolerogenic cytokines in the oral mucosa to the skin, mRNA expression of IL-10 and TGF- β is higher in all oral mucosal regions, and percentage of IL-10 and TGF- β -producing T cells is enhanced (11). In addition, the mRNA as well as protein expression of the Th1 cytokine interferon- γ (IFN- γ) is much higher in the oral mucosa when compared to the skin, implying that soluble mediators known to be able to suppress immune responses are constitutively expressed in the oral mucosa.

Target cells and structures for allergens applied during SLIT

Because of their close neighbourhood to the surface and their capacity to take up and present antigens to T cells as well as to prime T cell responses, DCs within the oral mucosa are considered as primary target cells of SLIT. This hypothesis is further supported by a study tracing sublingually applied antigen within the oral mucosal tissue in mice. In this context, sublingual challenge of ovalbumin (OVA) in BALB/c mice resulted in an accumulation of OVA within mucosal/submucosal interface, which disappeared later on and was most likely captured by CD11c⁺/CD11b⁺ and CD11c⁻/CD11b⁺ DCs, which are located within this zone (13). Moreover, oral mucosal DCs (oDCs) in humans constitutively express the high-affinity receptor for IgE (Fc ϵ RI), which might enable oDCs to take up allergens in a specific way via IgE bound to Fc ϵ RI on the cell surface. Fc ϵ RI is detectable

on the surface of oDCs of allergic as well as nonallergic individuals, and its expression as well as its occupation with IgE correlates with their serum IgE levels (14, 15). In humans, noninflamed, healthy oral mucosa comprises mainly myeloid DCs, which are by the expression of Langerin classified as oral mucosal Langerhans cells (oLCs) (14). Inflammatory DC subtypes as well as plasmacytoid DCs are not detectable within healthy oral mucosa (15). Number of myeloid DCs varies depending on the oral mucosal region. When compared to their counterparts in the noninflamed epidermis of the skin, oLCs express FcγR (i.e. CD16, CD32 and CD64) in higher amounts as well as the low-affinity receptor for IgE (CD23). Although CD83 expression mirroring the maturation stage of oLCs is comparable to the level of expression on LCs in the epidermis, expression of major histocompatibility class I and II molecules, costimulatory molecules CD80 and CD86 and CD40 is higher in oLCs than in LCs in the skin (Table 1) (14).

Studies on *ex vivo* isolated human oral mucosal tissue using dye labelled grass allergen phleum pratense (Phl p)5 revealed that the capacity of oLCs to take up allergens reaches a threshold after a defined time of allergen contact and at a specific allergen concentration applied (16). Up to this threshold, a further increase in the applied dose does not enhance the amount of allergen uptake by oLCs any further. This is in line with dose-dependent efficacy seen in clinical studies performed with grass pollen tablets (17, 18). Moreover, the capacity of oLCs of allergic and nonallergic donors to take up allergens does not differ very much *in vitro*. These observations imply that besides FcεRI, other receptors, such as maybe lectins or other pathways might play a role for allergen uptake by oLC during SLIT (Fig. 1A) (19).

Interestingly, only part of the oLCs take up the allergen in this *ex vivo* model, and oLCs that have taken up the allergen do not express the maturation marker CD83 to the same degree as oLCs migrating out of the tissue, which have not taken up allergens. Moreover, oLCs that have taken up allergens do not express chemokine receptor CCR7 in the same intensity as oLCs migrating without any allergens. Those findings imply that allergen uptake of oLCs attenuates their maturation and expression of chemokine receptor (CCR)7, which is essential for the recruitment of DCs to the peripheral lymphoid organs. Lower maturation stage of DCs was

described for a long time as a characteristic in particular of DCs with tolerogenic properties. Interestingly, LCs isolated of the skin, which have taken up allergens mature in the same manner as LCs migrating out of the skin without allergen uptake so that the lower maturation stage of allergen-loaded oLCs might provide a link to their constitutive protolerogenic properties (16). Moreover, lower CCR7 expression and the resulting migration of oLCs to the regional lymphoid tissue might further support the hypothesis of antigen presentation of oLCs to T cells outside the local draining lymphoid tissue for example within oral lymphoid foci, i.e. locally in the mucosa (Fig. 1B). This is underlined by the fact that oLCs are in close sterical contact to T cells in the oral mucosa (20).

