

Induction of Allergen-Specific Tolerance via Mucosal Routes

Laurent Mascarell, Aline Zimmer, Laurence Van Overtvelt,
Sophie Tourdot and Philippe Moingeon

Abstract Allergen-specific immunotherapy is the only curative treatment of allergies against insect venom, house dust mites, tree/grass pollens, or cat dander. Subcutaneous immunotherapy is successful to reorient the immune system and re-establish long-term tolerance. However, major drawbacks for using this route include: repeated injections, as well as the risk of anaphylaxis. In this context, alternative mucosal routes of administration are being considered together with the combined use of adjuvants/vector systems and recombinant allergens or peptide fragments. Herein, we review the current status in the use of mucosal routes (i.e., sublingual, oral, intranasal) for allergen-specific immunotherapy, as well as the latest understanding with respect to underlying mechanisms of action.

L. Mascarell · A. Zimmer · L. Van Overtvelt · S. Tourdot · P. Moingeon (✉)

Research and Development, Stallergènes, 6 rue Alexis de Tocqueville,
92160 Antony, France

e-mail: pmoingeon@stallergenes.fr

L. Mascarell

e-mail: lmascarell@stallergenes.fr

A. Zimmer

e-mail: azimmer@stallergenes.fr

L. Van Overtvelt

e-mail: lvanovervelt@stallergenes.fr

S. Tourdot

e-mail: stourdot@stallergenes.fr

Contents

1	Introduction.....	86
2	Tolerance Induction by Mucosal Immunotherapy	87
2.1	General Comments	87
2.2	Intranasal Immunotherapy	88
2.3	Oral Immunotherapy.....	93
2.4	Sublingual Immunotherapy	95
3	Interest in Adjuvants and Vector Systems for Mucosal Immunization	96
4	Conclusions.....	97
	References	98

1 Introduction

The first successful studies of allergen-immunotherapy were conducted in patients with hay fever in 1911, by Noon et al. As of today, many double blind, placebo-controlled (DBPC) studies have shown that subcutaneous allergen-specific immunotherapy (SCIT) is effective in decreasing both immediate and late-phase symptoms as well as the need for medication, when applied to a variety of type I allergies involving either insect venom, house dust mites, grass and tree pollens, or animal epithelia (Bousquet et al. 1998; Larche 2000; Gehlhar et al. 1999; Michils et al. 1998; Pichler and Pichler 2001; Moller et al. 2002; Powell et al. 2007). In terms of mechanisms, successful SCIT is often associated with a decrease in seric allergen-specific IgE antibodies together with an increase in IgG4, including potential blocking antibodies (Wachholz 2003; Pierson-Mullany et al. 2000). Successful SCIT redirects allergen-specific Th2 CD4⁺ cells (producing IL4, IL5, and IL13) (Durham and Till 1998; El Biaze et al. 2003) toward the Th1 type (secreting IL12/IFN γ) (Mavroleon 1998; Hamid et al. 1997; Benjaponpitak et al. 1999; Ebner et al. 1997; Secrist et al. 1993). In addition, SCIT promotes allergen-specific CD4⁺ regulatory T cells (i.e., IL10-producing Tr1 or CD4⁺CD25⁺ cells) (Robinson et al. 2004; Meiler et al. 2008). Several drawbacks currently limit the application of SCIT. First of all, repeated injections make this treatment cumbersome, most particularly for children. In addition, systemic reactions to SCIT, albeit infrequent, may occur, which include anaphylactic shocks (Medicines 1986). In this context, tolerance induction via non-invasive mucosal routes has raised considerable interest over the last two decades. Also, various non-mutually exclusive immune mechanisms have been discovered including (i) the development of active suppression via regulatory T cells, (ii) the inhibition of inappropriate immune responses through cellular anergy or clonal deletion, (iii) the generation of blocking antibodies (Faria and Weiner 2005, 2006a, b).

Herein, we review the pertinence of various mucosal routes (i.e., nasal, oral, and sublingual) of allergen administration as alternatives to SCIT. Out of these mucosal routes, only the sublingual one has clearly been documented as

efficacious for establishing antigen-specific tolerance in humans, in the context of large double blind placebo-controlled (DBPC) clinical studies conducted both in adults and children. Immune mechanisms associated with successful specific mucosal immunotherapy are discussed in the context of new strategies based on vectors/adjuvant molecules in order to better target antigen-presenting cells with tolerogenic capacity.

2 Tolerance Induction by Mucosal Immunotherapy

2.1 General Comments

Increasing evidence suggests that dendritic cells (DCs) play a crucial role in the regulation of mucosal immunity and tolerance, as a consequence of their capacity to direct the fate of T cells. The physiology of mucosal APCs, most particularly their capacity to support the differentiation of regulatory T cells, likely explains why the default response is usually tolerogenic at mucosal surfaces. The level of costimulatory molecules as well as the cytokine milieu influence the degree of antigen-presenting cell (APC) maturation and activation, leading to systemic or suppressive T cell responses (MacPherson et al. 2004). In most circumstances, mature DCs (mDCs) trigger specific effector T cells, whereas immature or semi-mature DCs (iDCs or smDCs) rather induce regulatory T cells, clonal deletion or hypo responsiveness (Steinman et al. 2003; Hawiger et al. 2001). Most particularly, iDCs continuously capture, transport, and present self-antigens as well as micro-environmental proteins from mucosal tissues to lymphoid organs to maintain tolerance (Pugh et al. 1983; Liu et al. 1998; Huang et al. 2000). Recent studies suggest that IL10-producing iDCs prime immunological tolerance through Tr1⁺ regulatory T cells in both mice and humans (Jonuleit et al. 2000; Menges et al. 2002).

APCs from the lamina propria of the small intestine, including CD11b⁺ CD11c⁺ DCs and CD11b⁺ F4/80⁺ CD11c⁻ macrophages, promote the development of extrathymic Foxp3⁺ Treg due to the presence of TGF β and retinoic acid (RA), i.e., a vitamin A metabolite highly expressed in gut-associated lymphoid tissue (GALT) (Sun et al. 2007; Denning et al. 2007). Together, these data demonstrate that the intestinal immune system has evolved to promote tolerance over inflammation. Although the oral immune system is prone as well to induce specific tolerance (Iwasaki and Kelsall 1999; Mascarell et al. 2008; Sun et al. 2008), it also comprises local effector mechanisms in the form of resident mucosal CD4⁺ T cells able to promote Th1, Th2 and Th17 patterns of polarization upon polyclonal activation (Mascarell et al. 2009). In agreement with these observations, recent data further demonstrate that the oral immune system retains the capacity to promote protective Th1 effector responses in draining lymph nodes (Cuburu et al. 2007; Song et al. 2008), and that IL17 producing CD4⁺ T cells are found within the oral cavity (Conti et al. 2009).

2.2 Intranasal Immunotherapy

Biodistribution studies in humans of radiolabeled *Parietaria judaica* allergen (Par j 1) following intranasal administration suggest that the allergen quickly disappears from the nasal cavity, is transported to the pharynx, and then absorbed through the gastrointestinal tract, reaching a plasmatic peak between 1.5 and 3 h after administration (Bagnasco et al. 1997; Passalacqua et al. 2005). Interestingly, nasal clearance is superior in allergic patients when compared to healthy volunteers, suggesting an IgE-mediated capture mechanism in Par j 1 sensitized patients.

