

BIBLIOGRAFÍA

MECANISMOS DE ACCIÓN DE LA ALERGIA

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IgE antibody plays an important role in allergic diseases. IgE synthesis by B cells requires two signals. The first signal is delivered by the cytokines IL-4 or IL-13, which target the Cepsilon gene for switch recombination. The second signal is delivered by interaction of the B cell surface antigen CD40 with its ligand (CD40L) expressed on activated T cells. This activates deletion switch recombination. We review the molecular mechanisms of IL-4 and CD40 signaling that lead to IgE isotype switching and discuss the implications for intervening to abort or suppress the IgE antibody response.

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The prevalence of immunoglobulin E (IgE)-mediated allergic diseases has been increasing for the last four decades. In this review determinants for an increased IgE synthesis are discussed on both an epidemiological and on an immunological level with special emphasis on the differentiation of the B cell to an IgE-producing plasma cell. Factors that favor an IgE immune response are low antigen doses and immunization via mucous membranes, but it is highly likely that other environmental factors besides exposure to the allergenic sources play a role. Important factors in the formation of the Thelper type 2 (Th2) T cell subset are the actions of thymic stromal lymphopoietin (TSLP) on dendritic cells and the OX40 ligand on CD4+ T cells. In order for a B lymphocyte to switch to IgE production it needs two signals provided by a Th2 cell in the form of the cytokines interleukin (IL-) 4/IL-13 and ligation of the CD40. In spite of a half-life of only a few days, there is evidence that the IgE response may last for years even without allergen stimulation. This is likely to be caused by long-lived IgE-producing plasma cells, and such cells may be difficult to target therapeutically thus emphasizing the need for more knowledge on preventable causes of IgE- and allergy development.

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In the last few decades, basic biology and immunology have thrived, largely thanks to the use of model organisms that allow exploration of complex functions in ideal experimental conditions and genetically defined backgrounds. IgE regulation studies are no exception to this rule. The current challenge is to anchor what we are learning in test tubes and animals to mechanisms of disease in patients with allergy. With information about the human genome rapidly piling up, and strong associations between single nucleotide polymorphisms and disease phenotypes reported more and more often, it is becoming clear that such anchoring cannot occur without a robust integration between the biology of model systems and the biology of natural genetic variants. Here we will argue that an essential component of this integration is the functional analysis of the mechanisms through which natural variation affects pathways relevant to the pathogenesis of IgE-dependent inflammation.

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The interaction of immunoglobulin E (IgE) and its receptors is critical for the manifestation of allergic disease. Currently, IgE receptors include the high-affinity Fc epsilonRI and the low-affinity Fc epsilonRII. Fc epsilonRI is a tetrameric or trimeric complex, and each has distinct expression patterns and unique functional consequences. In general, serum levels of IgE regulate Fc epsilonRI expression, and novel therapies that lower the concentration of IgE have provided insights into the contribution of this receptor in allergic disease. Newer therapies targeting Fc epsilonRI-signaling elements and the low-affinity IgE receptor, Fc epsilonRII (CD23), are being developed.

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Immunoglobulin E (IgE) plays a central role in the pathogenesis of allergic diseases by interacting with two membrane receptors: high-affinity Fc epsilonRI and low-affinity Fc epsilonRII (CD23). Allergen-induced IgE-occupied Fc epsilonRI aggregation on the mast cell or basophil cell surface leads to the activation of intracellular signaling events and eventually the release of pre-formed and de novo synthesized inflammatory mediators. The role of Fc epsilonRII in allergic diseases has been proposed to include regulation of IgE synthesis, enhanced histamine release from basophils, and a contribution to Ag-IgE complex presentation but the exact function of CD23 remains poorly understood. This review summarizes some new developments in IgE Fc-receptor studies with an emphasis on regulation of Fc epsilonRI expression and signal transduction, including monomeric IgE, lipid raft segregation, and some recently identified negative regulators. A better understanding of signaling events following IgE FcR aggregation will shed new light on how allergy patients might be treated more safely and effectively.

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MECANISMOS DE ACCIÓN DE LA INMUNOTERAPIA SUBLINGUAL

13. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2007;119:780-9.

Allergen-specific immunotherapy (SIT) has been used for almost a century as a desensitizing therapy for allergic diseases and represents the only curative and specific method of treatment. Administration of appropriate concentrations of allergen extracts has been shown to be reproducibly effective when patients are carefully selected. The mechanisms by which allergen-SIT has its effects include the modulation of T-cell and B-cell responses and related antibody isotypes as well as effector cells of allergic inflammation, such as eosinophils, basophils, and mast cells. The balance between allergen-specific T-regulatory (Treg) and T(H)2 cells appears to be decisive in the development of allergic and healthy immune responses against allergens. Treg cells consistently represent the dominant subset specific for common environmental allergens in sensitized healthy individuals. In contrast, there is a high frequency of allergen-specific T(H)2 cells in patients with allergy. The induction of a tolerant state in peripheral T cells represents an essential step in allergen-SIT. Peripheral T-cell tolerance is characterized mainly by generation of allergen-specific Treg cells leading to suppressed T-cell proliferation and T(H)1 and T(H)2 cytokine responses against the allergen. This is accompanied by a significant increase in allergen-specific IgG(4), and also IgG(1) and IgA, and a decrease in IgE in the late stage of the disease. In addition, decreased tissue infiltration of mast cells and eosinophils and their mediator release including circulating basophils takes place. Current understanding of mechanisms of allergen-SIT, particularly the role of Treg cells in peripheral tolerance, may enable novel treatment strategies.

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It remains unclear how and where unresponsiveness to fed antigens is induced. This "oral tolerance" is probably necessary to prevent the array of immune effector mechanisms required to counteract pathogens of the mucosae from being misdirected against food antigens or commensal flora. It will obviously be important to dissect where, when, and how such immunological homeostasis is maintained in the gut, but it will also be necessary to determine whether similar inductive and effector mechanisms are required for the therapeutic applications of oral tolerance systemically. This may be influenced by anatomical and microenvironmental effects on the phenotype and/or activation state of the antigen-presenting cell (APC), which presents orally delivered antigen. Fed antigen passes from the intestinal lumen either via the villus epithelium and M cells in the Peyer's patches (PP) or the mucosal lamina propria to the organized lymphoid tissues of the PP and mesenteric lymph nodes (MLN). In addition, there is evidence that mucosally administered antigen also gains access directly to peripheral lymphoid organs. Each of these sites contains distinctive populations of APCs and has unique local microenvironments that may influence the immune response in different ways. We propose that feeding antigen in high doses may induce clonal anergy, deletion, or altered differentiation because it gains direct access to resting APCs in the T cell areas of both the gut-associated lymphoid tissues (GALT) and peripheral lymphoid organs, with presentation occurring in the absence of productive costimulation. By contrast, low doses of tolerizing antigen may be taken up and presented preferentially by APCs in the GALT, where the local environment may favor the induction of regulatory T cells. This is consistent with our own and others findings, using adoptive transfer of TcR tg T cells. These studies have shown that antigen-specific CD4(+) T cells are activated simultaneously in all peripheral and gut-associated lymphoid organs after feeding high doses of proteins, but that this may be more restricted to local tissues when lower doses are used. Another level of anatomical control is imposed within lymphoid organs, where migration of T cells through distinct anatomical compartments can affect their differentiation. We find that, in contrast to orally primed T cells, orally tolerized T cells are unable to migrate into B cell follicles during their initial exposure to antigen. This affects their differen-

tiation as upon subsequent challenge with antigen in adjuvant, tolerized T cells can be found in follicles but are unable to provide the B cell help that primed T cells can deliver. We hypothesize that the initial defective migration of tolerized T cells prevents them from receiving signals from antigen-specific B cells in follicles and results in abortive differentiation. Thus, both gross and fine anatomical location of fed antigen presentation may be important in mucosal immunoregulation.

