

ORIGINAL ARTICLE

Evaluation of therapeutic sublingual vaccines in a murine model of chronic house dust mite allergic airway inflammation

S. Tourdot¹, S. Airouche¹, N. Berjont¹, A. Da Silveira¹, L. Mascarelli¹, A. Jacquet², L. Caplier³, M. Langelot⁴, V. Baron-Bodo¹ and P. Moingeon¹

¹Research and Development, Stallergenes SA, Antony, France, ²Chulalongkorn University, Bangkok, Thailand, ³BiodOxis, Romainville, France and ⁴Inserm UMR 915, Nantes, France

Clinical & Experimental Allergy

Summary

Background Second generation therapeutic vaccines based upon recombinant allergens or natural extracts, potentially formulated in vector systems or adjuvants, are being developed. To this aim, preclinical studies in relevant animal models are needed to select proper allergens, formulations and administration schemes.

Objective To develop a chronic house dust mite (HDM) allergy model to evaluate sublingual therapeutic vaccine candidates.

Methods The BABL/c mice that were used were sensitized with *Dermatophagoides pteronyssinus* (Dpte) and *Dermatophagoides farinae* (Dfar) mite extracts by intraperitoneal injections followed by aerosol exposures. Animals subsequently underwent sublingual immunotherapy (SLIT) with either Dpte, Dfar or Dpte/Dfar extracts, twice a week for 8 weeks. SLIT efficacy was assessed by whole body plethysmography, lung histology and broncho-alveolar lavages cell counts. Specific T cell and antibody responses to major and minor HDM allergens were monitored in tissues and serum/saliva, respectively.

Results Mice sensitized to Dpte and Dfar allergens exhibited strong airway hyperresponsiveness (AHR) and lung inflammatory infiltrates including eosinophils. Sensitized animals mounted Th2-biased cellular and humoral responses specific for group 1 and 2 major allergens, as well as group 5, 7 and 10 minor allergens. This phenotype was sustained for at least 2 months, allowing the evaluation of immunotherapeutic protocols with HDM extracts-based vaccines. In this model, SLIT decreased AHR and Th2 responses and induced HDM-specific IgAs in saliva. The Dpte/Dfar mix proved the most efficacious when compared to Dpte or Dfar extracts alone.

Conclusions and Clinical Relevance The efficacy of a sublingual vaccine based on a Dpte/Dfar allergen extract mix was demonstrated in a well standardized murine model of chronic allergic airway inflammation based on clinically relevant mite allergens. The latter will be used as a benchmark for evaluation of future vaccines, including recombinant allergens. This HDM allergic airway inflammation animal model is a useful tool to design and select candidate vaccines to be tested in humans.

Keywords allergy vaccines, HDM, sublingual immunotherapy

Submitted 14 June 2011; revised 1 August 2011; accepted 5 August 2011

Correspondence:

Philippe Moingeon, 6, rue Alexis de Tocqueville, 92183 Antony Cedex, France.

E-mail: pmoingeon@stallergenes.fr

Introduction

Allergen-specific immunotherapy (AIT) is a safe and effective treatment for type I respiratory allergies [1], which can be performed not only via either the subcutaneous, sublingual [2–4], but also via either the epicutaneous or intralymphatic routes [5–8]. AIT optimization is still ongoing, mostly as it relates to the use

of simplified and shortened treatment schemes, as well as the replacement of biological extracts by recombinant allergens [4, 9, 10]. AIT efficacy can also be further enhanced by the use of adjuvants and delivery systems [11–13]. In this context, animal models are critically needed to help selecting allergens, formulations and administration schemes at the preclinical stage. To this aim, animal models should ideally be

based on clinically relevant allergens, as opposed to conventional ovalbumine (OVA). Furthermore, a chronic inflammation should be established to allow testing long-term therapeutic protocols, as foreseen in human applications.

Hence, a growing number of clinically relevant allergens-based model are being developed [14]. Currently available preclinical models of house dust mite (HDM) sensitization are based on either the *Dermatophagoides pteronyssinus* (Dpte) or *Dermatophagoides farinae* (Dfar) common mite species [15, 16]. However, most allergic individuals in Europe, North America and Asia are co-exposed and co-sensitized to both species [17–19]. Thus, in the present study, we set up a mouse model of allergic airway inflammation recapitulating the main features of HDM allergy to investigate sublingual immunotherapy (SLIT) approaches. Herein, we demonstrate that mice sensitized to both Dpte/Dfar develop strong airway hyperresponsiveness (AHR), lung inflammation and eosinophilia, together with Th2-biased T and B cell responses specific for major and minor mite allergens. In those animals, SLIT with an equal w/w mix of aqueous extracts from both species proved the most successful compared to single Dpte or Dfar extracts at down-regulating both lung inflammation and HDM-specific Th2 responses.