Local nasal immunotherapy (LNIT) has been explored during the last three decades, with some encouraging results, both in terms of safety and efficacy in patients with allergic rhinitis (Canonica and Passalacqua 2003) (Table 1). Pioneer studies using either natural biological or chemically-modified (allergoid) forms demonstrated a significant reduction in both symptoms and requirement for symptomatic medication in patients allergic to ragweed and grass pollens (Deuschl and Johansson 1977; Nickelsen et al. 1981a, b; Georgitis et al. 1983). However, no cumulative effect of LNIT could be confirmed, since the administration of allergoid grass extracts was ineffective in the second year of treatment (Nickelsen et al. 1983). In addition, macronized powder forms of pollens (e.g., parietaria, birch, and grass) or mites as well as strips coated with mite extracts have been used in LNIT and shown to improve clinical efficacy, while reducing local side effects in patients with rhinitis (Andri et al. 1992, 1993, 1996; D'Amato et al. 1995; Andri and Senna 1995; Bellussi et al. 2002; Tsai et al. 2009). Some clinical studies suggested a long lasting protective effect (i.e., up to three or four years after the end of the treatment), most particularly on nasal symptoms (Passalacqua et al. 1997; Andri and Falagiani 2003). Adverse effects were rarely observed (Georgitis et al. 1983) and were limited to the upper respiratory tract (Andri et al. 1992).

Regarding immunological changes associated in humans with LNIT, some studies have shown an increase in serum or nasal IgE, IgG, and IgA antibodies, (Deuschl and Johansson 1977; Georgitis et al. 1983; Nickelsen et al. 1981a, b) whereas others failed to detect any differences in antibody responses (D'Amato et al. 1995). Locally, a reduction of inflammatory cellular infiltrates together with a decrease in histamine and eosinophil cationic protein (ECP) levels in nasal secretions have been observed (Passalacqua et al. 1997; Andri and Falagiani 2003).

To further decipher immune mechanisms associated with intranasal immunotherapy, murine models of established asthma have been developed using prophylactic or therapeutic intranasal treatment with either allergen extracts (from pollen or mite), purified/recombinant major allergens (Bet v 1, Der p 1) or polypeptide fragments (Bet v 1, Phl p 1, and Phl p 5) alone or together with an adjuvant (CpG-ODN, Flagelin, fungal immunomodulatory protein) or a vector system such as probiotics (e.g., *Lactobacillus plantarum*). In those pre-clinical models, LNIT reduced both airway hyperresponsiveness (AHR), cellular infiltration in lungs, specific seric IgE, the IgG1/IgG2a antibody ratio, and IL5 production in T cells.

Table 1 Comparative properties of mucosal immunization routes

Route of immunization	Allergens	Comments	References
Local nasal immunotherapy (LNIT)	Pollens (ragweed, grasses, parietaria, birch), house dust mites	Clinical efficacy: Local nasal immunotherapy (LNIT) is safe and induces a reduction in both symptoms and medication scores in patients with allergic rhinitis to house dust mite (HDM) and pollens (tree and grass). Chemically modified allergens (allergoids) are better-tolerated than aqueous extracts, but the therapeutic benefit is decreased. Some studies demonstrate a long lasting protective effect on nasal symptoms (grass allergy; three to four years after the end of the treatment). Nevertheless, LNIT requires technical skills to control the dose administered, thus impeding its large utilization in allergen-specific immunotherapy	Canonica and Passalacqua (2003); Deuschl and Johansson (1977); Nickelsen et al. (1981a, b, 1983); Georgitis et al. (1983); Andri et al. (1992, 1993, 1996); D'Amato et al. (1995); Andri and Senna (1995); Bellussi et al. (2002); Tsai et al. (2009); Passalacqua et al. (1997); Andri and Falagiani (2003)
	Used as: natural extracts, allergoids, macronized powder forms, strips coated extracts	Immune mechanisms: <i>Antibody responses:</i> LNIT with aqueous extracts or allergoids (ragweed and grass) is associated with an increase in seric or nasal allergen-specific IgA, IgE, and IgG antibodies. <i>Cellular responses:</i> Successful LNIT to parietaria significantly reduces cellular infiltration as well as histamine and eosinophil cationic protein (ECP) nasal secretion	Deuschl and Johansson (1977); Georgitis et al. (1983); D'Amato et al. (1995); Nickelsen et al. (1981a, b) Passalacqua et al. (1997); Andri and Falagiani (2003)
		Pre-clinical models and candidate vaccines: In murine models of established asthma, prophylactic or therapeutic intranasal treatments with either allergen extracts (pollen, mite), purified/recombinant major allergen (i.e., Bet v 1, Der p 1) or polypeptide fragments (i.e., rBet v 1, rPhl p 1, and rPhl p 5) alone or together with an immunopotentiator (CpG-ODN, Flagelin, fungal immunomodulatory protein, <i>Lactobacillus plantarum</i>) decrease the Th2 polarization (i.e., reduced AHR, cellular infiltration, seric IgE, IgG1/IgG2a ratio, and IL-5 production) together with an increased expression of IgA in saliva or TGFβ, IL10, and Foxp3 in CD4⁺ T cells	Liu and Tsai (2005); Daniel et al. (2006); Hisbergues et al. (2007); Robinson et al. (2004); Lee et al. (2008); Hufnagl et al. (2008); Winkler et al. (2006)

(continued)

Table 1 (continued)

Route of immunization	Allergens	Comments	References
Oral immunotherapy (OIT)	Pollens (ragweed, grasses, artemisia, birch), house dust mites, food allergens (milk, egg), autoimmune disease related antigen (type I diabetes, multiple sclerosis, rheumatoid arthritis, uveitis, and thyroiditis)	Clinical efficacy: Grass pollen OIT (low or high doses) is ineffective in terms of therapeutic effects, whereas OIT with birch, ragweed, artemisia pollens or HDM extracts (high doses) reduces asthma/rhinitis symptoms both in adults and children In OIT with food allergen, children received increasing doses of milk or egg which re-install tolerance toward the allergens in most cases. One placebo-controlled study of OIT for cow's milk and egg allergies does not report an enhanced tolerance recovery. A major drawback of those studies is that they report outcomes in small cohorts of patients often lacking a control group to analyze spontaneous improvement	Rebien et al. (1982); Taudorf and Weeke (1983); Mosbech et al. (1987); Urbanek et al. (1990); Taudorf et al. (1987, 1989); Moller et al. (1986); Leng et al. (1990); Litwin et al. (1997); Giovane et al. (1994); Meglio et al. (2004, 2008) Buchanan et al. (2007); Staden et al. (2007); Longo et al. (2008)
	Used as: natural extract in aqueous, freeze-dried, powder forms, enterosoluble capsules	OIT for autoimmune diseases has been tested in humans using antigenic peptides or purified/recombinant proteins but failed to demonstrate significant effects over the placebo group Immune mechanisms: <i>Antibody responses:</i> OIT with grass pollen allergen extracts does not alter humoral (IgE, IgG) responses. OIT with birch, ragweed, artemisia pollens, or mites extracts decreases seric IgE and up-regulates seric IgG1/IgG4 antibodies in allergic patients (adults and children) Successful OIT as well as spontaneous re-establishment of oral tolerance to cow's milk or egg allergens are associated with an increase in specific IgG4 and a reduction of food-specific IgE antibody levels	Faria and Weiner (2006) Rebien et al. (1982); Taudorf and Weeke (1983); Taudorf et al. (1987); Moller et al. (1986); Taudorf et al. (1989); Leng et al. (1990); Litwin et al. (1997); Giovane et al. (1994) Burks et al. (2008); Staden et al. (2007); Longo et al. (2008); Patriarca et al. (2003); Skripak et al. (2008)

(continued)

Table 1 (continued)