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Aero-allergens, including plant pollens, house dust mite particles, fungal spores, and mycelium fragments, are continuously inhaled and deposited on the airway mucosa. These particles and their soluble components actively interact with innate recognition systems present in the mucosal layer (e.g., surfactant proteins) and with various receptors present on a diversity of cells in the airways. Deposited particles are first removed by active transportation, the rate of which is either enhanced or inhibited by components present in the inhaled substances. Cleaning further depends on innate recognition, beginning with (a) soluble factors released into the mucosal surface layer that bind different bio-organic components; (b) innate receptors on phagocytic cells, alveolar macrophages, and dendritic monocytes; and (c) innate receptors on airway epithelial cells. Different innate receptor families (Toll-like receptors [TLRs], nucleotide-binding oligomerization domain receptors, and protein-activated receptors [PARs]) have been demonstrated on airway cells, including alveolar macrophages, monocytes, dendritic cells, and airway tissue cells (e.g., epithelial cells and mast cells). However, although the functional role of these receptors has been studied for infectious diseases, the functional role for reaction of airways to inhaled bio-organic substances, including allergens, is largely unexplored. Indirect evidence for functional interactions has come from in vivo animal studies, as well as in vitro studies with monocytes, macrophages, and epithelial cells, which have demonstrated release of cytokines and chemokines after contact with allergens such as house dust mite, cat, pollen, and fungi. Most allergens show direct activation of airway epithelial cells, suggesting a role for the innate receptors. However, the role of TLRs, PARs, and other receptors was studied for only a limited number of allergens. Current studies indicate synergistic interactions between members of the same receptor family (TLRs) as well as synergistic interactions between members of different families (TLRs, PARs, and nucleotide-binding oligomerization domain receptors), modulating responses into feed-forward or inhibitory actions. Study of these synergistic interactions and their genetic variations will provide insight regarding how the innate immune system determines the inflammatory reactions of the airways and the outcome of the T-helper-1- and T-helper-2-type responses to inhaled allergens.

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Sublingual immunotherapy has been shown in some clinical studies to modulate allergen-specific antibody responses [with a decrease in the immunoglobulin E/immunoglobulin G4 (IgE/IgG4) ratio] and to reduce the recruitment and activation of proinflammatory cells in target mucosa. Whereas a central paradigm for successful immunotherapy has been to reorient the pattern of allergen-specific T-cell responses in atopic patients from a T helper (Th)2 to Th1 profile, there is currently a growing interest in eliciting regulatory T cells, capable of downregulating both Th1 and Th2 responses through the production of interleukin (IL)-10 and/or transforming growth factor (TGF)-beta. We discuss herein immune mechanisms involved during allergen-specific sublingual immunotherapy (SLIT), in comparison with subcutaneous immunotherapy. During SLIT, the allergen is captured within the oral mucosa by Langerhans-like dendritic cells expressing high-affinity IgE receptors, producing IL-10 and TGF-beta, and upregulating indoleamine dioxygenase (IDO), suggesting that such cells are prone to induce tolerance. The oral mucosa contains limited number of proinflammatory cells, such as mast cells, thereby explaining the well-established safety profile of SLIT. In this context, second-gen-

eration vaccines based on recombinant allergens in a native conformation formulated with adjuvants are designed to target Langerhans-like cells in the sublingual mucosa, with the aim to induce allergen-specific regulatory T cells. Importantly, such recombinant vaccines should facilitate the identification of biological markers of SLIT efficacy in humans.

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BACKGROUND AND OBJECTIVE: The clinical effectiveness of noninjectable routes for specific immunotherapy has been demonstrated in many studies, but no data are available on the kinetics of allergens administered by these routes. Therefore we studied the kinetics of the radiolabeled purified major *Parietaria judaica* allergen (Par j 1) after sublingual, oral, and intranasal administration to healthy human beings. **METHODS:** After tracer administration (10 to 12.5 microg of Par j 1 labeled with iodine 123) to nonallergic volunteers, scintigraphic images were recorded at various times. Blood samples were also obtained at serial intervals to evaluate the absorption and distribution of radioactivity in plasma and to identify circulating radioactive species by molecular exclusion gel chromatography. **RESULTS:** When the sublingual route was used, no circulating radioactivity was detected until the tracer was kept under the tongue. The labeled allergen was rapidly degraded and absorbed in the gastrointestinal tract after swallowing. Plasma radioactivity peaked at about 1.5 to 3 hours and was mostly represented by free radioiodine and small radiolabeled peptides. Some activity not caused by free ¹²³I remained associated with the oral mucosa up to 18 to 20 hours after administration. When the oral route was used, the results were similar to those observed after swallowing the sublingually administered allergen but without any persistence of the tracer in the mouth. When the intranasal route was used, the pattern of plasma radioactivity mimicked that of the sublingual and oral routes, with absorption of activity from the radiolabeled allergen occurring in the gastrointestinal tract after transport to the pharynx by mucociliary clearance. A relevant fraction of the tracer was retained on the nasal mucosa up to 48 hours after administration. **CONCLUSION:** The data in this study provide the first experimental basis for exploring the in vivo kinetics of allergen administered through noninjectable routes for specific immunotherapy in human beings.

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BACKGROUND AND OBJECTIVE: Little is known about the pharmacokinetics of allergens for local immunotherapy. Thus, we studied the pharmacokinetics in allergic volunteers of a commercial allergenic vaccine in orosoluble tablets (LAIS(R), Lofarma S.p.A). **METHODS:** The carbamylated monomeric allergoid derived from *Parietaria judaica* major allergen (Par j 1), characterized by maintenance of the original molecular size, and the native allergen, were radiolabeled with ¹²³I, then incorporated into the commercial soluble tablets and administered to allergic subjects. Early sequential and late static scintigraphic acquisitions were performed, and plasma radioactivity was measured at different time intervals. **RESULTS:** No difference in local pharmacokinetics was observed between the allergen and the allergoid: part of the tracer was retained in the mouth for at least 2 h after swallowing. No direct absorption through the oral mucosa could be detected, as plasma radioactivity increased only after swallowing and peaked at 2 h. However, the plasma peak attained with the allergoid in tablets was significantly higher with respect to the native allergen. Finally, some undegraded allergoid, but not the allergen, could be constantly detected in the bloodstream at plasma peak. **CONCLUSIONS:**

The results showed a similar behaviour of the allergoid and the allergen in tablets as far as their local kinetics are concerned, whereas plasma peak was higher with the allergoid than with the allergen. Therefore we conclude that the chemical modification of the allergen may affect its pharmacokinetics, by making it less susceptible to enzymatic degradation.