Local and systemic immunological effects of SLIT

The reason why it has been decided that AIT via the oral mucosa should be conducted by applying allergens to the sublingual region of the mouth and has to be consequently called sublingual immunotherapy is that a lot of medications in particular from internal medicine have been applied to the submucosal membranes for a long time. Based on the fact that the epithelium of the sublingual region is thinner than that of other oral mucosal sites, resorption of drugs is higher at this site. However, in view of the anatomy and heterogeneity of the oral cavity, other aspects, might be important for the efficacy of SLIT (Table 2). Quantitative distribution and relation of oLCs, which are regarded as good guys of SLIT capable to take up the allergens as opposed to mast cells, which are rather considered to be bad guys during SLIT, since they are in part responsible for the side-effects is quite different. Lowest number of oLCs and highest number of mast cells were detectable in the sublingual region, while the ratio of oLCs to mast cells was much higher at other oral mucosal sites including the vestibulum (20). As a consequence, application of the allergens to the vestibular region instead of the sublingual region might be helpful in patients with sublingual swelling and itching in the clinical practice to circumvent or minimize mast cell-related side-effects of SLIT. There are only few data on the local changes within the mucosa evaluated using biopsies from patients under SLIT. Lower number of subepithelial mast cells but higher number of T cells was detected in patients under SLIT (21). In parallel, the amount of T cells expressing the marker of activated and regulatory T-cell forkhead box protein (Foxp)3 was significantly higher in the oral epithelium of SLIT-treated patients when compared to the placebo group (Fig. 1C) (22).

Enhancement of FoxP3⁺CD4⁺CD25⁺ T regulatory cells (Treg) and IL-10 in the peripheral blood as well as IL-18 and the signalling lymphocytic activation molecule (SLAM) in PBMCs was observed under SLIT (23, 24) (Fig. 1D). Those data imply a downregulation of Th2 immune responses as well as induction of tolerogenic pathways during SLIT (25, 26). Moreover, the level of antigen-specific regulatory T cells correlated negatively with clinical symptoms in patients under SLIT in one study (27). In addition, percentage of programmed cell death ligand (PDL)-1-expressing

Table 1 Comparison of epidermal LCs and LCs in the oral mucosa (noninflamed tissue) according to the data of (8, 10)

	LCs epidermal skin	LCs oral mucosa
CD1a	+++	++
MHC II	++	+++
FcεRI	(-/+)	+++
Langerin/CD207	+++	++
CD83	No difference	No difference
TLR2	(-/+)	++
TLR4	(-/+)	++
CD14	(-/+)	++

LCs, Langerhans cells; TLR, toll-like receptor; FcεRI, high-affinity receptor for IgE; MHC, major histocompatibility class.