Route of immunization	Allergens	Comments	References
Sublingual immunotherapy (SLIT)	Venoms, pollens (ragweed, birch, grasses, parietaria, olive), house dust mites	<i>Cellular responses:</i> No changes in histamine secretion by basophils following OIT with grass pollen allergen extracts. In the absence of clinical effect, TGFβ-producing Th3 regulatory cells were induced in patients treated for diabetes or multiple sclerosis	Faria and Weiner (2006); Taudorf and Weeke (1983)
		Pre-clinical models and candidate vaccines: Experimental animal models of autoimmune diseases show that CD4 ⁺ regulatory T cells involved in oral tolerance induction are highly heterogeneous and include Th3 cells (producing TGFβ and/or IL10), Tr1 cells (producing IL10) and CD4 ⁺ CD25 ⁺ cells secreting TGFβ or mediating their suppressive function through CTLA-4, PD-1, or latency-associated peptide (LAP) molecules. For example, oral administration of antigen coupled with non toxic cholera toxin B subunit (CTB) as a mucosal adjuvant promotes peripheral tolerance induction through the induction of both CD4 ⁺ CD25 ⁺ Foxp3 ⁺ and CD4 ⁺ Foxp3 ⁻ LAP ⁺ TGFβ ⁺ regulatory T cells	Faria and Weiner (2006); Sun et al. (2008); Nakamura et al. (2001); Oida et al. (2003); Chen et al. (2008)
		Clinical efficacy: SLIT is efficient in treating type I allergies to either house dust mites or pollens (from grass, parietaria, olive, ragweed, cupressus). It reduces both symptoms and medication requirements in adults and children. SLIT exhibits a favorable safety profile and long lasting effects in adults and children with rhinitis. SLIT using grass pollen or HDM sublingual tablets has shown a significant reduction in rhinitis symptoms together with an excellent safety profile in the context of large scale placebo-controlled phase III studies	Wilson et al. (2003); Khinchi et al. (2004); Wilson et al. (2005); Olaguibel and Alvarez Puebla (2005); Calamita et al. (2006); Penagos et al. (2006); Larenas-Linnemann (2009); Passalacqua et al. (2004); Pajno et al. (2003); Mascarell et al. (2006); Antunez et al. (2008); Baena-Cagnani et al. (2005); Fiocchi et al. (2005); Didier et al. (2006); Dahl et al. (2006); Ibanez et al. (2007); Dahl et al. (2008); Wahn et al. (2009); Didier et al. (2009); Horak et al. (2009); Malling et al. (2009)

(continued)

Table 1 (continued)

Route of immunization	Allergens	Comments	References
	Used as: natural biological extracts, allergoids, tablets	Immune mechanisms: <i>Antibody responses:</i> SLIT enhances allergen-specific IgG4 antibodies while decreasing IgE antibodies. In some studies, allergen-specific IgA antibodies are induced	Mascarell et al. (2006); Bahceciler et al. (2005)
		<i>Cellular responses:</i> SLIT to grass pollen or HDM decreases ECP and tryptase nasal secretion. In some studies SLIT used against house dust mites, birch, and grass pollens elicit both Th1 and regulatory T cells (i.e., IL10-producing Tr1 or Foxp3 ⁺ CD4 ⁺ CD25 ⁺ cells) in peripheral blood or in the nasal mucosa	Meiler et al. (2008); Marucci et al. (2001, 2003); Passalacqua et al. (1998); Jutel et al. (2003); Gardner et al. (2004); Taylor et al. (2004); Ciprandi et al. (2005); Bohle et al. (2007); Radulovic et al. (2008); Cosmi et al. (2006)
		Pre-clinical models and candidate vaccines: The lingual immune system is prone to induce specific tolerance since mucosal APCs including plasmacytoid, CD11b ⁺ CD11c ⁺ and CD11b ⁺ CD11c ⁻ DCs as well as Langerhans cells promote Th1/Treg responses in draining lymph nodes. Targeting lingual APCs with adjuvants (i.e., selected strains of Lactobacilli, Pam3CSK4, OM-294-BA-MP as well as immunosuppressive drugs e.g., glucocorticoids plus vitamin D3) or with mucoadhesive particles (polysaccharidic core nanoparticles (PSC) or chitosan) as vector systems improve SLIT efficacy in a therapeutic murine model of asthma by inhibiting Th2 responses (reduction of AHR, lung inflammation, eosinophils in bronchoalveolar lavages, IL5, and IL13 production) while inducing IFN-γ (Th1) and/or IL10 (Treg) producing T cells. In humans, the Th1 adjuvant monophosphoryl lipid A (MPL) is currently under investigation in sublingual immunotherapy	Mascarell et al. (2007, 2008); Allam et al. (2003, 2008a); Van Overtvelt et al. (2008); Lombardi et al. (2008); Razafindratsita et al. (2007); Saint-Lu et al. (2009)

It also increased allergen-specific IgA in saliva and elicited IFN γ and IL10 production by circulating CD4⁺ T cells (Liu and Tsai 2005; Daniel et al. 2006; Hisbergues et al. 2007; Mousavi et al. 2008; Lee et al. 2008; Hufnagl et al. 2008). In a murine model of birch pollen allergy, immunological tolerance to Bet v 1 correlated with an increased expression of both transforming growth factor-beta (TGF β), and IL10, as well as the induction of Foxp3⁺ CD4⁺ T cells, thus highlighting an active regulatory mechanism (Winkler et al. 2006).

Altogether, LNIT appears safe and efficient in treating allergic rhinitis linked with pollen and mite. As of today, however, LNIT efficacy has not yet been documented in the context of large clinical studies versus placebo. Also, nasal application of allergen extracts makes it difficult to control the exact dose administered to patients, thus currently limiting its broad use in allergen-specific immunotherapy.

2.3 Oral Immunotherapy

In the 1980s, oral immunotherapy (OIT) has been studied in both adults and children with pollen-induced allergic rhinitis (Rebien et al. 1982; Taudorf and Weeke 1983; Mosbech et al. 1987; Urbanek et al. 1990; Taudorf et al. 1987, 1989; Moller et al. 1986; Leng et al. 1990; Litwin et al. 1997) (Table 1). A number of these studies were performed with grass pollen allergens presented either as an aqueous solution, or as powder or enterosoluble forms (Rebien et al. 1982; Taudorf and Weeke 1983; Mosbech et al. 1987; Urbanek et al. 1990). Although OIT with grass pollen was well-tolerated, it has been shown to be clinically ineffective. In addition, no significant changes in seric allergen-specific IgG or IgE levels were detected in comparison to placebo (Rebien et al. 1982; Taudorf and Weeke 1983). Conversely, OIT with high doses of selected allergen aqueous extracts (e.g., *Dermatophagoides pteronyssinus* or *Artemisia*) (Leng et al. 1990; Giovane et al. 1994) or using encapsulated allergens (e.g., birch or ragweed pollens) (Taudorf et al. 1987, 1989; Moller et al. 1986; Litwin et al. 1997) showed evidence of safety and efficacy associated with a significant decrease in serum-specific IgE antibodies, and an upregulation of serum-specific IgG1/IgG4 antibody levels in allergic patients (adults and children).

OIT is currently being evaluated to re-establish the tolerance to dietary proteins in children who have developed food allergies, such as cow's milk and egg allergies (Burks et al. 2008). Several clinical studies have been conducted in children failing to outgrown those allergies with food allergens (Meglio et al. 2004, 2008; Buchanan et al. 2007; Staden et al. 2007; Longo et al. 2008). Due to the risk of severe reactions, desensitization protocols rely upon the administration of small incremental doses of the food allergen in order to reach amounts normally tolerated (from micrograms to milligrams to grams) followed by a food challenge at the end of the study protocol to evaluate efficacy. In a standardized OIT protocol applied to cow's milk allergy, desensitization was totally or partially successful in

85% of cases with an acceptable safety profile (Meglio et al. 2004). In addition, a long lasting efficacy was observed for up to 4–5 years after the end of treatment (Meglio et al. 2008). Also, encouraging results were obtained in egg OIT (Buchanan et al. 2007). One limitation of those studies is that they rely upon small cohorts of patients, they often lack a control group and do not measure spontaneous outgrowth. For example, a randomized, placebo-controlled study of OIT for cow's milk and egg allergies comparing a control group (with allergen eviction) with the tolerance induction group, concluded that both groups exhibited tolerance to allergens, thus, pointing out that OIT for food allergy should take into consideration the spontaneous rate of recovery of patients (Staden et al. 2007). Nonetheless, a recent study of children with a severe allergy to cow's milk showed a significant 90% increase in tolerated milk intake, only in the group receiving the therapy, whereas the eviction control group failed the food challenge after 1 year (Longo et al. 2008). These studies raise questions regarding criteria for patient selection, dose incrementation, and treatment duration.