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BACKGROUND: The safety and efficacy of sublingual immunotherapy have been demonstrated in moderate allergic asthma and seasonal rhinitis. However, not much is known about the precise mechanism of action of the allergen when it crosses the oral mucosa. OBJECTIVE: To define this mechanism, we investigated the role of Langerhans' cells in the capture and internalization of allergens. METHODS: We generated dendritic cells in vitro with the phenotypic characteristics of Langerhans-like dendritic cells (LLDCs) from cord blood CD34(+) progenitors. We used two recombinant major allergens: Bet v 1 and Phl p 1 labeled with FITC. RESULTS: Internalization of allergens and control proteins was dose- and time-dependent and related to the immature state of the cells. LLDCs internalized allergens with a high efficiency in comparison with control molecules. Allergens were only internalized by macropinocytosis, as demonstrated by the use of various inhibitors. Addition of intracellular pH-modifying molecules indicated that only a part of the allergens was accumulated in acidic vesicles, whereas the majority remained in other cytoplasmic structures. Pulse-chase experiments calculated a half-life of 4 hours, suggesting that part of the molecules were not metabolized in the lysosome. Allergen internalization by LLDCs might be followed by processing in some experiments, as demonstrated by activation of autologous T lymphocytes in 4 of 9 experiments. CONCLUSION: These elements showed that Langerhans' cells present in mucosa might play an active role in immune responses to allergens.

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In the skin and in the epithelium of the oral mucosa a comparable network of Langerhans cells can be found. Antigen application on either epithelium leads to rapid emigration of Langerhans cells to the draining lymph nodes. Application on the oral mucosa leads to tolerance induction while application on the skin results in sensitization of the animal. Here we show that there are no differences in the antigen presentation capacity of oral mucosa- and skin-derived dendritic cells. However, measurement of IFN-gamma and IL-5 production, as representatives of Th1 and Th2 cytokines respectively, in total lymph node suspensions after sensitization via the skin or oral mucosa demonstrated a skewing of the response towards Th2 after antigen application on the oral mucosa. Together with our previous studies, in which it was shown that oral tolerance induction is not inherent to oral mucosa-derived dendritic cells, we postulate that oral tolerance is determined at the level of draining lymph nodes influenced by local cytokine profiles.

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Respiratory exposure to allergen induces T cell tolerance and protection against the develop-

ment of airway hyperreactivity and asthma. However, the specific mechanisms by which tolerance is induced by respiratory allergen are not clear. We report here that pulmonary dendritic cells (DCs) from mice exposed to respiratory antigen transiently produced interleukin 10 (IL-10). These phenotypically mature pulmonary DCs, which were B-7(hi) as well as producing IL-10, stimulated the development of CD4(+) T regulatory 1--like cells that also produced high amounts of IL-10. In addition, adoptive transfer of pulmonary DCs from IL-10(+/-), but not IL-10(-/-), mice exposed to respiratory antigen induced antigen-specific unresponsiveness in recipient mice. These studies show that IL-10 production by DCs is critical for the induction of tolerance, and that phenotypically mature pulmonary DCs mediate tolerance induced by respiratory exposure to antigen.

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Active suppression is mediated by a subpopulation of CD4(+) T cells that prevents autoimmunity. However, the mechanisms involved in their differentiation in vivo are currently under intensive research. Here we show that in vitro culture of bone marrow cells in the presence of IL-10 induces the differentiation of a distinct subset of dendritic cells with a specific expression of CD45RB. These CD11c(low)CD45RB(high) DCs are present in the spleen and lymph nodes of normal mice and are significantly enriched in the spleen of IL-10 Tg mice. These natural or in vitro-derived DCs display plasmacytoid morphology and an immature-like phenotype, and secrete high levels of IL-10 after activation. OVA peptide-pulsed CD11c(low)CD45RB(high) DCs specifically induce tolerance through the differentiation of Tr1 cells in vitro and in vivo. Our findings identify a natural DC subset that induces the differentiation of Tr1 cells and suggest their therapeutic use.

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BACKGROUND: Mucosal dendritic cells (DC) play a crucial role in tolerance induction as seen in mucosal immunotherapy of atopic diseases. Nevertheless little is known about the phenotypical differences of oral and nasal mucosal DC (nmDC). Recently, we could show that oral mucosal myeloid CD1a(+) DC (omDC) differ from their skin counterparts especially by the expression of high affinity receptor for immunoglobulin E (IgE; FcepsilonRI). However, expression pattern of FcepsilonRI and phenotypical characteristics of CD1a(+) nmDC have not been elucidated in detailed yet. **METHODS:** We performed detailed phenotypical comparison of nmDC and omDC of atopic and nonatopic individuals. **RESULTS:** As reported for omDC, FcepsilonRI on nmDC of atopic donors was elevated and mostly occupied by IgE while FcepsilonRI was present only in low amounts on nmDC of nonatopic donors. Nevertheless, the highest FcepsilonRI expression has been observed on omDC. Furthermore, significant amounts of costimulatory molecules CD40, CD80 and CD86 could be detected on nmDC that expressed more CD80 compared with omDC. Moreover, nmDC displayed less major histocompatibility complex (MHC) class I and II molecules than omDC. In addition, nmDC expressed more C-type lectins CD205, CD206 as well as myeloid marker CD11b while omDC displayed increased expression of CD207 and lipopolysaccharide (LPS) receptor CD14. **CONCLUSION:** Together these data imply that nmDC phenotypical differ from omDC which might result in diverse functional properties and might be of relevance for selecting routes for immunotherapy of atopic diseases. Moreover these data provide a basis for further studies investigating immunological mechanisms underlying mucosal immunotherapy.

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BACKGROUND: The oral mucosa represents a unique immunologic unit with a high frequency of native allergen contact within the gastrointestinal tract in which immune tolerance is the natural outcome of allergen contact. Although Langerhans' cells (LC), known to play a crucial role in initiating allergen-dependent immune responses in the skin, have also been detected in the oral mucosa, little is known about their phenotype and exact physiologic role. **OBJECTIVE:** To elucidate whether LC from oral mucosa (oLC) differ from skin LC (sLC), these cells were subjected to detailed comparative analysis. **METHODS:** Crude epidermal and oral mucosa cell suspensions were prepared by trypsinization. oLC and sLC were compared phenotypically by flow cytometry techniques and functionally in T-cell proliferation assays. **RESULTS:** In contrast to sLC, freshly isolated oLC expressed significantly higher amounts of MHC class I and II, as well as costimulatory molecules CD40, CD80/B7.1, and CD86/B7.2. oLC displayed FcγRIII/CD16 and FcγRI/CD64. Most surprisingly, oLC constitutively expressed the high affinity receptor for IgE (FcεRI) even in nonatopic donors. FcεRI expression on oLC was further increased and correlated with the serum IgE levels in atopic individuals. oLC showed a higher allogeneic stimulatory activity than sLC, whereas the activation of autologous T cells correlated to the FcεRI expression. **CONCLUSION:** Taken together, our results strongly indicate that oLC profoundly differ from their skin counterparts. The constitutive high expression of FcεRI on oLC could point to particular skills of these cells within the regional immune system of the oral mucosa