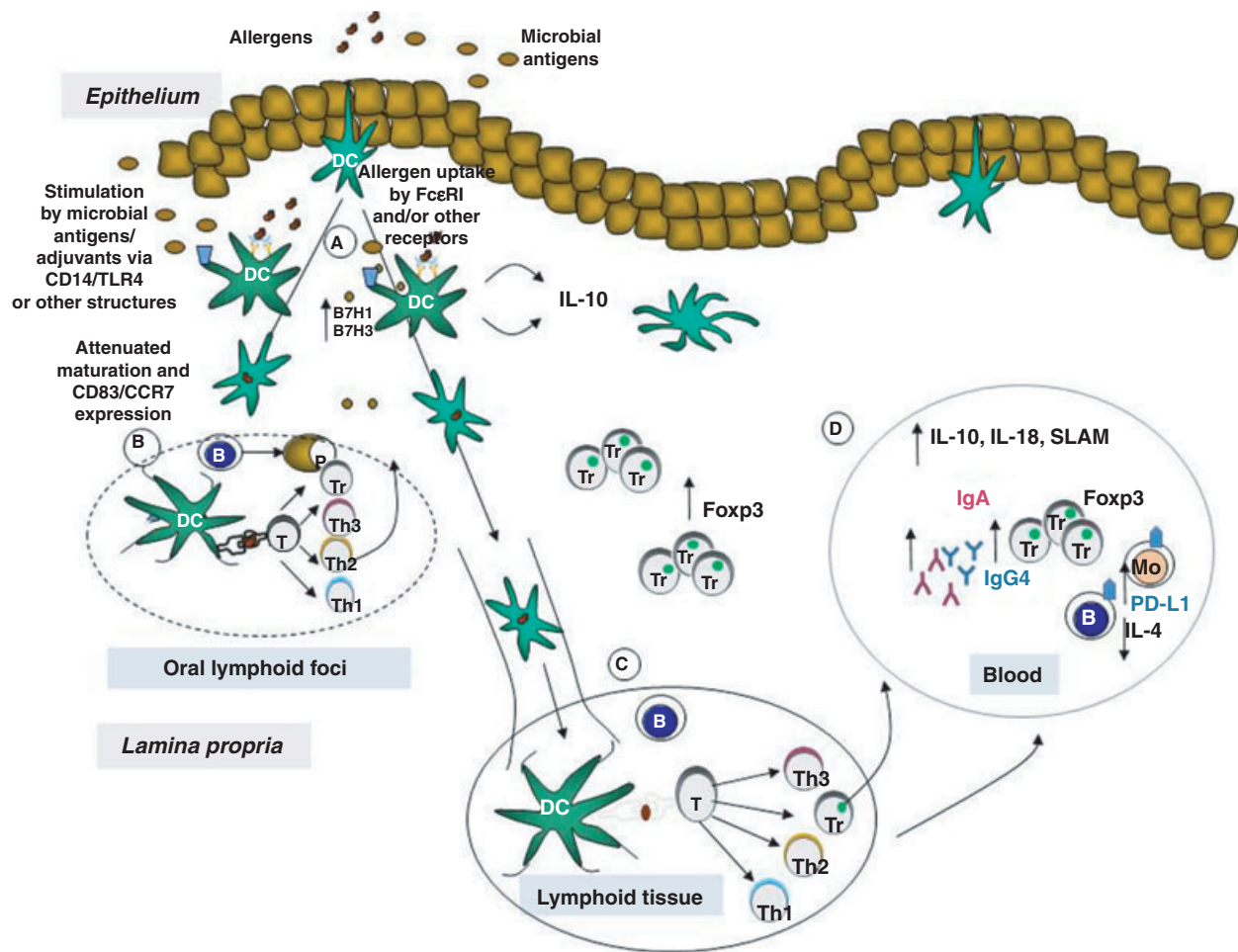


Figure 1 Proposed local and systemic mechanisms of sublingual allergen-specific immunotherapy (SLIT). (A) Allergens are taken up by FcεRI and/or other structures expressed by oral mucosal DCs. Oral mucosal DCs are concomitantly stimulated by microbial antigens present under physiological conditions in the oral mucosa, which induce protolerogenic mechanisms in oral mucosal DCs, such as upregulation of co-inhibitory molecule expression (B7H1 and B7H3) or release of IL-10. These tolerogenic properties could be targeted by substitution of allergens with adjuvants, which employ the same structures on oral DCs to enhance the effect of SLIT. (B) Attenuated maturation and slower upregulation of CD83 and CCR7 of oral DCs after allergen uptake during migration to the lymphoid tissue might provide evidence for local contact of oral

mucosal DCs with T cells, despite classical contact with T cells in the lymphoid tissue. Oral mucosal DCs are capable to prime regulatory T cell subtypes, including forkhead box protein 3 (Foxp3)-expressing T cells, which increase in the oral mucosa during SLIT. (D) IL-10, IL-18 and signalling lymphocytic activation molecule (SLAM) expression of PBMC as well as IgG4 serum levels and amount of Foxp3-expressing T cells increases in the blood during SLIT. Moreover, expression of programmed cell death ligand (PD-L1) is upregulated on B cells and monocytes in the peripheral blood of SLIT-treated patients in particular during the allergen season, while IL-4 production decreases. Altogether, the mechanisms depicted might go along with induction of allergen-specific tolerance and clinical effect of SLIT.

monocytes and B cells increased during the pollen season in patients under SLIT (28). Moreover, percentage of IL-4-producing monocytes, B cells and T cells decreased in season, while percentage of IL-10-producing monocytes, B cells and T cells increased in season in SLIT-treated patients (28). The same was true for IgG4 serum levels, which increased also during season under SLIT when compared to individuals without SLIT.