Materials and methods

Animals, culture medium and reagents

The BABL/c mice used were 6–8-week-old female ones purchased from Charles River (L'Arbresle, France). International levels of ethical standards were applied for animal handling. Experimental protocols were approved by Stallergenes internal ethical committee for animal experimentation. Culture medium consisted of RPMI 1640 supplemented with 10% foetal calf serum, 1% L-glutamine, 200 U/mL penicillin and 200 µg/mL streptomycin (all from Invitrogen, Carlsbad, CA, USA). Phosphate-buffered saline (PBS) and alum were purchased from Lonza (Basel, Switzerland) and Pierce (Rockford, IL, USA), respectively. Dpte and Dfar mite extracts were obtained from Stallergenes SA (Antony, France). Recombinant Der p 1 (rDer p 1) was produced in tobacco plants as previously described [20], natural Der p 2 (nDer p 2) was purified from Dpte extracts at Stallergenes SA, natural Der f 1 (nDer f 1) and Der f 2 (nDer f 2) were purchased from Indoor Biotechnologies Inc (Charlottesville, VA, USA). Recombinant allergens, including Der p 5 (rDer p 5) produced in *Pichia pastoris*, and Der p 7 (rDer p 7) as well as Der p 10 (rDer p 10), both produced in *Escherichia coli*, were obtained from the Université Libre de Bruxelles, Gosselies, Belgium.

Induction of allergic airway inflammation and sublingual immunotherapy

Mice were sensitized by i.p. injections on days 0 and 14 of Dpte and Dfar extracts (using doses equivalent to 10 µg allergen group 1 per mouse) in presence of 2 mg alum, then exposed to aerosolized Dpte/Dfar extracts (using similar doses as above) 20 min/day, from days 21 to 24. On day 25, AHR was evaluated by whole body plethysmography (Buxco Europe Ltd, Winchester, UK) and expressed as enhanced pause (Penh) as previously described [21]. Penh indexes were obtained as a ratio between Penh values measured after either exposure to increasing doses of methacholine (i.e. 12.5, 25, 50, 100 mg/mL, Sigma, St Louis, MO, USA) or nebulized PBS. Bronchial resistance and dynamic compliance were measured using a Flexivent® system (Emka Technologies, Falls Church, VA, USA) after exposure to increasing doses of methacholine (i.e. 12.5, 25, 50, 100 mg/mL), as described elsewhere [22]. Mice were randomized into control (PBS) and treatment groups (receiving either Dpte, Dfar, or Dpte/Dfar 50–50% w/w mix, respectively), prior to undergoing SLIT twice a week for 8 weeks using doses equivalent to 50 µg group 2 allergen for all groups (with 25 µg equivalent from group 2 Dpte extracts + 25 µg equivalent from group 2 Dfar extracts in the Dpte/Dfar 50–50% w/w mix group). For SLIT administration, mice were held on their back for 1 min to prevent immediate swallowing of the antigen. After treatment completion, mice were challenged by aerosol re-exposure to Dpte/Dfar extracts for two consecutive days. AHR was measured 24 h after the last aerosol and mice killed the following day for further analysis. In a second experiment, Dpte/Dfar 50–50% w/w mix extracts were administrated at doses containing either 1, 10 or 50 µg total equivalent group 2 allergen originating from both species.

Lung inflammation

For tissue histology, lungs were recovered, fixed in phosphate-buffered formalin-zinc and embedded in paraffin wax. Tissue sections were stained with haematoxylin, eosin and safran for the determination of cellular infiltrates. For analysis of inflammatory cells in broncho-alveolar lavages (BAL), mice were anesthetized by intraperitoneal (i.p.) injection of pentobarbital (50 mg/kg body weight; Vetoquinol, Lure, France), and BAL performed with 1200 µL PBS. BAL fluid was centrifuged at 800 g for 5 min at 4°C. Cell pellets were resuspended in PBS, spun onto glass slides by cyto-centrifugation, fixed and stained with May–Grünwald Giemsa (Réactifs RAL, Martillac, France) for differential cell counting. Supernatants were stored at –80°C for cytokine analysis.

Antibody and T cell responses

Blood was collected by cardiac puncture under terminal anaesthesia and sera stored at -20°C . Salivation was induced by intraperitoneal injection of $50\text{ }\mu\text{g}/\text{mouse}$ pilocarpine (Sigma) 15 min before saliva collection. HDM-specific antibody responses were evaluated using ELISA. Briefly, sera and saliva samples were incubated for 24 h at 37°C in 96-well plates coated overnight with either purified rDer p 1, nDer p 2, rDer p 5, rDer p 7, rDer p 10, nDer f 1, nDer f 2 allergens ($10\text{ }\mu\text{g}/\text{mL}$), or Dpte and Dfar extracts (at doses equivalent to $10\text{ }\mu\text{g}/\text{mL}$ Der p 2). Plates were washed and revealed with biotinylated secondary antibody anti-mouse IgG1, IgG2a, IgA (BD Biosciences, San Jose, CA, USA), or anti-IgE-HRP antibodies. After washing, specific antibody binding was detected with streptavidin-peroxydase/2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) substrate tandem (Roche Diagnostics, Meylan, France) or ABTS alone after reading the plates at 405 nm on a multiskan Ascent spectrophotometer (Thermo Labsystems, Waltham, MA, USA). Antibody titres were defined as the reverse of the last dilution for which the optical value was twofold higher than the mean of control mice.