In terms of serological changes, OIT for cow's milk leads to a significant decrease in food-specific IgE levels (Longo et al. 2008; Patriarca et al. 2003) concomitantly with an increase in specific IgG4 levels (Longo et al. 2008; Patriarca et al. 2003; Skripak et al. 2008). In the case of spontaneous re-establishment of oral tolerance to cow's milk or egg allergens in children, allergen-specific IgE levels also decrease significantly (Staden et al. 2007). Accordingly, children whose allergy resolves spontaneously exhibit a reorientation of preexisting Th2 responses toward Th1 and regulatory T cells (Burks et al. 2008).

Oral tolerance has been further tested in human autoimmune diseases such as type I diabetes, multiple sclerosis, rheumatoid arthritis, uveitis and thyroiditis, using antigenic peptides as well as purified or recombinant proteins (Faria and Weiner 2006a). Although the induction of regulatory mechanisms such as TGF β -producing Th3 cells was observed in patients treated for diabetes or multiple sclerosis, phase II/III trials showed no significant effect over the placebo group (Faria and Weiner 2006b). In contrast, specific oral tolerance regimens have demonstrated promising results in experimental animal models of autoimmune diseases. In those experiments, high doses of antigen administered orally promote T cell depletion or anergy (Faria and Weiner 2005), whereas low doses rather induce suppressive mechanisms involving regulatory T cells. Such regulatory T cells are highly heterogeneous and include Th3 cells (producing TGF β and/or IL10), Tr1 cells (producing IL10), and CD4⁺ CD25⁺ T cells mediating their suppressive function through cytokine secretion, or cell contact-dependent mechanisms involving the CTLA-4 or PD-1 molecules (Faria and Weiner 2006a). In addition, new subsets (CD4⁺ CD25⁻ or CD4⁺ CD25⁺) of regulatory T cells have been described which express latency-associated peptide (LAP), secrete TGF β and corresponding surface receptors at the cell surface, thus mediating regulatory functions through both soluble factors and cell contact (Nakamura et al. 2001; Oida et al. 2003; Chen et al. 2008).

Altogether, although oral tolerance induction has been successful in a number of animal models, the clinical efficacy of OIT remains to be firmly established in humans.

The inability of the protein to bypass the gastrointestinal barrier remains problematic in the oral administration of protein drugs. In order to overcome problems of low allergen/protein bioavailability and absorption after OIT, current investigations are focusing on novel and enhanced delivery systems for a safe oral administration of the antigen.

2.4 Sublingual Immunotherapy

Allergen-specific sublingual immunotherapy (SLIT) has been investigated in allergic patients since 1984 (Bousquet et al. 1999; La Rosa et al. 1999; Wilson et al. 2003; Clavel et al. 1998; Vourdas et al. 1998; Bousquet et al. 2001; Pajno et al. 2000; Marogna et al. 2005) (Table 1). During SLIT, the allergen extract is applied as a solution or as a tablet and kept under the tongue for at least 1–2 min, thus facilitating allergen capture by oral Langerhans cells (oLCs). As of today, more than one billion doses have been administered to humans with an excellent safety profile, demonstrating that SLIT is a valid alternative to SCIT (Khinchi et al. 2004). SLIT is efficient in treating type I allergies to either house dust mites, pollens (from grass, parietaria, olive, ragweed, cupressus) or cat dander in adults and children, with a reduction of both symptoms and medication requirements. As such, SLIT can be used in patients with rhinitis with or without mild-asthma (Wilson et al. 2003, 2005; Olaguibel and Alvarez Puebla 2005; Calamita et al. 2006; Penagos et al. 2006; Larenas-Linnemann 2009; Passalacqua et al. 2004; Pajno et al. 2003; Mascarell et al. 2006). Comparative clinical trials in birch pollen-allergic adults or mite-allergic children revealed no statistical difference in terms of efficacy between SCIT and SLIT when high-dose SLIT regimens were used (Khinchi et al. 2004; Antunez et al. 2008). Also, long-term clinical benefits are observed following SLIT in adults and children with rhinitis (Wilson et al. 2003). The safety and efficacy profile of high-dose immunotherapy has been confirmed in children younger than 5 years (Baena-Cagnani et al. 2005; Fiocchi et al. 2005). As of today, the pertinence of SLIT for tolerance induction in humans has been definitively established by multiple DBPC phase III studies conducted in large cohorts of patients allergic to grass pollen (Didier et al. 2006, 2009; Dahl et al. 2006, 2008; Ibanez et al. 2007; Wahn et al. 2009; Horak et al. 2009; Malling et al. 2009). In addition, house dust mite sublingual tablets tested in 509 adults patients in a pivotal phase IIb/III clinical trial induced a significant decrease in rhinitis symptoms while demonstrating a good safety profile after only 1 year of treatment (our unpublished data).

Similarly to SCIT, SLIT reduces allergen-specific inflammatory responses at various levels. For example, it decreases ECP and tryptase nasal secretion (Marcucci et al. 2001, 2003; Passalacqua et al. 1998). It also enhances specific seric IgG4 while decreasing IgE antibodies (Mascarell et al. 2006; Bahceciler et al. 2005), thus inhibiting basophil or mast cell (MC) degranulation. SLIT elicits both Th1 and regulatory T cells (i.e., IL10-producing Tr1 or Foxp3⁺ CD4⁺ CD25⁺ T cells)

in peripheral blood or in the nasal mucosa (Meiler et al. 2008; Jutel et al. 2003; Gardner et al. 2004; Taylor et al. 2004; Ciprandi et al. 2005; Bohle et al. 2007; Radulovic et al. 2008; Cosmi et al. 2006).

APCs identified thus far in the lingual oral mucosa of both mice and humans, consist of oLCs, myeloid CD11b⁺ CD11c⁻, CD11b⁺ CD11c⁺ as well as plasmacytoid DCs (Mascarell et al. 2008; Allam et al. 2003) with a semi-mature phenotype (CD40⁻ CD80⁻ CD86⁺), consistent with a tolerogenic function (Steinman et al. 2003; Hawiger et al. 2001). Those oral APCs located at the mucosal/sub-mucosal junction are likely involved in antigen capture during SLIT (Mascarell et al. 2008) and represent a privileged target for tolerance induction, since they induce IFN γ and/or IL10 producing CD4⁺ T cells mediating suppressive functions (Mascarell et al. 2008; Allam et al. 2008a). Also, cross-linking of high affinity receptors for IgE (Fc ϵ RI) on human oLCs induces regulatory molecules such as TGF β , IL10, or indoleamine 2,3-dioxygenase (IDO) (Allam et al. 2003; Novak et al. 2001). In contrast, pro-inflammatory cells, i.e., MCs and eosinophils, are located in submucosal tissues in humans and mice (Marcucci et al. 2007; Allam et al. 2008a), suggesting that allergen contact with such cells might be limited during SLIT. Differences in distribution of oLCs and MCs have been observed within the oral cavity, with the vestibular region identified as the most favorable site for allergen application during sublingual immunotherapy, given its high oLCs and low MCs density (Allam et al. 2008b). In mice, resident mucosal CD4⁺ T cells co-localize with CD11b⁺ tolerogenic APCs in the lamina propria, suggesting that the lingual immune system is organized in a similar manner to the small intestine, another critical site for tolerance induction (Denning et al. 2007; Mascarell et al. 2008). Resident mucosal CD4⁺ T cells express the mucosal homing receptor α E β 7 integrin CD103, also described as a regulatory marker (Suffia et al. 2005; Allakhverdi et al. 2006).