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Antigen-pulsed dendritic cells (DCs) are used as natural adjuvants for vaccination, but the factors that influence the efficacy of this treatment are poorly understood. We investigated the parameters that affect the migration of subcutaneously injected mouse-mature DCs to the draining lymph node. We found that the efficiency of DC migration varied with the number of injected DCs and that CCR7^{+/+} DCs migrating to the draining lymph node, but not CCR7^{-/-} DCs that failed to do so, efficiently induced a rapid increase in lymph node cellularity, which was observed before the onset of T cell proliferation. We also report that DC migration could be increased up to 10-fold by preinjection of inflammatory cytokines that increased the expression of the CCR7 ligand CCL21 in lymphatic endothelial cells. The magnitude and quality of CD4⁺ T cell response was proportional to the number of antigen-carrying DCs that reached the lymph node and could be boosted up to 40-fold by preinjection of tumor necrosis factor that conditioned the tissue for increased DC migration. These results indicate that DC number and tissue inflammation are critical parameters for DC-based vaccination.

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BACKGROUND: Non-injective routes of immunotherapy are thought to be valuable therapeutic options for respiratory allergy. We investigated the clinical efficacy and the effects of sublingual/oral immunotherapy on conjunctival allergic inflammation in patients with mite-induced respiratory allergy. **METHODS:** We used a double-blind placebo-controlled design. 20 patients with mite-induced rhinoconjunctivitis (six of whom also had mild asthma) were randomly assigned sublingual/oral immunotherapy (n=10) or placebo (n=10) for 2 years. We assessed symptom score by diary cards and inflammatory-cell infiltrate, and expression of intercellular adhesion molecule 1 (ICAM-1) in the conjunctiva after specific allergen challenge at enrollment and after 12 and 24 months of treatment. **FINDINGS:** We found significantly lower symptom scores in the immunotherapy group than in the placebo group in most of the winter months (p=0.05). Compared with the placebo group, inflammatory-cell infiltration after conjunctival challenge, and ICAM-1 expression on conjunctival epithelium decreased significant-

ly in the first year of treatment in the immunotherapy group ($p=0.04$ and $p=0.02$, respectively). These effects were also seen for the minimum persistent inflammation, in symptom-free patients exposed constantly to allergens ($p=0.02$). Serum concentrations of eosinophil cationic protein decreased significantly ($p=0.04$). Immunotherapy was well tolerated and compliance was good. **INTERPRETATION:** Our results suggest that this immunotherapy is clinically effective in rhinoconjunctivitis and that it decreases the immune-mediated inflammatory responses to the allergen.

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Induction of intranasal tolerance prevents the body from eliciting unwanted immune responses against harmless antigens that enter the body through the nasal mucosa. To study the intrinsic capacities of the cervical, nose draining lymph nodes (CLN), which are essential for tolerance induction, genes that are differentially expressed in CLN and not in peripheral lymph nodes (PLN) were characterized. The gene that is predominantly overexpressed in CLN codes for IgG2b. This is confirmed by a higher percentage of IgG2b+ B220+ cells in CLN compared with any PLN. However, this predominance of IgG2b-positive B cells in the CLN is not specific for the lymph node itself but rather determined by the region drained by lymph nodes at the cervical site, as transplanted PLN at these locations show a comparable predominance. It was demonstrated that IgG2b, when compared with IgG1, led to differential activation of dendritic cells (DC) through Fc receptor signalling. The results point to a unique local combination of cells and factors in the nose draining CLN leading to highly specialized immune reactivity. The results point out that predominance of a distinct IgG isotype in a lymphoid environment may lead to highly specialized immune reactivity.

29. Von Bubnoff D, Fimmers R, Bogdanow M, Matz H, Koch S, Bieber T. Asymptomatic atopy is associated with increased indoleamine 2,3-dioxygenase activity and interleukin-10 production during seasonal allergen exposure. *Clin Exp Allergy*. 2004 Jul;34(7):1056-63.

BACKGROUND: Indoleamine 2,3-dioxygenase (IDO) is a tryptophan (TRP)-catabolizing enzyme with regulatory effects on T cells. T cell inhibition is achieved through both TRP depletion and TRP metabolite accumulation in specific local tissue microenvironments. The expression of IDO activity by different types of antigen-presenting cells (APCs) has been shown to play a role in many instances of immunoregulation and tolerance induction. Induction of IDO after the engagement of the high-affinity receptor for IgE, FcεRI, on atopic monocytes has been suggested to regulate T cell responses in atopic disorders. Interleukin-10 (IL-10), a cytokine known for its down-regulatory functions in the immune system, has recently been associated with the stable expression of IDO in mature tolerogenic dendritic cells. **OBJECTIVE:** This study was devised to understand the role of systemic IDO and IL-10 in the prevention of clinical apparent allergy. **METHODS:** The concentration of TRP and the break-down product kynurenine were measured by high-performance liquid chromatography in- and off-season in sera from patients with seasonal allergic rhinitis ($n=12$) and from clinically asymptomatic atopic patients sensitized to specific aeroallergens ($n=12$). Non-atopic (NA) individuals ($n=12$) served as control. The concentration of plasma IL-10 was determined in parallel from these donors by ELISA in- and off-season. **RESULTS:** In clinically unresponsive but aeroallergen-sensitized atopic individuals significantly higher systemic activity of IDO and increased plasma IL-10 levels were found during allergen exposure but not off-season compared to symptomatic atopic individuals with allergic rhinitis and NA individuals. **CONCLUSION:** Enhanced systemic IDO activity as well as increased systemic levels of IL-10 may contribute to the containment of allergic T cell responses and could be involved in the maintenance of a state of clinical unresponsiveness.

30. Aoyama-Kondo T, Yoshida T, Tsobe K, Nakayama A, Asai J, Oka T, Nakashima I. Characterization of antibody responses of local lymph nodes to antigen given under the oral submucosa. *Immunobiology*. 1992 Apr;184(4-5):372-83.

We studied the function of submandibular lymph nodes (MLN) in the oral mucosa immune system as compared with that of inguinal lymph nodes (ILN) in the cutaneous one. Primary IgM, IgG and IgA antibody responses in MLN to sheep red blood cells (SRBC) as a model antigen given submucosally occurred more extensively than those in ILN to the antigen injected subcutaneously. Particularly, definite IgA synthesis was seen in MLN but not in ILN. This IgA synthesis was shown to be originated locally in oral submucosal lymphoid tissue or MLN but not in gut-associated lymphoid tissue (GALT). This suggested that the oral mucosal tissue including MLN acts like Peyer's patches in GALT for IgA synthesis. When mice were administered with SRBC and bacterial lipopolysaccharide (LPS) submucosally, the adjuvant effect of LPS was only observed on the capacity of MLN cells for secondary antibody response in vitro. This contrasted to the marked augmentation by LPS of both the primary antibody response in ILN and capacity for in vitro secondary antibody response of ILN cells of mice given SRBC and LPS subcutaneously. The radioactivities detected in the local lymph nodes and other tissues of mice given ⁵¹Cr labeled SRBC submucosally or subcutaneously were comparable with each other. MLN, however, contained more Ig+/B220+ B cells and less Thyl+/Ly-1+ T cells than ILN did, and the L3T4/Ly-2 ratio of T cell subpopulations in MLN was lower than that in ILN. Partially corresponding to this observation, the B cell-dependent area was developed more extensively in MLN than in ILN. This difference in cellular composition and organization might in part explain the reason why MLN and ILN display distinct modes of response and sensitivity to the action of LPS.