To assess T cell responses, lungs and spleen were removed. To recover cells from lung tissues, one lobe was incubated for 1 h in RPMI supplemented with digest reagent (collagenase $75\text{ U}/\text{mL}$; Roche, Basel, Switzerland). Isolated cells were filtered through a $70\text{ }\mu\text{m}$ sieve and washed twice before re-suspension in culture medium. Cells were plated at 10^6 cells per 96-well plate well and stimulated with $10\text{ }\mu\text{g}/\text{mL}$ of either recombinant, natural purified mite allergens, Dpte/Dfar extracts equivalent to $10\text{ }\mu\text{g}/\text{mL}$ Der p 2, or medium alone, in a final volume of $200\text{ }\mu\text{L}$. After 72 h at 37°C in 5% CO_2 /95% air, IFN- γ , IL-5, IL-10 and IL-13 were measured in culture supernatants using a CBA Flex kit (BD Biosciences) according to the manufacturer's instructions.

Statistical analysis

Results are presented as mean $\pm$ SEM values. Data were analysed using non-parametric Mann-Whitney tests when comparing sensitized mice with controls, and Kruskal-Wallis non-parametric tests for intergroup comparisons. P values <0.05 was considered as significant. Values of $*P < 0.05$ and $**P < 0.01$.

Results

House dust mite allergens induce a strong airway inflammation and Th2 responses in BALB/c mice

To induce allergic airway inflammation to common mites, mice were exposed to equal quantities of Dpte

and Dfar extracts via a combination of administration routes. Briefly, mice received a mixture of crude allergen extracts i.p. at days 0 and 14, prior to being exposed to aerosolized allergens for four consecutive days, from day 21 to 24. AHR was assessed by whole body plethysmography 24 h after the last aerosol exposure. Sensitized mice showed strong AHR to methacholine when compared to control group (Fig. 1a), in association with acute inflammation in the lung (Fig. 1b, lower panel). Using invasive methods, both an increase in bronchial resistance (Fig. 1c) and a significant decrease in dynamic compliance (Fig. 1d) were confirmed in sensitized animals. BAL analyses revealed both eosinophilic and neutrophilic infiltrates (Fig. 1e), and a local cytokine secretion profile unambiguously biased towards Th2 when compared to control group (Fig. 1f). Th2 cytokine production was also observed upon re-stimulation of splenocytes and lung cells from HDM-sensitized mice with each of rDer p 1, nDer p 2, nDer f 1 or nDer f 2 major mite allergens, with responses against Dpte-derived allergens being noticeably the strongest (Fig. 2a). Sensitized mice also mounted Th2 responses against each of group 5, 7 and 10 minor allergens (Fig. 2b). Such responses were not detectable in control mice (data not shown). Serum antibody responses correlated with the Th2-biased cellular response observed in the lung, as higher levels of HDM-specific IgG1 vs. IgG2a were consistently measured in sensitized animals (Fig. 2c). Detection of seric HDM-specific IgE confirmed the establishment of a type I respiratory allergy in such animals, with sensitization to both major and minor allergens (Figs 2d and e see Methods for IgE levels in the control group).

Sublingual immunotherapy with house dust mite decreases airway hyperresponsiveness and inflammation

To assess the efficacy of sublingual vaccination with HDM extracts, HDM-sensitized mice were randomized to receive SLIT with Dpte, Dfar, a Dpte/Dfar 50–50% w/w mix, or PBS. As shown in Fig. 3, upon re-challenge with allergens, mice treated with the Dpte/Dfar mix vaccine showed a significant decrease in AHR when compared with the PBS-treated group. A non-statistically significant decrease of AHR was observed following treatment with either Dpte or Dfar alone (Fig. 3a).

To confirm the efficacy of the Dpte/Dfar vaccine, SLIT was also conducted with various doses of the w/w mix (ranging from 1 to $50\text{ }\mu\text{g}$ total equivalent group 2 allergen), demonstrating a dose-dependent down-regulation of AHR in Dpte/Dfar-treated groups (Fig. 3b). AHR reduction for control and $50\text{ }\mu\text{g}$ groups was accompanied by a decrease in eosinophils in BAL and in lung inflammatory infiltrates (Figs 4a and b). In such chronic therapeutic settings, neutrophilia was no longer