3 Interest in Adjuvants and Vector Systems for Mucosal Immunization

Given the dual capacity of mucosal immune systems, appropriate signalling to APCs e.g., with immunopotentiators (i.e., synthetic or biological adjuvant molecules) or enhanced targeting via vector systems are needed to promote tolerance over inflammation. For example, selected strains of Lactobacilli have been shown to enhance SLIT efficacy in a therapeutic murine model of asthma (Van Overtvelt et al. 2008). Triggering Toll like receptors (TLR) by lipid A derivatives or lipopeptides (Pam3CSK4 and OM-294-BA-MP, respectively) as well as immunosuppressive drugs (e.g., glucocorticoids with vitamin D3) are also effective in improving SLIT efficacy (Van Overtvelt et al. 2008; Mascarell et al. 2007; Lombardi et al. 2008). In allergen-specific immunotherapy, the use of Th1 adjuvants to potentiate tolerance induction is also currently under investigation. Cytosine-guanine oligonucleotides (CpG) (Simons et al. 2004) and monophosphoryl lipid A (MPL)

(Drachenberg et al. 2001, 2003) have been shown to be effective in ragweed and grass pollen subcutaneous immunotherapy, respectively. The latter is also presently being tested in sublingual immunotherapy regimens in humans.

Selected formulations can be used to present allergens in a particulate form, thereby targeting APCs with a phagocytic property. For example, targeting oral APCs following sublingual administration of OVA formulated within mucoadhesive particles (e.g., polysaccharidic core nanoparticles or chitosan) induces the proliferation of antigen specific suppressive T cells producing IFN γ /IL10 in draining cervical lymph nodes. Sublingual administration of OVA formulated with such particules enhances tolerance induction in mice with established asthma, with a dramatic reduction of both AHR, lung inflammation, eosinophils numbers in bronchoalveolar lavages, as well as antigen-specific Th2 responses (Razafindratsita et al. 2007; Saint-Lu et al. 2009). Other delivery systems targeting DCs are currently being considered (Bohle et al. 2004; El Azami El Idrissi et al. 2002; Jeannin et al. 2000; Goetsch et al. 2001; Haicheur et al. 2003; Bonifaz et al. 2004; Santeliz et al. 2002; Tighe et al. 2000). For instance, the adenylate cyclase (CyaA) from *Bordetella pertussis* (El Azami El Idrissi et al. 2002), the B subunit of shiga toxin (STxB) (Haicheur et al. 2003), the outer membrane protein A from *Klebsiella pneumoniae* (kpOmpA) (Jeannin et al. 2000; Goetsch et al. 2001) or the bacterial cell surface protein of *Geobacillus stearothermophilus* (S-layer) (Bohle et al. 2004) target DCs and prime efficient Th1 cellular responses. Also, anti-DEC-205 antibodies coupled with antigen target DCs, and induce strong T cell responses in mice when administered together with anti-CD40 antibodies (Bonifaz et al. 2004). Whether or not such delivery systems are relevant to induce allergen-specific tolerance by mucosal routes remain to be addressed.

4 Conclusions

The development of second generation mucosal based-vaccines will benefit from a better understanding of mucosal immune systems. The latter comprise various subsets of tolerogenic APCs organized in a compartmentalized manner and programmed to induce Th1/Treg responses. Based on placebo-controlled clinical studies conducted in large cohort of patients, the sublingual route is as of today the only firmly proven mucosal route to establish tolerance in humans. Targeting the allergen to tolerogenic mucosal DCs using vector systems, mucoadhesive particulate formulations or adjuvant molecules represent a promising strategy to enhance tolerance induction. Until now, the development of allergy vaccines has been exclusively based on natural or modified allergen extracts, but, there is a growing interest in using recombinant allergens including native or hypoallergenic forms, mixture of allergens or allergen-derived peptides (Niederberger et al. 2004; Gafvelin et al. 2005; Purohit et al. 2008; Jutel et al. 2005; Muller et al. 1998; Himly et al. 2009; Pauli et al. 2008; Verhoef et al. 2005; Larche and Wraith 2005; Edlmayr et al. 2009). Thus, the development of relevant recombinant major

allergens together with appropriate presentation systems suitable for mucosal application will help to develop more efficient and safe allergy vaccines in the future.

References

- Allakhverdi Z, Fitzpatrick D, Boisvert A et al (2006) Expression of CD103 identifies human regulatory T-cell subsets. *J Allergy Clin Immunol* 118:1342–1349
- Allam JP, Novak N, Fuchs C et al (2003) Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive Fc ϵ RI expression. *J Allergy Clin Immunol* 112:141–148
- Allam JP, Peng WM, Appel T et al (2008a) Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells. *J Allergy Clin Immunol* 121:368–374 e1
- Allam JP, Stojanovski G, Friedrichs N et al (2008b) Distribution of Langerhans cells and mast cells within the human oral mucosa: new application sites of allergens in sublingual immunotherapy? *Allergy* 63:720–727
- Andri L, Falagiani P (2003) Symptomatic relief after grass nasal immunotherapy: lasting efficacy after 4–5 years. *J Investig Allergol Clin Immunol* 13:228–231
- Andri L, Senna G (1995) Local nasal immunotherapy. *Allergy* 50:190
- Andri L, Senna GE, Betteli C et al (1992) Local nasal immunotherapy in allergic rhinitis to *Parietaria*. A double-blind controlled study. *Allergy* 47:318–323
- Andri L, Senna G, Betteli C et al (1993) Local nasal immunotherapy for *Dermatophagoides*-induced rhinitis: efficacy of a powder extract. *J Allergy Clin Immunol* 91:987–996
- Andri L, Senna G, Betteli C et al (1996) Local nasal immunotherapy with extract in powder form is effective and safe in grass pollen rhinitis: a double-blind study. *J Allergy Clin Immunol* 97:34–41
- Antunez C, Mayorga C, Corzo JL, Jurado A, Torres MJ (2008) Two year follow-up of immunological response in mite-allergic children treated with sublingual immunotherapy. Comparison with subcutaneous administration. *Pediatr Allergy Immunol* 19:210–218
- Baena-Cagnani CE, Passalacqua G, Baena-Cagnani RC, Croce VH, Canonica WG (2005) Sublingual immunotherapy in pediatric patients: beyond clinical efficacy. *Curr Opin Allergy Clin Immunol* 5:173–177
- Bagnasco M, Mariani G, Passalacqua G et al (1997) Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 100:122–129
- Bahceciiler NN, Arikian C, Taylor A et al (2005) Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int Arch Allergy Immunol* 136:287–294
- Bellussi L, Bologna M, Di Stanislao C et al (2002) Simplified local nasal immunotherapy in mite dust allergic rhinitis. *J Investig Allergol Clin Immunol* 12:42–47
- Benjaponpitak S, Oro A, Maguire P et al (1999) The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J Allergy Clin Immunol* 103:468–475
- Bohle B, Breitwieser A, Zwolfer B et al (2004) A novel approach to specific allergy treatment: the recombinant fusion protein of a bacterial cell surface (S-layer) protein and the major birch pollen allergen Bet v 1 (rSbsC-Bet v 1) combines reduced allergenicity with immunomodulating capacity. *J Immunol* 172:6642–6648
- Bohle B, Kinaciyan T, Gerstmayr M et al (2007) Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 120:707–713
- Bonifaz LC, Bonnyay DP, Charalambous A et al (2004) In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* 199: 815–824