31. Bahceciler NN, Arikan C, Taylor A, Akdis M, Blaser K, Barlan IB, Akdis CA. Int Arch Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Allergy Immunol*. 2005 Mar;136(3):287-94. Epub 2005 Feb 16.

OBJECTIVE: To evaluate the clinical outcome and changes in allergen-specific antibodies during sublingual immunotherapy (SLIT) in house dust mite (HDM)-allergic asthma patients and to compare levels of allergen-specific antibodies in HDM-allergic patients before and after treatment with that of healthy controls. **METHOD:** Thirty-one asthma patients allergic to HDM were studied. Patients in groups I (n=17) and II (n=14) received SLIT with a standardized *Dermatophagoides pteronyssinus* plus *Dermatophagoides farinae* 50/50 extract for 6 and 12 months, respectively. A group of healthy children (n=8) were enrolled as controls. Patients in both groups were evaluated at the start and at the end of treatment according to daily symptom and medication scores, lung function and skin prick tests, PC20, blood eosinophil count, and Der-p-1-specific IgE, IgA, IgG1 and IgG4 levels. **RESULTS:** Drug consumption decreased significantly in both groups. Furthermore, PC20 and forced expiratory flow between 25 and 75% of vital capacity of patients in group II improved significantly. Although specific IgA, IgG1 and IgG4 levels did not change throughout the treatment period, total eosinophil count and specific IgE decreased significantly in both groups. According to baseline measurements, specific IgA levels of patients in groups I and II were significantly lower than that of controls. This difference disappeared at the end of the treatment period in both groups. **CONCLUSION:** SLIT seems to be effective in ameliorating clinical symptoms, drug consumption and bronchial hyperreactivity, and results in downregulation of Der-p-1-specific IgE production. Furthermore, at the end of SLIT, specific IgA levels, which were decreased compared to healthy controls initially, did no longer differ between patients and controls. Copyright (c) 2005 S. Karger AG, Basel.

32. Ciprandi G, Fenoglio D, Cirillo I, Vizzaccaro A, Ferrera A, Tosca MA, Puppo F. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol*. 2005 Jul;95(1):38-44.

BACKGROUND: Subcutaneous specific immunotherapy has been demonstrated to be capable of inducing T-cell regulatory response. Interleukin 10 (IL-10) plays a crucial role in inducing allergen-specific tolerance; however, no previous studies have examined IL-10 production after sublingual immunotherapy (SLIT). **OBJECTIVE:** To evaluate T-cell proliferation and IL-10 production in patients successfully treated with SLIT for house dust mites (HDMs). **METHODS:** Peripheral blood mononuclear cells were isolated from patients after at least 3 years of successful HDM SLIT and from matched untreated allergic patients and healthy control subjects. After 3 and 6 days of in vitro stimulation with phytohemagglutinin (PHA), *Candida albicans*, and *Dermatophagoides farinae*, proliferation and production of IL-10 were measured. **RESULTS:** Patients treated with SLIT showed a significant reduction of proliferation induced by *C. albicans* compared with untreated atopic patients ($P < .001$), but a significant reduction was also demonstrated in healthy controls compared with untreated atopic patients ($P < .001$). Patients treated with SLIT also showed a significant increase of IL-10 production after *Candida* and PHA stimuli compared with patients with untreated rhinitis ($P < .001$ for both). Patients with untreated rhinitis did not produce IL-10. **CONCLUSION:** This preliminary study confirms reduced T-cell proliferation and preliminarily provides the first evidence, to our knowledge, of peripheral IL-10 production in allergic patients successfully treated with HDM SLIT.

33. Ciprandi G, Fenoglio D, Cirillo I, Milanese M, Minuti P. Sublingual Immunotherapy and Tregs. *Allergy* 2006;61: 511-3.

34. Fanta C, Bohle B, Hirt W, Siemann U, Horak F, Kraft D, Ebner H, Ebner C. Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int Arch Allergy Immunol.* 1999 Nov;120(3):218-24.

Twenty-four patients suffering from grass pollen allergy underwent sublingual immunotherapy (SLIT) with standardized grass pollen extract for 1 year. In order to investigate immunological changes induced by the administration of allergens via the oral mucosa, the SLIT-spit method was applied. The cumulative dose of approximately 80 microg of major allergen (grass group 5 allergen), was relatively low. During the time of treatment, we could observe a significant increase in the levels of specific IgG and IgG4 antibodies. However, the titers of allergen-specific IgE antibodies showed a significant increase in the course of SLIT as well. Analyzing lymphoproliferative responses, a significant decrease in reactivity in response to stimulation with complete grass pollen extract ($p = 0.001$) and to recombinant Phl p 1 (a major allergen of timothy grass, $p < 0.001$) could be observed, indicating the induction of immunological tolerance. Proliferative responses to a control antigen (tetanus toxoid) were not influenced by the treatment. At different time points during SLIT, allergen (Phl p 1)-specific T cell clones (TCC) were established from the peripheral blood of the patients. Cytokine production by allergen-stimulated T cells did not reveal any changes consistent with immune deviation, i.e. the ratio of Th1/Th2 TCC did not change during SLIT. In conclusion, we provide evidence that sublingual treatment leads to systemic changes in immunoreactivity to the administered allergen.

35. Fenoglio D, Puppo F, Cirillo I, Vizzaccaro A, Ferrera A, Tosca MA, Marseglia G, Ciprandi G. Sublingual specific immunotherapy reduces PBMC proliferations. *Allergy Immunol (Paris).* 2005 Apr;37(4):147-51.

BACKGROUND: Subcutaneous specific immunotherapy has been demonstrated capable of inducing T regulatory response. There is few evidence concerning immunological changes induced by sublingual immunotherapy. **OBJECTIVE:** The aim of this study was to evaluate T cell proliferation in subjects successfully treated with SLIT for HDM. **METHODS:** PBMCs were isolated from patients after at least 3 years of successful HDM SLIT and from matched untreated allergic and healthy control subjects. After 3 and 6 days of in vitro stimulation with PHA, *Candida albicans*, *Dermatophagoides farinae*, grasses, *Parietaria judaica*, and cat, prolifera-

tion. **RESULTS:** Subjects treated with SLIT showed significant reduction of proliferation induced by *Candida albicans*, *Parietaria*, and grasses in comparison with untreated atopics ($p=0.0002$, 0.0033 , and 0.009 respectively). **CONCLUSION:** This pilot study confirms reduced T cell proliferation in allergic subjects treated with SLIT.