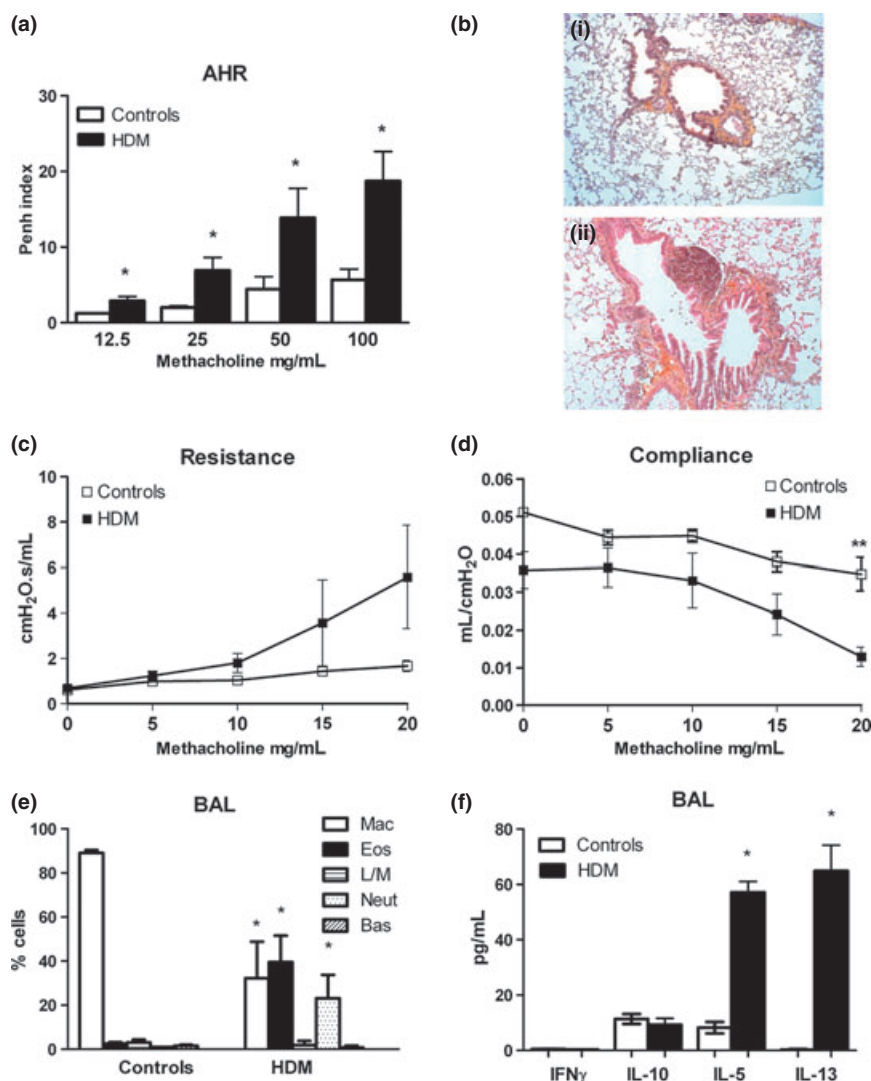


Fig. 1. House dust mite (HDM)-sensitized mice exhibit strong airway hyperresponsiveness (AHR), lung inflammation and a Th2 cytokine profile (a) AHR to methacholine was measured by whole body pletysmography at day 25 and expressed as a Penh index. Results are expressed as mean values $\pm$ SEM, with $n = 4$ –8 mice per group. A value of $*P < 0.05$ in comparison to control mice. (b) Lungs from control (1) and HDM-sensitized mice (2) were collected for histology studies on haematoxylin, eosin and safran-stained, wax-embedded tissue sections. Data shown are representative of four mice evaluated in each group. (c) Bronchial resistance and (d) dynamic compliance were measured using a flexivent system 24 h after the last aerosol. Results are expressed as mean values $\pm$ SEM, with $n = 6$ mice per group. (e) Differential cell counts were performed after May–Grünwald Giemsa staining of broncho-alveolar lavages (BAL) cytopspins. Results are expressed as mean values $\pm$ SEM, with $n = 4$ –8 mice per group. (f) Cytokine profiles were determined in BALs from HDM-sensitized mice by CBA. Results are expressed as mean values $\pm$ SEM, with $n = 4$ –8 mice per group. Data are representative of three independent experiments.

observed in BAL even in PBS-treated groups (data not shown).

Airway hyperresponsiveness and lung inflammation decreases are associated with changes in CD4⁺ T cell polarization

The T cell responses were investigated in animal groups having received SLIT with 10 or 50 μ g equivalent group 2 allergen per dose of the Dpte/Dfar mix, as described in Fig. 3b. SLIT with the Dpte/Dfar mix led to

a dose-dependent reduction of IL-5 production in the lung in response to stimulation with all HDM major allergens, when compared to animals receiving PBS (Fig. 4c, left panel). In line with previous studies [23–25], a significant increase in IL-10 secretion by nDer p 2- and nDer f 1-specific T cells was observed in animals receiving the Dpte/Dfar 50 μ g dose mix, suggesting a potential induction of regulatory T cells (Fig. 4c, right panel).

As we previously observed in an OVA-induced chronic asthma model [24], SLIT had no major impact