Data obtained with the help of murine model systems revealed that the amount of allergen-specific IgE in the nasal mucosa decreases during SLIT (29). Mice sensitized against

the timothy grass allergen phleum pratense challenged intranasally after SLIT, displayed reduced sneezing and eosinophil recruitment to the nasal mucosa as well as allergen-specific IgE in the nasopharyngeal lavage fluid (30). Increase in serum IgG4 and peak seasonal IgA₁/IgA₂ reduced the number of inflammatory cells infiltrating target organs, as well as reduction in eosinophilic cationic protein went along with SLIT (31–33). However, data on serum IgA levels under SLIT are still conflicting, there are also sincere reports about enhanced levels of IgA in serum, bronchoalveolar and nasal lavage fluid after SLIT (34).

Table 2 Comparison of different parameters in the sublingual vs the vestibular region according to the data of (8, 16)

	Sublingual region	Vestibulum
Thickness of lining mucosa	+	++
Number of oral LCs	++	+++
Number of mast cells	+++	++
Number of CD3+ T cells	++	+++
Distribution of FcεRI on oLCs	++	++
TLR2*	++	++
TLR4*	++	+++
TGF-β*	+	++
IL-10*	++	++
IFN-γ*	++	++
IL-17A*	+	+++
IL-17F*	+	+++
IL-26*	+	+++
IL-6*	++	++
Foxp3*	++	++

IFN-γ, interferon-γ; Foxp3, forkhead box transcription factor protein 3; oLCs, oral Langerhans cells; TGF-β, transforming growth factor-β.

*Relative mRNA expression in the whole tissue.

The putative role of adjuvants in SLIT

Using the routes of bacterial recognition by the innate immune system with the help of ligands to pattern recognition receptors, which are widely expressed by oLCs, represents an attractive strategy to increase the clinical effect. The main idea behind targeting those structures in the oral mucosa is to enhance priming of Th1 immune responses, which counteract Th2 immune responses and to take advantage of the fact that the oral mucosal immune network contains very sophisticated ways to maintain the immunohomeostasis by actively upregulating protolerogenic immune responses in case of incoming danger signals. As TLR such as TLR2 and TLR4 are highly expressed by oLCs in contrast to epidermal LCs, and ligands to TLR4 have been used as adjuvants in subcutaneous allergen-specific immunotherapy before, effect of TLR4 stimulation of *ex vivo* isolated oLCs has been evaluated intensively. TLR4 expression on oLCs correlates with the expression of the LPS receptor CD14, both of which are only expressed in very low amounts by epidermal LCs isolated from nonlesional skin (12). Stimulation of oLCs via TLR4 increases their expression of co-inhibitory surface molecules as well as production of IL-10 and TGF-β (12). Moreover, oLCs preactivated via TLR4 and co-cultured with naïve T cells prime more T cells releasing IL-10 and TGF-β and increase the fraction of Foxp3-expressing T cells (12). In a small phase I/IIa clinical study, the same TLR4 ligand has been used as adjuvant together with grass pollen extract during SLIT when compared to SLIT with grass pollen extract alone or placebo. Highest proportion of negative nasal challenge tests and earlier increase in IgG together with smaller IgE increase was observed in the group treated with the adjuvant–allergen combination (35), indicating that adjuvants might improve the efficacy of SLIT, too.