36. Marcucci F, Sensi L, Frati F, Bernardini R, Novembre E, Barbato A, Pecora S. Effects on inflammation parameters of a double-blind, placebo controlled one-year course of SLIT in children monosensitized to mites. *Allergy* 2003 Jul;58(7):657-62.

BACKGROUND: Clinical documentation about effects on local markers of inflammation of sublingual immunotherapy (SLIT) in children is still poor. **METHODS:** Twenty-four children (age range 4-16 years, average 8.5 years) monosensitized to house dust mites (HDMs) were randomized to receive active or placebo SLIT for this allergen according to a double-blind, placebo-controlled design. Before treatment and 10-12 months later the following parameters were checked: ECP and tryptase in sputum and nasal secretion, serum and nasal mite-specific IgE (sIgE), allergen-specific nasal challenge test (sNCT), nasal symptoms and tryptase after sNCT. **RESULTS:** Nasal tryptase and nasal IgE in basal conditions were unchanged in treated children but significantly increased in untreated children ($P = 0.0156$ and $P = 0.0313$, respectively). The threshold for sNCT was unchanged in both groups of children, but the symptom score after sNCT was unchanged in the placebo group and significantly decreased in the active group ($P = 0.0084$). The nasal tryptase after sNCT was unchanged in the active group and significantly increased in the placebo group ($P = 0.0218$). Intergroup comparison showed a significant difference in oral tryptase and nasal tryptase after sNCT in favour of the active group. **CONCLUSIONS:** These interim results after only 1 year of treatment show that SLIT in children monosensitized to HDMs is able to avoid the spontaneous increase in both nasal sIgE antibodies and in local allergic inflammation in basal conditions. These outcomes are confirmed and supported by the decrease of symptoms in the active group combined with the increase of nasal tryptase only in the control group in both cases after sNCT.

37. Marcucci F, Sensi L, Frati F, Senna GE, Canonica GW, Parmiani S, Passalacqua G. Sublingual tryptase and ECP in children treated with grass pollen sublingual immunotherapy (SLIT): safety and immunologic implications. *Allergy*. 2001 Nov;56(11):1091-5.

BACKGROUND: The clinical safety of sublingual immunotherapy (SLIT) has been repeatedly confirmed; nevertheless, the possible onset of local oral symptoms is still a concern, and nothing is known about the pathogenesis of this effect. We aimed to determine whether the administration of SLIT in allergic children can evoke an IgE-mediated reaction, by measuring the levels of sublingual tryptase and ECP. **METHODS:** Thirty children (7-12 years old) with allergic rhinitis/asthma due to grass pollen were prescribed SLIT. In these children, an allergen-specific nasal challenge was performed, and nasal tryptase and ECP were measured before and after. Sublingual ECP and tryptase were also assessed before the SLIT, after 1 month, and after 6 months of treatment. Ten matched allergic children and 10 healthy ones served as controls for the baseline levels of sublingual ECP and tryptase. **RESULTS:** The levels of nasal tryptase and ECP significantly increased after nasal challenge ($P<0.001$), whereas no change during the SLIT course (at the beginning, after 1 month, and after 6 months) could be detected in sublingual tryptase either before or after SLIT administration. The sublingual ECP significantly decreased after 6 months of SLIT. The baseline levels of nasal tryptase and ECP were significantly higher in allergic subjects than in healthy controls, as was the level of sublingual ECP. **CONCLUSIONS:** In the presence of an IgE-mediated reaction (ASNC), a significant increase of tryptase and ECP can be seen. When SLIT is administered, such a phenomenon does not occur; therefore, SLIT does not elicit any IgE reaction in the mouth. It is noteworthy that allergic subjects display higher levels of nasal ECP and tryptase than healthy subjects, even when symptom-free, and these observations may indicate the presence of subclinical inflammation.

38. Passalacqua G, Albano M, Fregonese L, Riccio A, Pronzato C, Mela GS, Canonica GW. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet*. 1998 Feb 28;351(9103):629-32.

BACKGROUND: Non-injective routes of immunotherapy are thought to be valuable therapeutic options for respiratory allergy. We investigated the clinical efficacy and the effects of sublingual/oral immunotherapy on conjunctival allergic inflammation in patients with mite-induced respiratory allergy. **METHODS:** We used a double-blind placebo-controlled design. 20 patients with mite-induced rhinoconjunctivitis (six of whom also had mild asthma) were randomly assigned sublingual/oral immunotherapy (n=10) or placebo (n=10) for 2 years. We assessed symptom score by diary cards and inflammatory-cell infiltrate, and expression of intercellular adhesion molecule 1 (ICAM-1) in the conjunctiva after specific allergen challenge at enrollment and after 12 and 24 months of treatment. **FINDINGS:** We found significantly lower symptom scores in the immunotherapy group than in the placebo group in most of the winter months ($p=0.05$). Compared with the placebo group, inflammatory-cell infiltration after conjunctival challenge, and ICAM-1 expression on conjunctival epithelium decreased significantly in the first year of treatment in the immunotherapy group ($p=0.04$ and $p=0.02$, respectively). These effects were also seen for the minimum persistent inflammation, in symptom-free patients exposed constantly to allergens ($p=0.02$). Serum concentrations of eosinophil cationic protein decreased significantly ($p=0.04$). Immunotherapy was well tolerated and compliance was good. **INTERPRETATION:** Our results suggest that this immunotherapy is clinically effective in rhinoconjunctivitis and that it decreases the immune-mediated inflammatory responses to the allergen.

39. Bousquet J, Scheinmann P, Guinnee MT, Perrin-Fayolle M, Sauvaget J, Tonnel AB, Pauli G, Caillaud D, Dubost R, Leynadier F, Vervloet D, Herman D, Galvain S, André. Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy*. 1999 Mar;54(3):249-60.

A double-blind, placebo-controlled study was carried out in 85 patients with a well-documented history of perennial asthma caused by house-dust mites. Patients received either placebo or sublingual immunotherapy (SLIT) with a standardized *Dermatophagoides pteronyssinus* (DP)-*D. farinae* (DF) 50/50 extract. After a run-in period, patients received increasing doses up to 300 IR every day for 4 weeks and then three times a week for the following 24 months. The cumulative dose was about 104000 IR, equivalent to 4.2 mg Der p 1 and 7.3 mg Der f 1. Symptom and medication scores and respiratory function were assessed throughout the trial. Serum specific IgE and IgG4 were determined before SLIT (t_0) and after 6 (t_1), 11 (t_2), 17 (t_3), and 25 months (t_4) of SLIT. Mite exposure was evaluated at t_0 , t_2 , and t_4 by semiquantitative guanine determinations. Patients aged 15 years and older were asked to assess their quality of life (QoL) by completing the SF20 (Short Form Health Status Survey) plus two items at t_0 , t_2 , and t_4 . Use of inhaled corticosteroids and beta2-agonists was significantly decreased after 25 months of treatment in both groups ($P<0.03$). SLIT patients showed significant improvements in respiratory function at t_4 (% predicted FEV1 ($P = 0.01$), VC ($P = 0.002$), morning ($P = 0.01$) and evening ($P = 0.03$) PEFR), and reduction in daytime asthma score ($P = 0.02$). In the SLIT group, the post-treatment PD20 was 1.75 times higher than the baseline value. There was no change in PD20 in the placebo group. Compared to the placebo group, the SLIT group showed a significant increase in specific IgE DP ($P = 0.05$), IgE DF ($P = 0.02$), IgG4 DP ($P = 0.001$), and IgG4 DF ($P = 0.001$) levels after SLIT. QoL scores were similar in both groups at t_0 and t_2 . At t_4 , all scores were better in the SLIT group than in the placebo group, with the differences being most marked for the general perception of health ($P = 0.01$) and physical pain ($P = 0.02$). Adverse events were similar in the two groups. This study shows that SLIT in house-dust-mite-related asthma has a good safety profile and improves respiratory function, bronchial hyperreactivity, and QoL.