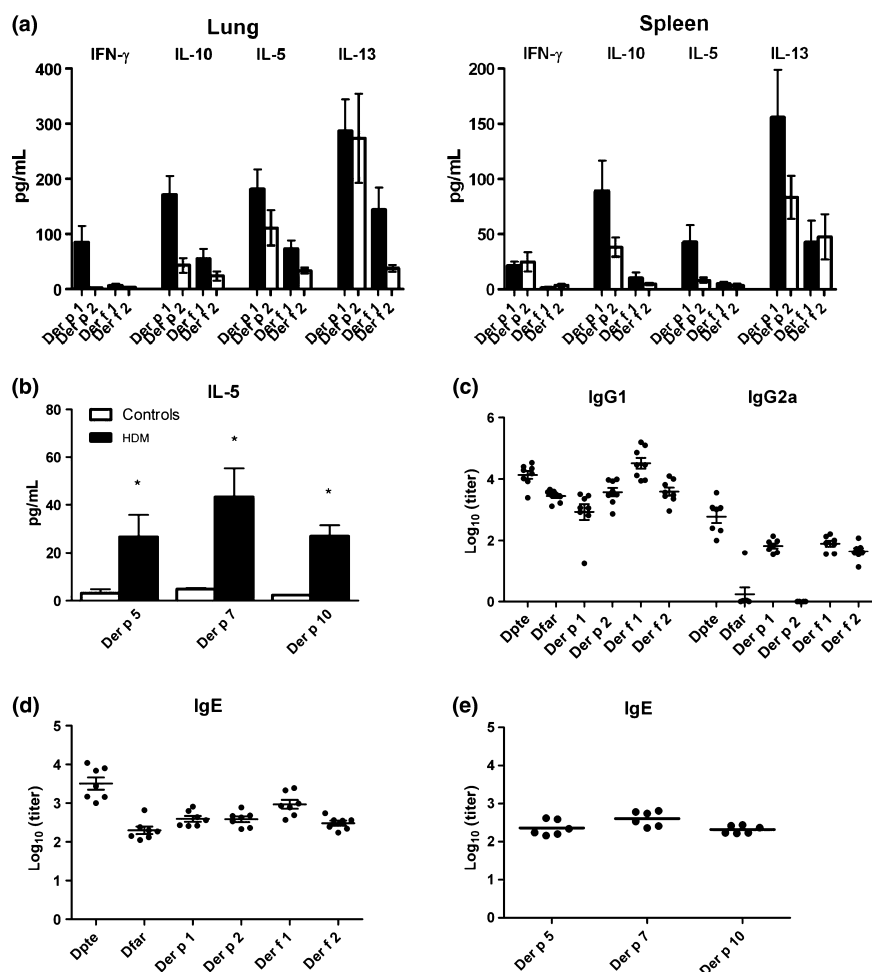


Fig. 2. Lung inflammation in house dust mite (HDM)-sensitized mice is associated with mite specific Th2-biased T cell and antibody responses (a) Lung cells and splenocytes from HDM-sensitized were restimulated *in vitro* with 10 µg/mL purified rDer p 1, rDer p 2, nDer f 1, or nDer f 2 allergens for 72 h. IFN-γ, IL-5, IL-10 and IL-13 cytokine levels were measured by CBA in culture supernatants. Results are expressed as mean values ± SEM, with $n = 6-8$ mice per group. (d) Lung cells were recovered from tissues as described in Methods and cultured *in vitro* with 10 µg of either Der p 5, Der p 7, or Der p 10 for 72 h. IL-5 levels were measured by CBA in culture supernatants. Results are expressed as mean values ± SEM, with $n = 3-4$ mice per group. (b, c, e) Blood was collected by cardiac puncture under terminal anaesthesia and seric antibody responses were assessed by ELISA using HDM extracts or purified allergens-coated plates. Results are expressed as mean values ± SEM, with $n = 6-8$ mice per group. Data are representative of three independent experiments. Background signals detected in non-sensitized animals from the control group were included into the calculation of antibody titres, as described in Methods.

on allergen-IgG1, IgG2a and IgE levels in serum (Figs 5a and b). In contrast, SLIT induced strong IgA responses against both mite species in saliva (Fig. 5c).

Discussion

An increasing number of allergic airway inflammation models based on clinically relevant allergens are being developed for preclinical testing of allergy vaccines [14]. However, most animal models of respiratory allergy document the efficacy of prophylactic vaccines, whereas very few allow the investigation of therapeutic protocols. Yet, the mechanisms underlying prophylactic and therapeutic immunotherapy may differ greatly,

since numerous features of acute vs. chronic airway inflammation are dissimilar [26, 27]. For example, inhibition of specific IgE induction is a general feature of prophylactic SLIT, whereas more limited effects on established seric IgE responses are observed in therapeutic settings. Similar discrepancies regarding IgG1 and IgG2a levels have also been reported [28, 29]. In addition, the challenge of a therapeutic vaccine mainly lies in its ability to redirect the immune response from an established Th2 to a Th1/Treg pattern [30-32]. In prophylactic settings on the contrary, Th1/Treg responses are induced *de novo* from a pool of naïve T cells, which might therefore lead to an overestimation of vaccine efficacy [33].