- Bousquet J, Lockey R, Malling HJ (1998) Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 102:558–562
- Bousquet J, Scheinmann P, Guinnee MT et al (1999) Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy* 54:249–260
- Bousquet J, Van Cauwenberge P, Khaltaev N (2001) Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 108:S147–S334
- Buchanan AD, Green TD, Jones SM et al (2007) Egg oral immunotherapy in nonanaphylactic children with egg allergy. *J Allergy Clin Immunol* 119:199–205
- Burks AW, Laubach S, Jones SM (2008) Oral tolerance, food allergy, and immunotherapy: implications for future treatment. *J Allergy Clin Immunol* 121:1344–1350
- Calamita Z, Sacconato H, Pela AB, Atallah AN (2006) Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. *Allergy* 61:1162–1172
- Canonica GW, Passalacqua G (2003) Noninjection routes for immunotherapy. *J Allergy Clin Immunol* 111:437–448 quiz 49
- Chen ML, Yan BS, Bando Y, Kuchroo VK, Weiner HL (2008) Latency-associated peptide identifies a novel CD4⁺CD25⁺ regulatory T cell subset with TGFβ-mediated function and enhanced suppression of experimental autoimmune encephalomyelitis. *J Immunol* 180:7327–7337
- Ciprandi G, Fenoglio D, Cirillo I et al (2005) Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 95:38–44
- Clavel R, Bousquet J, Andre C (1998) Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. *Allergy* 53:493–498
- Conti HR, Shen F, Nayyar N et al (2009) Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Exp Med* 206:299–311
- Cosmi L, Santarlasci V, Angeli R et al (2006) Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon-γ and interleukin 10-production. *Clin Exp Allergy* 36:261–272
- Cuburu N, Kweon MN, Song JH et al (2007) Sublingual immunization induces broad-based systemic and mucosal immune responses in mice. *Vaccine* 25:8598–8610
- D'Amato G, Lobefalo G, Liccardi G, Cazzola M (1995) A double-blind, placebo-controlled trial of local nasal immunotherapy in allergic rhinitis to Parietaria pollen. *Clin Exp Allergy* 25:141–148
- Dahl R, Kapp A, Colombo G et al (2006) Efficacy and safety of sublingual immunotherapy with grass allergen tablets for seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 118:434–440
- Dahl R, Kapp A, Colombo G et al (2008) Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol* 121:512–518 e2
- Daniel C, Repa A, Wild C et al (2006) Modulation of allergic immune responses by mucosal application of recombinant lactic acid bacteria producing the major birch pollen allergen Bet v 1. *Allergy* 61:812–819
- Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B (2007) Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 8:1086–1094
- Deuschl H, Johansson GO (1977) Hyposensitization of patients with allergic rhinitis by intranasal administration of chemically modified grass pollen allergen. A pilot study. *Acta Allergol* 32:248–262
- Didier A, Melac M, Combebias A, Andre C (2006) Efficacy and safety of sublingual immunotherapy grass pollen tablets in grass pollen rhinoconjunctivitis. *J Allergy Clin Immunol* 117(Suppl LB5):721

- Didier A, Melac M, Montagut A et al (2009) Agreement of efficacy assessments for five-grass pollen sublingual tablet immunotherapy. *Allergy* 64:166–171
- Drachenberg KJ, Wheeler AW, Stuebner P, Horak F (2001) A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 56:498–505
- Drachenberg KJ, Heinzkill M, Urban E, Woroniecki SR (2003) Efficacy and tolerability of short-term specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A (MPL) for children and adolescents. *Allergol Immunopathol (Madr)* 31:270–277
- Durham SR, Till SJ (1998) Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 102:157–164
- Ebner C, Siemann U, Bohle B et al (1997) Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 27:1007–1015
- Edlmayr J, Niespodziana K, Linhart B et al (2009) A combination vaccine for allergy and rhinovirus infections based on rhinovirus-derived surface protein VP1 and a nonallergenic peptide of the major timothy grass pollen allergen Phl p 1. *J Immunol* 182:6298–6306
- El Azami El Idrissi M, Ladant D, Leclerc C (2002) The adenylate cyclase of *Bordetella pertussis*: a vector to target antigen presenting cells. *Toxicon* 40:1661–1665
- El Biaze M, Boniface S, Koscher V et al (2003) T cell activation, from atopy to asthma: more a paradox than a paradigm. *Allergy* 58:844–853
- Faria AM, Weiner HL (2005) Oral tolerance. *Immunol Rev* 206:232–259
- Faria AM, Weiner HL (2006a) Oral tolerance: therapeutic implications for autoimmune diseases. *Clin Dev Immunol* 13:143–157
- Faria AM, Weiner HL (2006b) Oral tolerance and TGF-beta-producing cells. *Inflamm Allergy Drug Targets* 5:179–190
- Fiocchi A, Pajno G, La Grutta S et al (2005) Safety of sublingual-swallow immunotherapy in children aged 3–7 years. *Ann Allergy Asthma Immunol* 95:254–258
- Gafvelin G, Thunberg S, Kronqvist M et al (2005) Cytokine and antibody responses in birch-pollen-allergic patients treated with genetically modified derivatives of the major birch pollen allergen Bet v 1. *Int Arch Allergy Immunol* 138:59–66
- Gardner LM, Thien FC, Douglass JA, Rolland JM, O'Hehir RE (2004) Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4⁺ CD25⁺ interleukin-10⁺ T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 34:1209–1219
- Gehlhar K, Schlaak M, Becker W, Bufe A (1999) Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen-specific IgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy* 29:497–506
- Georgitis JW, Reisman RE, Clayton WF et al (1983) Local intranasal immunotherapy for grass-allergic rhinitis. *J Allergy Clin Immunol* 71:71–76
- Giovane AL, Bardare M, Passalacqua G et al (1994) A three year double-blind placebo-controlled study with specific oral immunotherapy to *Dermatophagoides*: evidence of safety and efficacy in paediatric patients. *Clin Exp Allergy* 24:53–59
- Goetsch L, Gonzalez A, Plotnicky-Gilquin H et al (2001) Targeting of nasal mucosa-associated antigen-presenting cells in vivo with an outer membrane protein A derived from *Klebsiella pneumoniae*. *Infect Immun* 69:6434–6444
- Haicheur N, Benchetrit F, Amessou M et al (2003) The B subunit of Shiga toxin coupled to full-size antigenic protein elicits humoral and cell-mediated immune responses associated with a Th1-dominant polarization. *Int Immunol* 15:1161–1171
- Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR (1997) Increases in IL-12 messenger RNA⁺ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 99:254–260
- Hawiger D, Inaba K, Dorsett Y et al (2001) Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J Exp Med* 194:769–779