40. La Rosa M, Ranno C, André 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 Aug;104(2 Pt 1):425-32

BACKGROUND: Sublingual-swallow immunotherapy was recently recognized in the World Health Organization Position Paper (Allergen immunotherapy: therapeutic vaccines for allergic diseases) "as a viable alternative to parenteral injection therapy to treat allergic diseases" in adults. More controlled studies were required to assess the efficacy and safety of this treatment in children. **OBJECTIVE:** This study was carried out to assess the clinical efficacy and safety profile of sublingual-swallow immunotherapy with high-dose allergen in children with allergies. **METHODS:** We used a double-blind placebo-controlled design. Forty-one children with *Parietaria* -induced rhinoconjunctivitis were randomized to receive sublingual standardized *Parietaria judaica* extract (n = 20) or placebo (n = 21) for 2 years. The cumulative dose of allergen was 375 times higher than that used in parenteral immunotherapy and the cumulative dose of Par j 1 major allergen was 52.5 mg over 2 years. The main efficacy assessment criteria were symptoms and rescue medication scores recorded on the patients' diary cards. Secondary criteria were changes in skin and conjunctival specific reactivity as well as blood parameters, analyzed after 1 and 2 years of immunotherapy. The safety of the treatment was assessed by evaluating the frequency and severity of adverse effects. **RESULTS:** A significant reduction in rhinitis symptoms was observed in the active treatment group during the second season ($P = .02$), with no difference in medication scores. A significant decrease in skin reactivity ($P = .002$ after 2 years of treatment) and an increase in the threshold dose for conjunctival allergen provocation test ($P = .02$) were observed in the active treatment group compared with the group receiving placebo. A significant increase in specific IgG(4) levels ($P = .02$) was also observed in the active group. Immunotherapy was well tolerated. **CONCLUSION:** Sublingual-swallow immunotherapy in *Parietaria* -allergic children provided a clinical benefit and a decreased specific reactivity to the allergen. The safety profile of this treatment, which constitutes an important issue, indicated good tolerance and compliance.

41. Clavel R, Bousquet J, André C. Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. *Allergy*. 1998 May;53(5):493-8.

Sublingual-swallow immunotherapy (SLIT) using high doses of standardized allergen extracts has been found to be effective in reducing allergic symptoms and medication needs. A double-blind, placebo-controlled study was carried out in a large number of patients to determine whether medication needs can be reduced by SLIT. Some 136 patients with grass-pollen rhinitis with or without mild asthma were studied. Patients received either placebo or SLIT with a standardized grass-pollen extract administered daily with increasing doses up to 300 IR (index of reactivity) from January to the end of July 1994. During the grass-pollen season, patients were instructed to use medications as required and to visit their doctors in case of asthma. Symptom-medication scores were assessed during the pollen season, and serum-specific IgG4 was measured before and at the end of SLIT. In the SLIT group, drug consumption dropped significantly throughout the pollen season ($P < 0.02$). Moreover, at the peak of the pollen season, betamethasone consumption was significantly reduced in the SLIT group ($P < 0.02$). Only one patient in the SLIT group had an asthma attack compared to eight patients in the placebo group ($P < 0.02$). IgG4 levels increased significantly in the SLIT group ($P < 0.001$) but without correlation with symptoms. Side-effects were comparable in both groups. This study indicates that SLIT in grass-pollen rhinitis is well tolerated, improves overall clinical symptoms, and reduces drug consumption and the need for oral corticosteroids.

42. Cosmi L, Santarlaschi V, Angeli R, Liotta F, Maggi L, Frosali F, et al. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-gamma- and interleukin-10-production. Clin Exp Allergy. 2006 Mar;36(3):261-72.

BACKGROUND: The clinical efficacy and safety of sublingual immunotherapy (SLIT) for aeroallergens has been demonstrated in several trials, whereas the immunological changes induced by this treatment, which may account for the clinical improvement, are still unclear. **OBJECTIVE:** To investigate the effects of a successful SLIT on the in vitro allergen-driven T cell response and cytokine secretion as well as on the serum levels of chemokines and of IgE, IgG1 and IgG4 antibodies (Abs). **MATERIALS AND METHODS:** Twenty-five Dermatophagoides pteronyssinus (Dp)-sensitive patients with perennial rhinitic and/or rhinitic and asthmatic symptoms were randomized into two groups (13 untreated (UT) and 12 SLIT-treated) for a 1 year and half study. The proliferative response of peripheral blood mononuclear cell (PBMC) to purified Der p1 allergen, their cytokines (IFN-gamma, IL-4, IL-10 and TGF-beta) production and serum levels of chemokines associated with T helper type 1 (Th1) (CXCL10) or T helper type 2 (Th2) (CCL22) responses and of Dp-specific IgE, IgG1 and IgG4 Abs were evaluated before and after 6 months of treatment. **RESULTS:** SLIT induced a significant reduction of symptom medication scores after 6, 12 and 18 months of treatment in comparison with UT patients. SLIT-treated patients showed a significant decrease in serum levels of DP-specific IgE Abs, whereas total IgE, and specific IgG1 and IgG4 Abs remained unchanged. The proliferative response of allergen-specific T cells to Der p1 in vitro after 6 months of treatment was reduced, while no effect was observed on T cell proliferation to recall antigen (streptokinase). Moreover, Der p1-driven IFN-gamma and IL-10 were significantly increased in culture supernatants of PBMC from 6 month-treated patients in comparison with those detected at the beginning of therapy. **CONCLUSIONS:** These data suggest that the allergen-driven enhancement of IL-10- and IFN-gamma-producing T cells precedes and associates with SLIT-induced down-regulation of specific IgE, thus providing a rationale to explain the clinical benefit of SLIT in allergic patients.

43. Ippoliti F, De Santis W, Volterrani A, Lenti L, Canitano N, Lucarelli S, Frediani T. Immunomodulation during sublingual therapy in allergic children. Pediatr Allergy Immunol. 2003 Jun;14(3):216-21.