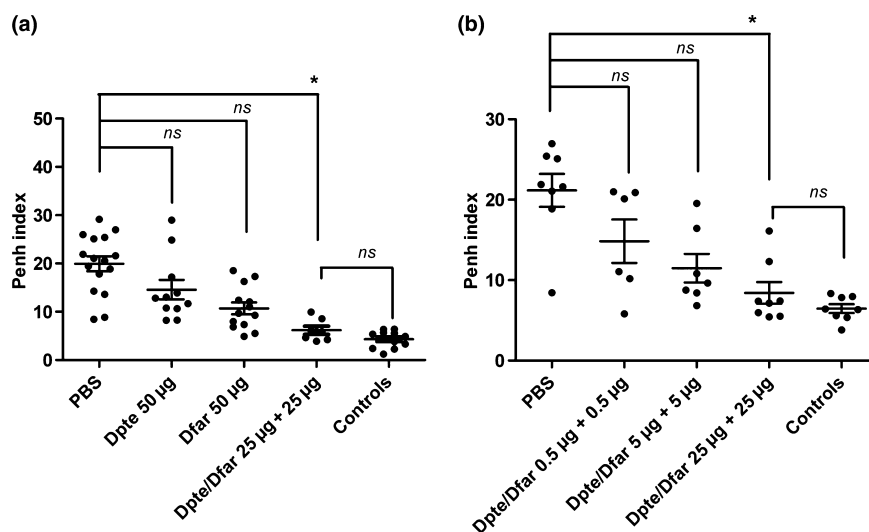


Fig. 3. Sublingual immunotherapy (SLIT) with house dust mite extracts decreases airway hyperresponsiveness (AHR) in a dose-dependent manner (a) After 8 weeks of SLIT with either phosphate-buffered saline (PBS), *Dermatophagoides pteronyssinus* (Dpte) (dose equivalent to 50 µg group 2 allergen), *Dermatophagoides farinae* (Dfar) (dose equivalent to 50 µg group 2 allergen) or Dpte/Dfar (dose equivalent to 25 µg group 2 allergen from Dpte + 25 µg group 2 allergen from Dfar), mice were re-exposed to allergen extracts for two consecutive days. AHR to methacholine was measured by whole body plethysmography 24 h after the last challenge and expressed as a Penh index. Results are shown for the 100 mg/mL methacholine dose and expressed as mean values $\pm$ SEM, with $n = 6$ –16 mice per group. * $P < 0.05$ in comparison to PBS-treated group. ns, Non-statistically different from control mice. (b) Same as in (a), with SLIT performed with PBS or Dpte/Dfar 50–50% w/w mix vaccines containing either 1, 10 or 50 µg total equivalent group 2 Dpte and Dfar allergen per dose. Data are representative of two independent experiments.

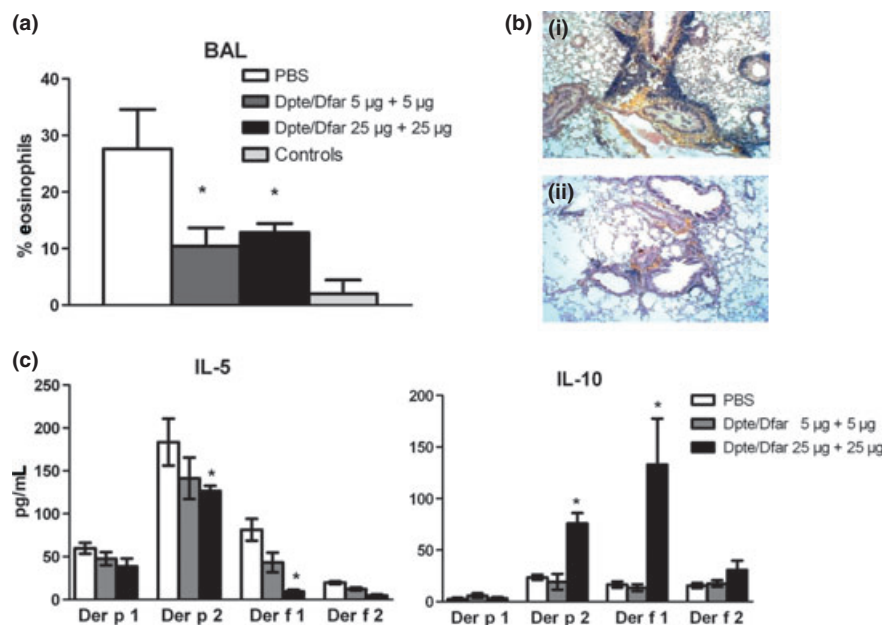


Fig. 4. Sublingual immunotherapy with *Dermatophagoides pteronyssinus*/*Dermatophagoides farinae* reduces lung inflammation and Th2 T cell responses, while increasing IL-10 production (a) Differential cells counts were performed after May–Grünwald Giemsa staining of broncho-alveolar lavages cytopins. Results are expressed as mean values $\pm$ SEM, with $n = 4$ –9 mice per group. * $P < 0.05$ in comparison to phosphate-buffered saline (PBS)-treated group. (b) Lungs were collected from PBS (1) and 50 µg dose (2) -treated groups for histology studies of haematoxylin, eosin and safran-stained, wax-embedded tissue sections. Data shown are representative of four mice evaluated in each group. (c) Lung cells were recovered as described in Methods and restimulated *in vitro* with 10 µg purified group 1 and group 2 allergens for 72 h. IL-5 and IL-10 cytokine levels were measured by CBA in culture supernatants. Results are expressed as mean values $\pm$ SEM, with $n = 6$ –8 mice per group. * $P < 0.05$ in comparison to PBS-treated group. Data are representative of three independent experiments.