- Himly M, Nony E, Chabre H et al (2009) Standardization of allergen products: 1. Detailed characterization of GMP-produced recombinant Bet v 1.0101 as biological reference preparation. *Allergy* 64(7):1038–1045
- Hisbergues M, Magi M, Rigaux P et al (2007) In vivo and in vitro immunomodulation of Der p 1 allergen-specific response by *Lactobacillus plantarum* bacteria. *Clin Exp Allergy* 37:1286–1295
- Horak F, Jaeger S, Worm M, Melac M, Didier A (2009) Implementation of pre-seasonal sublingual immunotherapy with a five-grass pollen tablet during optimal dosage assessment. *Clin Exp Allergy* 39:394–400
- Huang FP, Platt N, Wykes M et al (2000) A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med* 191:435–444
- Hufnagl K, Focke M, Gruber F et al (2008) Airway inflammation induced after allergic polysensitization can be prevented by mucosal but not by systemic administration of polypeptides. *Clin Exp Allergy* 38:1192–1202
- Ibanez MD, Kaiser F, Knecht R et al (2007) Safety of specific sublingual immunotherapy with SQ standardized grass allergen tablets in children. *Pediatr Allergy Immunol* 18:516–522
- Iwasaki A, Kelsall BL (1999) Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 190:229–239
- Jeannin P, Renno T, Goetsch L et al (2000) OmpA targets dendritic cells, induces their maturation and delivers antigen into the MHC class I presentation pathway. *Nat Immunol* 1:502–509
- Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH (2000) Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 192:1213–1222
- Jutel M, Akdis M, Budak F et al (2003) IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 33:1205–1214
- Jutel M, Jaeger L, Suck R et al (2005) Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 116:608–613
- Khinchi MS, Poulsen LK, Carat F et al (2004) Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* 59:45–53
- La Rosa M, Ranno C, Andre C et al (1999) Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 104:425–432
- Larche M (2000) Specific immunotherapy. *Br Med Bull* 56:1019–1036
- Larche M, Wraith DC (2005) Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat Med* 11:S69–S76
- Larenas-Linnemann D (2009) Sublingual immunotherapy in children: complete and updated review supporting evidence of effect. *Curr Opin Allergy Clin Immunol* 9:168–176
- Lee SE, Koh YI, Kim MK et al (2008) Inhibition of airway allergic disease by co-administration of flagellin with allergen. *J Clin Immunol* 28:157–165
- Leng X, Fu YX, Ye ST, Duan SQ (1990) A double-blind trial of oral immunotherapy for *Artemisia* pollen asthma with evaluation of bronchial response to the pollen allergen and serum-specific IgE antibody. *Ann Allergy* 64:27–31
- Litwin A, Flanagan M, Entis G et al (1997) Oral immunotherapy with short ragweed extract in a novel encapsulated preparation: a double-blind study. *J Allergy Clin Immunol* 100:30–38
- Liu YH, Tsai JJ (2005) Production of salivary immunoglobulin A and suppression of *Dermatophagoides pteronyssinus*-induced airway inflammation by local nasal immunotherapy. *Int Arch Allergy Immunol* 138:161–168

- Liu L, Zhang M, Jenkins C, MacPherson GG (1998) Dendritic cell heterogeneity in vivo: two functionally different dendritic cell populations in rat intestinal lymph can be distinguished by CD4 expression. *J Immunol* 161:1146–1155
- Lombardi V, Van Overtvelt L, Horiot S et al (2008) Toll-like receptor 2 agonist Pam3CSK4 enhances the induction of antigen-specific tolerance via the sublingual route. *Clin Exp Allergy* 38(11):1819–1829
- Longo G, Barbi E, Berti I et al (2008) Specific oral tolerance induction in children with very severe cow's milk-induced reactions. *J Allergy Clin Immunol* 121:343–347
- MacPherson G, Milling S, Yrlid U et al (2004) Uptake of antigens from the intestine by dendritic cells. *Ann N Y Acad Sci* 1029:75–82
- Malling HJ, Montagut A, Melac M et al (2009) Efficacy and safety of five-grass pollen sublingual immunotherapy tablets in patients with different clinical profiles of allergic rhinoconjunctivitis. *Clin Exp Allergy* 39:387–393
- Marcucci F, Sensi L, Frati F et al (2001) Sublingual tryptase and ECP in children treated with grass pollen sublingual immunotherapy (SLIT): safety and immunologic implications. *Allergy* 56:1091–1095
- Marcucci F, Sensi L, Frati F et al (2003) Effects on inflammation parameters of a double-blind, placebo controlled 1 year course of SLIT in children monosensitized to mites. *Allergy* 58:657–662
- Marcucci F, Sensi L, Incorvaia C et al (2007) Oral reactions to sublingual immunotherapy: a bioptic study. *Allergy* 62:1475–1477
- Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G (2005) Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: a 3 year randomized controlled study. *J Allergy Clin Immunol* 115:1184–1188
- Mascarell L, Van Overtvelt L, Moingeon P (2006) Novel ways for immune intervention in immunotherapy: mucosal allergy vaccines. *Immunol Allergy Clin North Am* 26:283–306 vii–viii
- Mascarell L, Van Overtvelt L, Lombardi V et al (2007) A synthetic triacylated pseudo-dipeptide molecule promotes Th1/TReg immune responses and enhances tolerance induction via the sublingual route. *Vaccine* 26:108–118
- Mascarell L, Lombardi V, Louise A et al (2008) Oral dendritic cells mediate antigen-specific tolerance by stimulating TH1 and regulatory CD4⁺ T cells. *J Allergy Clin Immunol* 122:603–609 e5
- Mascarell L, Lombardi V, Zimmer A et al (2009) Mapping of the lingual immune system reveals the presence of both regulatory and effector CD4⁺ T cells. *Clin Exp Allergy*
- Mavroleon G (1998) Restoration of cytokine imbalance by immunotherapy. *Clin Exp Allergy* 28:917–920
- Medicines CoSo (1986) Desensitising vaccines: an allergist's view. *Br Med J* 293:1169–1170
- Meglio P, Bartone E, Plantamura M, Arabito E (2004) Giampietro PG: A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. *Allergy* 59:980–987
- Meglio P, Giampietro PG, Gianni S, Galli E (2008) Oral desensitization in children with immunoglobulin E-mediated cow's milk allergy-follow-up at 4 year and 8 months. *Pediatr Allergy Immunol* 19:412–419
- Meiler F, Zumkehr J, Klunker S et al (2008) In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med* 205:2887–2898
- Menges M, Rossner S, Voigtlander C et al (2002) Repetitive injections of dendritic cells matured with tumor necrosis factor alpha induce antigen-specific protection of mice from autoimmunity. *J Exp Med* 195:15–21
- Michils A, Mairesse M, Ledent C et al (1998) Modified antigenic reactivity of anti-phospholipase A2 IgG antibodies in patients allergic to bee venom: conversion with immunotherapy and relation to subclass expression. *J Allergy Clin Immunol* 102:118–126
- Moller C, Dreborg S, Lanner A, Bjorksten B (1986) Oral immunotherapy of children with rhinoconjunctivitis due to birch pollen allergy. A double blind study. *Allergy* 41:271–279

- Moller C, Dreborg S, Ferdousi HA et al (2002) Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 109:251–256
- Mosbech H, Dreborg S, Madsen F et al (1987) High dose grass pollen tablets used for hyposensitization in hay fever patients. A one year double blind placebo-controlled study. *Allergy* 42:451–455
- Mousavi T, Tajik N, Moradi M, Radjabzadeh MF (2008) CpG Immunotherapy in *Chenopodium album* sensitized mice: The comparison of IFN- γ , IL-10 and IgE responses in intranasal and subcutaneous administrations. *Clin Mol Allergy* 6:10
- Muller U, Akdis CA, Fricker M et al (1998) Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 101:747–754
- Nakamura K, Kitani A, Strober W (2001) Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor β . *J Exp Med* 194:629–644
- Nickelsen JA, Goldstein S, Mueller U et al (1981a) Local intranasal immunotherapy for ragweed allergic rhinitis. I. Clinical response. *J Allergy Clin Immunol* 68:33–40
- Nickelsen JA, Goldstein S, Mueller U et al (1981b) Local intranasal immunotherapy for ragweed allergic rhinitis. II. Immunologic response. *J Allergy Clin Immunol* 68:41–45
- Nickelsen JA, Georgitis JW, Mueller UR et al (1983) Local nasal immunotherapy for ragweed-allergic rhinitis. III. A two year of treatment. *Clin Allergy* 13:509–519
- Niederberger V, Horak F, Vrtala S et al (2004) Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci U S A* 101(Suppl 2):14677–14682
- Novak N, Bieber T, Katoh N (2001) Engagement of Fc epsilon RI on human monocytes induces the production of IL-10 and prevents their differentiation in dendritic cells. *J Immunol* 167:797–804
- Oida T, Zhang X, Goto M et al (2003) CD4+CD25- T cells that express latency-associated peptide on the surface suppress CD4+CD45RB high-induced colitis by a TGF- β -dependent mechanism. *J Immunol* 170:2516–2522
- Olaguibel JM, Alvarez Puebla MJ (2005) Efficacy of sublingual allergen vaccination for respiratory allergy in children. Conclusions from one meta-analysis. *J Investig Allergol Clin Immunol* 15:9–16
- Pajno GB, Morabito L, Barberio G, Parmiani S (2000) Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. *Allergy* 55:842–849
- Pajno GB, Peroni DG, Vita D et al (2003) Safety of sublingual immunotherapy in children with asthma. *Paediatr Drugs* 5:777–781
- Passalacqua G, Albano M, Riccio AM, Scordamaglia A, Canonica GW (1997) Local nasal immunotherapy: experimental evidences and general considerations. *Allergy* 52:10–16
- Passalacqua G, Albano M, Fregonese L et al (1998) Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 351:629–632
- Passalacqua G, Guerra L, Pasquali M, Lombardi C, Canonica GW (2004) Efficacy and safety of sublingual immunotherapy. *Ann Allergy Asthma Immunol* 93:3–12 quiz 12-3, 103
- Passalacqua G, Altrinetti V, Mariani G et al (2005) Pharmacokinetics of radiolabelled Par j 1 administered intranasally to allergic and healthy subjects. *Clin Exp Allergy* 35:880–883
- Patriarca G, Nucera E, Roncallo C et al (2003) Oral desensitizing treatment in food allergy: clinical and immunological results. *Aliment Pharmacol Ther* 17:459–465
- Pauli G, Larsen TH, Rak S et al (2008) Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 122:951–960
- Penagos M, Compalati E, Tarantini F et al (2006) Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3–18 years of age: a meta-analysis of

- randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol* 97:141–148
- Pichler CE, Pichler WJ (2001) Three years of specific immunotherapy with house-dust-mite extracts in patients with rhinitis and asthma: significant improvement of allergen-specific parameters and of nonspecific bronchial hyperreactivity. *Allergy* 56:301–306
- Pierson-Mullany LK, Jackola D, Blumenthal M (2000) Rosenberg A: Altered allergen binding capacities of Amb a 1-specific IgE and IgG4 from ragweed-sensitive patients receiving immunotherapy. *Ann Allergy Asthma Immunol* 84:241–243
- Powell RJ, Frew AJ, Corrigan CJ (2007) Durham SR: Effect of grass pollen immunotherapy with Alutard SQ on quality of life in seasonal allergic rhinoconjunctivitis. *Allergy* 62:1335–1338
- Pugh CW, MacPherson GG, Steer HW (1983) Characterization of nonlymphoid cells derived from rat peripheral lymph. *J Exp Med* 157:1758–1779
- Purohit A, Niederberger V, Kronqvist M et al (2008) Clinical effects of immunotherapy with genetically modified recombinant birch pollen Bet v 1 derivatives. *Clin Exp Allergy* 38:1514–1525
- Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT (2008) Grass pollen immunotherapy induces Foxp3-expressing CD4⁺ CD25⁺ cells in the nasal mucosa. *J Allergy Clin Immunol* 121:1467–1472, 72 e1
- Razafindratsita A, Saint-Lu N, Mascarell L et al (2007) Improvement of sublingual immunotherapy efficacy with a mucoadhesive allergen formulation. *J Allergy Clin Immunol* 120:278–285
- Rebien W, Puttonen E, Maasch HJ, Stix E, Wahn U (1982) Clinical and immunological response to oral and subcutaneous immunotherapy with grass pollen extracts. A prospective study. *Eur J Pediatr* 138:341–344
- Robinson DS, Larche M, Durham SR (2004) Tregs and allergic disease. *J Clin Invest* 114:1389–1397
- Saint-Lu N, Tourdot S, Razafindratsita A et al (2009) Targeting the allergen to oral dendritic cells with mucoadhesive chitosan particles enhances tolerance induction. *Allergy* 64:1003–1013
- Santeliz JV, Van Nest G, Traquina P, Larsen E, Wills-Karp M (2002) Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol* 109:455–462
- Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT (1993) Allergen immunotherapy decreases interleukin 4 production in CD4⁺ T cells from allergic individuals. *J Exp Med* 178:2123–2130
- Simons FE, Shikishima Y, Van Nest G, Eiden JJ, HayGlass KT (2004) Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA. *J Allergy Clin Immunol* 113:1144–1151
- Skripak JM, Nash SD, Rowley H et al (2008) A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 122:1154–1160
- Song JH, Nguyen HH, Cuburu N et al (2008) Sublingual vaccination with influenza virus protects mice against lethal viral infection. *Proc Natl Acad Sci U S A* 105:1644–1649
- Staden U, Rolinck-Werninghaus C, Brewe F et al (2007) Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. *Allergy* 62:1261–1269
- Steinman RM, Hawiger D, Liu K et al (2003) Dendritic cell function in vivo during the steady state: a role in peripheral tolerance. *Ann N Y Acad Sci* 987:15–25
- Suffia I, Reckling SK, Salay G, Belkaid Y (2005) A role for CD103 in the retention of CD4⁺CD25⁺ Treg and control of *Leishmania major* infection. *J Immunol* 174:5444–5455
- Sun CM, Hall JA, Blank RB et al (2007) Small intestine lamina propria dendritic cells promote de novo generation of Foxp3⁺ T reg cells via retinoic acid. *J Exp Med* 204:1775–1785
- Sun JB, Flach CF, Czerkinsky C, Holmgren J (2008) B lymphocytes promote expansion of regulatory T cells in oral tolerance: powerful induction by antigen coupled to cholera toxin B subunit. *J Immunol* 181:8278–8287
- Taudorf E, Weeke B (1983) Orally administered grass pollen. *Allergy* 38:561–564

- Taudorf E, Laursen LC, Lanner A et al (1987) Oral immunotherapy in birch pollen hay fever. *J Allergy Clin Immunol* 80:153–161
- Taudorf E, Laursen L, Lanner A et al (1989) Specific IgE, IgG, and IgA antibody response to oral immunotherapy in birch pollinosis. *J Allergy Clin Immunol* 83:589–594
- Taylor A, Verhagen J, Akdis CA, Akdis M (2004) T regulatory cells in allergy and health: a question of allergen specificity and balance. *Int Arch Allergy Immunol* 135:73–82
- Tighe H, Takabayashi K, Schwartz D et al (2000) Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 106:124–134
- Tsai JJ, Liao EC, Tsai FH, Hsieh CC, Lee MF (2009) The effect of local nasal immunotherapy in allergic rhinitis: using strips of the allergen dermatophagoides pteronyssinus. *J Asthma* 46:165–170
- Urbanek R, Burgelin KH, Kahle S, Kuhn W, Wahn U (1990) Oral immunotherapy with grass pollen in enterosoluble capsules. A prospective study of the clinical and immunological response. *Eur J Pediatr* 149:545–550
- Van Overtvelt L, Lombardi V, Razafindratsita A et al (2008) IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. *Int Arch Allergy Immunol* 145:152–162
- Verhoef A, Alexander C, Kay AB, Larche M (2005) T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. *PLoS Med* 2:e78
- Vourdas D, Syrigou E, Potamianou P et al (1998) Double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. *Allergy* 53:662–672
- Wachholz PA (2003) Durham SR: Induction of ‘blocking’ IgG antibodies during immunotherapy. *Clin Exp Allergy* 33:1171–1174
- Wahn U, Tabar A, Kuna P et al (2009) Efficacy and safety of five-grass-pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 123:160–166 e3
- Wilson DR, Torres LI, Durham SR (2003) Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev* CD002893
- Wilson DR, Lima MT, Durham SR (2005) Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 60:4–12
- Winkler B, Hufnagl K, Spittler A et al (2006) The role of Foxp3+ T cells in long-term efficacy of prophylactic and therapeutic mucosal tolerance induction in mice. *Allergy* 61:173–180