The clinical efficacy of sublingual immunotherapy (SLIT) has been demonstrated, but its mechanism of action is still controversial. The most recent experimental observations suggest that a critical role in the modulation of immune response is sustained by Th2 cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13, by co-stimulatory molecules, such as CD40 on B cells, and by hormones and neuropeptides. To better understand whether SLIT affects immune responses we used a double-blind placebo-controlled design. Eighty-six children with mild asthma due to allergy to Dermatophagoides pteronyssinus (33 of whom also had rhinoconjunctivitis) were randomly assigned SLIT (n = 47) or placebo (n = 39). We assessed symptom scores using diary cards of each patient and determined the expression of CD40 on B cells and the serum concentration of ECP, IL-13, prolactin (PRL) and ACTH at enrolment and after 6 months of therapy. We observed a significant reduction in asthma and rhinitis scores in the immunotherapy group compared with the placebo group, no variation in CD40 and ACTH, but a significant decrease in ECP, IL-13 and PRL after 6 months of therapy (p <0.01). Our results confirm the efficacy and safety of SLIT, and lead us to believe that it could modulate the synthesis of Th2 cytokines, as revealed from the decrease of IL-13. In addition, the reduction of PRL might be a signal of reduced activation of T lymphocytes.

44. Kinaciyan T, Jahn-Schmid B, Radakovics A, Zwolfer B, Schreiber C, Francis JN, et al. Successful sublingual immunotherapy with birch pollen has limited effects on concomitant food allergy to apple and the immune response to the Bet v 1 homolog Mal d 1. J Allergy Clin Immunol. 2007 Apr;119(4):937-43.

BACKGROUND: Cross-reactivity between the major birch pollen allergen, Bet v 1, and the apple protein, Mal d 1, frequently causes food allergy. **OBJECTIVE:** To investigate the effects of successful sublingual immunotherapy (SLIT) with birch pollen extract on apple allergy and the immune response to Bet v 1 and Mal d 1. **METHODS:** Before and after 1 year of SLIT, Bet v 1-sensitized patients with oral allergy syndrome to apple underwent nasal challenges with birch pollen and double-blind placebo-controlled food challenges with apple. Bet v 1-specific and Mal d 1-specific serum antibody levels and proliferation in PBMCs and allergen-specific T-cell lines (TCLs) were determined. Bet v 1-specific TCLs were mapped for T-cell epitopes. **RESULTS:** In 9 patients with improved nasal provocation scores to birch pollen, apple-induced oral allergy syndrome was not significantly reduced. Bet v 1-specific IgE and IgG(4) levels significantly increased. Bet v 1-specific T-cell responses to all epitopes and those cross-reactive with Mal d 1 significantly decreased. However, neither Mal d 1-specific IgE and IgG(4) levels nor Mal d 1-induced T-cell proliferation changed significantly. In contrast, Mal d 1-specific TCLs showed increased responses to Mal d 1 after 1 year of SLIT. **CONCLUSION:** This longitudinal study indicates that pollen SLIT does not efficiently alter the immune response to pollen-related food allergens, which may explain why pollen-associated food allergy is frequently not ameliorated by pollen immunotherapy even if respiratory symptoms significantly improve. **CLINICAL IMPLICATIONS:** SLIT with birch pollen may have no clinical effect on associated apple allergy.

45. Passalacqua G, Albano M, Riccio A, Fregonese L, Puccinelli P, Parmiani S, Canonica GW. Clinical and immunologic effects of a rush sublingual immunotherapy to *Parietaria* species: A double-blind, placebo-controlled trial. *J Allergy Clin Immunol.* 1999 Nov;104(5):964-8.

BACKGROUND: The local (noninjection) routes of immunotherapy are presently regarded as viable therapeutic options for respiratory allergy, and their mechanisms of action are currently undergoing investigation. **OBJECTIVE:** We evaluated the clinical efficacy of a preseasonal rush sublingual-swallow immunotherapy and its effects on allergic inflammation in patients with seasonal rhinoconjunctivitis caused by *Parietaria* species. **METHODS:** Thirty patients with *Parietaria* species-induced rhinoconjunctivitis (13 with mild intermittent asthma) were randomly assigned sublingual-swallow immunotherapy or placebo in a rush preseasonal course. We assessed the seasonal symptom-drug intake score by diary card and the inflammatory infiltration and the intercellular adhesion molecule 1 expression on nasal epithelium after specific allergenic challenge before and after treatment. **RESULTS:** The investigated immunotherapy was well tolerated, and no side effects were recorded. A significant reduction of the symptom score ($P = .016$) and drug intake score ($P = .008$) after immunotherapy was observed only in the active group. A decrease of the cumulative score was observed also in the placebo group ($P = .046$), but the significance was clearly higher ($P = .006$) in the active group. In the active group a reduction of neutrophils ($P = .001$), eosinophils ($P = .01$), and intercellular adhesion molecule 1 expression ($P = .04$) after specific nasal challenge was also detected. **CONCLUSION:** The present results suggest that this sublingual-swallow immunotherapy administered through a rush schedule is clinically effective and safe and that it decreases the immune-mediated inflammatory responses to the allergen.

46. Savolainen J, Jacobsen L, Valovirta E. Sublingual immunotherapy in children modulates allergen-induced in vitro expression of cytokine mRNA in PBMC. *Allergy.* 2006 Oct;61(10):1184-90.

BACKGROUND: During subcutaneous immunotherapy (SCIT), there is a local mucosal shift from Th2 to Th1 type cytokine predominance and downregulation of interleukin (IL)-5 and eosinophilia. According to recent studies IL-10- and transforming growth factor (TGF)- β -induced tolerance is another key phenomenon in SCIT. Few data to date is available on mech-

anisms and roles of these cytokines in sublingual immunotherapy (SLIT). SCOPE: This study was undertaken to analyse the allergen-induced in vitro mRNA expression of IL-4, IL-5, IL-10, TGF-beta and interferon (IFN)-gamma during SLIT in peripheral blood mononuclear cells (PBMC) of children with allergic rhinitis (AR). METHODS: Ten patients with AR undergoing pollen SLIT with a weekly dose of 200,000 SQ-U, 10 with a weekly dose of 24,000 SQ-U of glycerinated mixture of *Betula verrucosa*, *Corylus avellana* and *Alnus glutinosa* and 10 with placebo were included in the study. Peripheral blood mononuclear cell samples were collected and stimulated with pollen allergen extract prior to the treatment, after 1 and 2 years of the treatment. The cytokine mRNA expression was assessed using kinetic real time reverse transcription polymerase chain reaction (RT-PCR; TaqMan). RESULTS: The in vitro allergen-induced mRNA expression of IL-5 by PBMC in the placebo group at 1 ($P = 0.0065$) and 2 ($P = 0.013$) years of therapy were increased in comparison with the highest dose. The expression of IL-10 mRNA was increased in the highest dose group ($P = 0.0016$) and the lower dose group ($P = 0.034$) at 2 years of therapy when compared with placebo. The change in the expression of allergen-induced TGF-beta had an inversed correlation with the change of IL-5 ($r = -0.38$, $P = 0.036$) and positive correlation with the change of IL-10 ($r = 0.58$, $P = 0.0019$). CONCLUSIONS: Sublingual immunotherapy induced a dose-dependent systemic allergen-specific immunological response in children with AR. During high-dose SLIT, there was activation of regulatory cytokine IL-10 and an inhibitory effect on IL-5 expression increase that was associated with TGF-beta.