Bacterial toxins are useful adjuvants to deliver antigens to major histocompatibility pathways (36). In a mouse model, conjugation of an antigen to cholera toxin B as adjuvant showed higher efficacy than antigen administered to the sublingual mucosa alone. Suppression of T-cell proliferation was much more effective, and TGF-β serum levels and percentage of antigen-specific Foxp3-expressing regulatory T cells were higher (37). However, detoxified or nontoxic variants have been generated within the last years to circumvent the problem of toxicity of most of such substances (36). A very physiological alternative is represented by nonpathogenic food-grade bacteria such as lactic acid bacteria (36). Co-application of allergens with lactic acid bacteria to the mucosa in a murine model induced increased levels of allergen-specific IgG₂ in the sera as well as higher IFN-γ production by Th1 cells. Furthermore, IL-12p70 and IL-10 production of DCs increased, while airway hyper-responsiveness, bronchial inflammation and proliferation of T cells in the lymph nodes decreased (38). Sublingual application of 1,25-dihydroxyvitamin D₃, dexamethason or lactobacillus plantarum as other potential adjuvants applicable during SLIT induced IL-10 production by DCs (39). Further on, approaches using virus-like particles and plasmid DNA (36) represent other strategies, which follow the physiological ways of pathogen transmission. PlasmidDNA–lipid complexes applied intranasally have been shown to induce effective immunity against pathogens transferred via mucosal surfaces and hold promise to be applied even to the oral mucosa in the future (40).

Considering the high dilution effect of saliva in the oral mucosal, recent approaches were aimed to optimize mucosal adhesion of antigens with the help of OVA formulated with maltodextrin. Increasing mucosal adhesion reduced airway hyper-responsiveness, IL-5 and IgE production in a mouse asthma model (41). Further on, longer contact times might be achieved by the use of mucoadhesive additives. This is of practical importance in view of the low amount of allergen, which remains locally in the tissue after sublingual administration (42).

The next 100 years of SLIT

In view of a multitude of research activities as well as clinical studies, together with continuous progress in the extraction and preparation of allergens as well as availability of recombinant allergens, it is very likely that SLIT will continue its career as one of the few rationale-based treatments we hold in our hands in the clinical practice to treat allergies. Addition of different adjuvants to the allergen preparations or targeting different regions within the oral mucosa such as the vestibulum might improve efficacy and reduce local or systemic side-effects of this treatment. Together with our growing experience while using SLIT, the type of allergens available with proven efficacy in clinical studies will be expanded. Moreover, besides asthma and allergic diseases, other allergen-triggered diseases might be included, and aspects of preventive treatment at early time points of life might be in particular of importance in the context of this

noninvasive, patient-friendly way to treat. Anyway, tools to control the compliance of the patients as well as efficacy of SLIT are still on our wish list to be discovered during the next 100 years of SLIT.

Acknowledgement

This work was supported by grants from the Deutsche Forschungsgemeinschaft (KFO 208 TPA1 and SFB704 TPA4) and a BONFOR grant of the University of Bonn. N.N. is supported by a Heisenberg-Professorship of the DFG NO454/5-2.