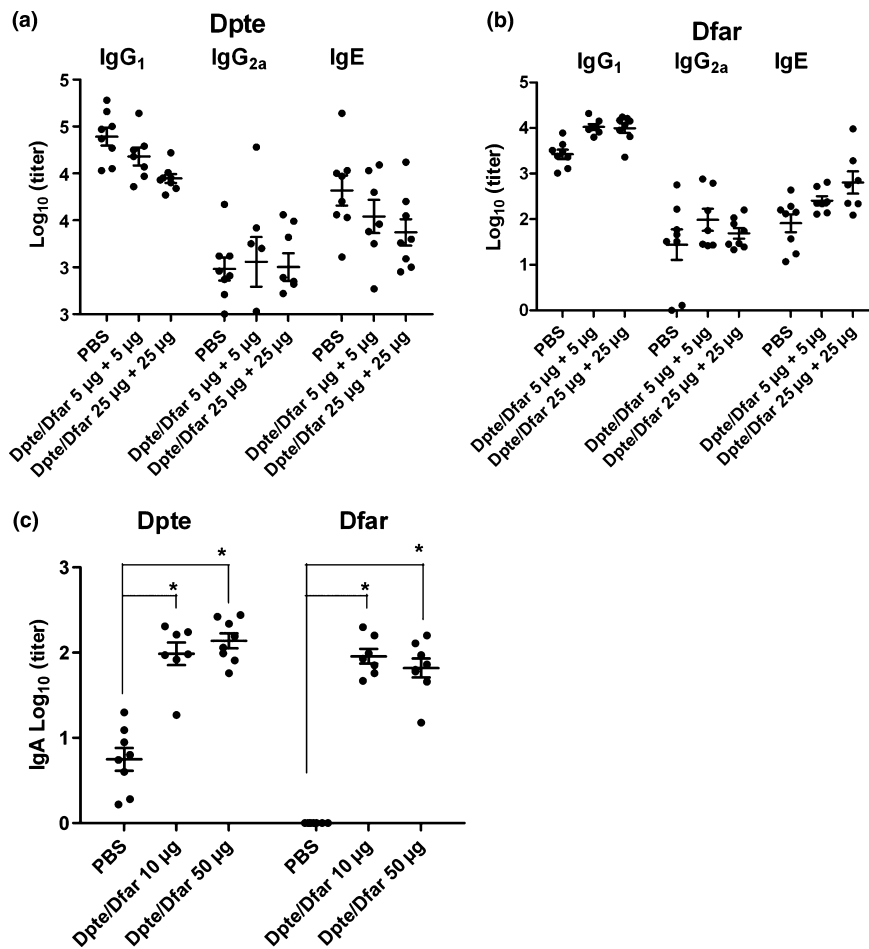


Fig. 5. *Dermatophagoides pteronyssinus* (Dpte) and *Dermatophagoides farinae* (Dfar)-specific antibody levels in serum and saliva after sublingual immunotherapy (a, b) Blood was collected by cardiac puncture under terminal anaesthesia from mice having received phosphate-buffered saline (PBS) or Dpte/Dfar mixes, as well as from control group. Seric anti-Dpte and anti-Dfar IgG1, IgG2a and IgE responses were assessed in sera by ELISA using plates coated with extracts as described in Methods. (c) Saliva was collected after induction with pilocarpine as described in Methods. Anti-Dpte IgA responses were assessed by ELISA using plates coated with extracts as described in Methods. (a, b, c) Titres were defined as the reverse of the last dilution for which the optical value was twofold higher to the one of control group. Results are expressed as mean values $\pm$ SEM, with $n = 7$ –8 mice per group. A value of $*P < 0.05$ in comparison to PBS-treated group. Data are representative of three independent experiments.

Herein, we developed a chronic HDM allergy model with the aim to evaluate sublingual therapeutic vaccine candidates. BALB/c mice exposed to Dpte and Dfar extracts developed lung inflammation, high AHR associated with Th2 biased mite-specific T cell responses in target organs and peripheral blood, as well as strong seric IgE responses against both major and minor mite allergens. Although airway remodelling was not investigated in the present study, the chronic inflammation phenotype induced was sustained for at least 8 weeks, allowing the evaluation of therapeutic desensitization protocols based on aqueous mite extracts. Hence, this model allowed us to explore the efficacy of one-species vs. mixed SLIT vaccines. We found that a Dpte/Dfar 50–50% w/w mix currently used to treat mite allergic

patients [34, 35] had a strong efficacy in our mouse model. Clinical efficacy was accompanied by induction of allergen-specific IgA at the site of administration.

Of note, the Dpte/Dfar mix proved more efficacious than single extracts at hampering allergy symptoms and dampening down HDM-specific Th2 responses in Dpte-/Dfar-sensitized animals. Most investigations regarding HDM-specific immunotherapy were carried out in animals sensitized to either one of the most prevalent mite species, i.e. Dpte or Dfar [15, 16, 36–38]. However, epidemiology studies confirmed that a large majority of HDM allergic subjects throughout the world exhibit antibody responses against both Dpte and Dfar allergens, suggesting that co-exposure to the two species leads to co-sensitization [17, 18]. Our preclinical

model recapitulates those features since HDM-sensitized mice develop antibody and T cell responses not only against group 1 and group 2 major allergens originating from the two mite species, but also against minor allergens [19]. The fact that the Dpte/Dfar mix proved more efficacious than single extracts in such settings suggests that despite a significant homology and cross-reactivity, allergens from both Dpte and Dfar species are required to reach maximum SLIT efficacy. Our results are consistent with the observation in a recent phase IIb/III clinical trial conducted in HDM-allergic patients that SLIT with Dpte/Dfar sublingual tablets induces a significant decrease in rhinitis symptoms after 1 year of treatment, with persisting benefit during a second treatment-free year [39].