References

- Valovirta E, Passalacqua G, Canonica WG. Non-injection routes for immunotherapy of allergic diseases. *Clin Allergy Immunol* 2004;**18**:607–623.
- Novak N, Haberkot J, Bieber T, Allam JP. The immune privilege of the oral mucosa. *Trends Mol Med* 2008;**14**:191–198.
- Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev* 2007; (1):CD001936.
- Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005;**60**:4–12.
- Penagos M, Compalati E, Tarantini F, Baena-Cagnani R, Huerta J, Passalacqua G et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: a meta-analysis of randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol* 2006;**97**:141–148.
- Horak F, Ziegelmayer P, Ziegelmayer R, Lemell P, Devillier P, Montagut A et al. Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J Allergy Clin Immunol* 2009;**124**:471–477.
- Durham SR, Emminger W, Kapp A, Colombo G, De Monchy JG, Rak S et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 2010;**125**:131–138.
- Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA et al. Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001;**183**:3770–3783.
- Lu FX, Jacobson RS. Oral mucosal immunity and HIV/SIV infection. *J Dent Res* 2007;**86**:216–226.
- Jotwani R, Palucka AK, Al-Quotub M, Noori-Shirazi M, Kim J, Bell D et al. Mature dendritic cells infiltrate the T cell-rich region of oral mucosa in chronic periodontitis: in situ, in vivo, and in vitro studies. *J Immunol* 2001;**167**:4693–4700.
- Allam JP, Duan Y, Winter J, Stojanovski G, Fronhoffs F, Wenghoefer M et al. Tolerogenic T cells, Th1/Th17 cytokines and TLR2/TLR4 expressing dendritic cells predominate the microenvironment within distinct oral mucosal sites. *Allergy* 2010; 10-9995. [pubmed ahead of print].
- Allam JP, Peng WM, Appel T, Wenghoefer M, Niederhagen B, Bieber T et al. Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. *J Allergy Clin Immunol* 2008;**121**:368–374.
- Rask C, Brimnes J, Lund K. Shorter dosing intervals of sublingual immunotherapy lead to more efficacious treatment in a mouse model of allergic inflammation. *Scand J Immunol* 2010;**71**:403–412.
- Allam JP, Novak N, Fuchs C, Asen S, Berge S, Appel T et al. Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcεpsilonRI expression. *J Allergy Clin Immunol* 2003;**112**:141–148.
- Allam JP, Niederhagen B, Bucheler M, Appel T, Betten H, Bieber T et al. Comparative analysis of nasal and oral mucosa dendritic cells. *Allergy* 2006;**61**:166–172.
- Allam JP, Wurtzen PA, Reinartz M, Winter J, Vrtala S, Chen KW et al. Phl p 5 resorption in human oral mucosa leads to dose-dependent and time-dependent allergen binding by oral mucosal Langerhans cells, attenuates their maturation, and enhances their migratory and TGF-beta1 and IL-10-producing properties. *J Allergy Clin Immunol* 2010;**126**:638–645.
- Didier A, Malling HJ, Worm M, Horak F, Jager S, Montagut A et al. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. *J Allergy Clin Immunol* 2007;**120**:1338–1345.
- Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006;**117**:802–809.
- Larche M. Immune mechanisms of sublingual immunotherapy: are oral Langerhans cells the masters of tolerance? *J Allergy Clin Immunol* 2010;**126**:646–647.
- Allam JP, Stojanovski G, Friedrichs N, Peng W, Bieber T, Wenzel J et al. Distribution of Langerhans cells and mast cells within the human oral mucosa: new application sites of allergens in sublingual immunotherapy? *Allergy* 2008;**63**:720–727.
- Scadding GW, Shamji MH, Jacobson MR, Lee DI, Wilson D, Lima MT et al. Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. *Clin Exp Allergy* 2010;**40**:598–606.
- Mosges R, Ritter B, Kayoko G, Allekotte S. Carbamylated monomeric allergoids as a therapeutic option for sublingual immunotherapy of dust mite- and grass pollen-induced allergic rhinoconjunctivitis: a systematic review of published trials with a meta-analysis of treatment using Lais tablets. *Acta Dermatovenereol Alp Panonica Adriat* 2010;**19**:3–10.
- Nieminen K, Laaksonen K, Savolainen J. Three-year follow-up study of allergen-induced in vitro cytokine and signalling lymphocytic activation molecule mRNA responses in peripheral blood mononuclear cells of allergic rhinitis patients undergoing specific immunotherapy. *Int Arch Allergy Immunol* 2009;**150**:370–376.
- Laaksonen K, Junikka M, Laheesmaa R, Terho EO, Savolainen J. In vitro allergen-induced mRNA expression of signalling lymphocytic activation molecule by PBMC of patients with allergic rhinitis is increased during specific pollen immunotherapy. *J Allergy Clin Immunol* 2003;**112**:1171–1177.

Authors' contributions

N.N., T.B. and J.-P.A. have written the manuscript.

Conflict of interest statement

N.N. has received research grants from Alk Abello and is speaker for Alk Abello, Bencard Allergy Therapeutics and Novartis/LetiPharma. J.-P.A. has received research grants from Alk Abello and is speaker for Bencard Allergy Therapeutics.

25. Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007;**120**: 707–713.
26. Savolainen J, Nieminen K, Laaksonen K, Laiho T, Jacobsen L, Lahesmaa R et al. Allergen-induced in vitro expression of IL-18, SLAM and GATA-3 mRNA in PBMC during sublingual immunotherapy. *Allergy* 2007;**62**:949–953.
27. Fujimura T, Yonekura S, Taniguchi Y, Horiguchi S, Saito A, Yasueda H et al. The induced regulatory T cell level, defined as the proportion of IL-10Foxp3(+) cells among CD25(+)CD4(+) leukocytes, is a potential therapeutic biomarker for sublingual immunotherapy: a preliminary report. *Int Arch Allergy Immunol* 2010;**153**:378–387.
28. Piconi S, Trabattini D, Rainone V, Borgonovo L, Passerini S, Rizzardini G et al. Immunological effects of sublingual immunotherapy: clinical efficacy is associated with modulation of programmed cell death ligand 1, IL-10, and IgG4. *J Immunol* 2010;**185**: 7723–7730.
29. Marcucci F, Sensi L, Di CG, Incorvaia C, Frati F. Dose dependence of immunological response to sublingual immunotherapy. *Allergy* 2005;**60**:952–956.
30. Brimnes J, Kildsgaard J, Jacobi H, Lund K. Sublingual immunotherapy reduces allergic symptoms in a mouse model of rhinitis. *Clin Exp Allergy* 2007;**37**:488–497.
31. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006;**6**:761–771.
32. Akdis CA, Barlan IB, Bahceciler N, Akdis M. Immunological mechanisms of sublingual immunotherapy. *Allergy* 2006;**61**(Suppl 81):11–14.
33. Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van OL. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006;**61**:151–165.
34. Kildsgaard J, Brimnes J, Jacobi H, Lund K. Sublingual immunotherapy in sensitized mice. *Ann Allergy Asthma Immunol* 2007;**98**:366–372.
35. Pfaar O, Barth C, Jaschke C, Hormann K, Klimek L. Sublingual allergen-specific immunotherapy adjuvanted with monophosphoryl lipid A: a phase I/IIa study. *Int Arch Allergy Immunol* 2010;**154**:336–344.
36. Mascarell L, Van OL, Moingeon P. Novel ways for immune intervention in immunotherapy: mucosal allergy vaccines. *Immunol Allergy Clin North Am* 2006;**26**:283–viii.
37. Sun JB, Cuburu N, Blomquist M, Li BL, Czerkinsky C, Holmgren J. Sublingual tolerance induction with antigen conjugated to cholera toxin B subunit induces Foxp3+CD25+CD4+ regulatory T cells and suppresses delayed-type hypersensitivity reactions. *Scand J Immunol* 2006;**64**: 251–259.
38. Van OL, Moussu H, Horiot S, Samson S, Lombardi V, Mascarell L et al. Lactic acid bacteria as adjuvants for sublingual allergy vaccines. *Vaccine* 2010;**28**:2986–2992.
39. Van OL, Lombardi V, Razafindratsita A, Saint-Lu N, Horiot S, Moussu H et al. IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. *Int Arch Allergy Immunol* 2007;**145**: 152–162.
40. Klavinskis LS, Barnfield C, Gao L, Parker S. Intranasal immunization with plasmid DNA-lipid complexes elicits mucosal immunity in the female genital and rectal tracts. *J Immunol* 1999;**162**:254–262.
41. Razafindratsita A, Saint-Lu N, Mascarell L, Berjont N, Bardot T, Betbeder D et al. Improvement of sublingual immunotherapy efficacy with a mucoadhesive allergen formulation. *J Allergy Clin Immunol* 2007;**120**:278–285.
42. Bagnasco M, Altrineti V, Pesce G, Caputo M, Mistrello G, Falagiani P et al. Pharmacokinetics of Der p 2 allergen and derived monomeric allergoid in allergic volunteers. *Int Arch Allergy Immunol* 2005;**138**: 197–202.