Importantly, our study also confirmed that successful SLIT in animals sensitized to natural allergens is associated with local immunological changes. Indeed, in agreement with our previous study [24], SLIT did not translate into dramatic variations of mite specific IgE, IgG1 or IgG2a titres in serum, likely due to the presence of long-lived plasma cells [40]. With respect to specific T cell responses, SLIT down-regulated Th2 responses against mite allergens and induced, at least when using high dose regimens, IL-10 producing T cells (possibly Tr1 regulatory cells) as previously reported [23, 41, 42]. In agreement with previous preclinical SLIT studies

conducted in OVA, HDM or timothy grass allergic mice [24, 28, 43], SLIT in the present model induced HDM-specific mucosal IgA. IgA is thought to play a role in tolerance induction [44, 45] and has been shown to increase in serum during the course of SLIT in grass pollen atopic patients [42].

Altogether, our model of HDM-sensitized mice mirrors the sensitization profiles found in HDM allergic patients. Thus, using the Dpte/Dfar mix vaccine as a benchmark, we are currently evaluating in this model second generation vaccines based on mite recombinant allergens, combined to various delivery systems.

Acknowledgements

We thank Martine Pinheiro for excellent secretarial assistance. Alain Jacquet was funded by National Science and Technology Development Agency (NSTDA, BIOTEC Grant P-09-00324), the National Research University Project of CHE and the Ratchadaphiseksomphot Endowment Fund (HR1164A) and the CU-Cluster-Emerging H-8-68-53.

Conflict of interest: Some authors (ST, SA, NB, ADS, LM, ML, VB, PM) are employees at Stallergenes SA. The authors declare no financial or commercial conflict of interest.

References

- 1 Bousquet J, Lockey R, Malling HJ *et al*. Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American Academy of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* 1998; **81**:401–5.
- 2 Gidaro GB, Marcucci F, Sensi L, Incorvaia C, Frati F, Ciprandi G. The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin Exp Allergy* 2005; **35**:565–71.
- 3 Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005; **60**:4–12.
- 4 Canonica GW, Bousquet J, Casale T *et al*. Sub-lingual immunotherapy: World Allergy Organization Position Paper 2009. *Allergy* 2009; **64** (Suppl. 91):1–59.
- 5 Senti G, Graf N, Haug S *et al*. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2009; **124**:997–1002.
- 6 Senti G, Johansen P, Kundig TM. Intralymphatic immunotherapy. *Curr Opin Allergy Clin Immunol* 2009; **9**:537–43.
- 7 Pipet A, Botturi K, Pinot D, Vervloet D, Magnan A. Allergen-specific immunotherapy in allergic rhinitis and asthma. Mechanisms and proof of efficacy. *Respir Med* 2009; **103**:800–12.
- 8 Carnes J, Robinson DS. New strategies for allergen immunotherapy. *Recent Pat Inflamm Allergy Drug Discov* 2008; **2**:92–101.
- 9 Passalacqua G, Lombardi C, Troise C, Canonica GW. Sublingual immunotherapy: certainties, unmet needs and future directions. *Eur Ann Allergy Clin Immunol* 2009; **41**:163–70.
- 10 Cox L, Cohn JR. Duration of allergen immunotherapy in respiratory allergy: when is enough, enough? *Ann Allergy Asthma Immunol* 2007; **98**:416–26.
- 11 Saint-Lu N, Tourdot S, Razafindratsita A *et al*. Targeting the allergen to oral dendritic cells with mucoadhesive chitosan particles enhances tolerance induction. *Allergy* 2009; **64**:1003–13.
- 12 Daniel C, Repa A, Wild C *et al*. Modulation of allergic immune responses by mucosal application of recombinant lactic acid bacteria producing the major birch pollen allergen Bet v 1. *Allergy* 2006; **61**:812–9.
- 13 Hirose I, Tanaka H, Takahashi G *et al*. Immunomodulatory effects of CpG oligodeoxynucleotides on house dust mite-induced airway inflammation in mice. *Int Arch Allergy Immunol* 2008; **147**:6–16.
- 14 Fuchs B, Braun A. Improved mouse models of allergy and allergic asthma – chances beyond ovalbumin. *Curr Drug Targets* 2008; **9**:495–502.
- 15 Ulrich K, Hincks JS, Walsh R *et al*. Anti-inflammatory modulation of chronic airway inflammation in the murine house dust mite model. *Pulm Pharmacol Ther* 2008; **21**:637–47.
- 16 Wakahara K, Tanaka H, Takahashi G *et al*. Repeated instillations of *Derma-tophaagoides farinae* into the airways can induce Th2-dependent airway hyperresponsiveness, eosinophilia and remodeling in mice: effect of intratracheal treatment of fluticasone propio-

- nate. *Eur J Pharmacol* 2008; **578**: 87–96.
- 17 Pittner G, Vrtala S, Thomas WR *et al.* Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 2004; **34**:597–603.
 - 18 Thomas WR, Hales BJ. T and B cell responses to HDM allergens and antigens. *Immunol Res* 2007; **37**:187–99.
 - 19 Weghofer M, Thomas WR, Kronqvist M *et al.* Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest* 2008; **38**:959–65.
 - 20 Burtin D, Chabre H, Olganier B *et al.* Production of native and modified recombinant Der p 1 molecules in tobacco plants. *Clin Exp Allergy* 2009; **39**:760–70.
 - 21 Hamelmann E, Schwarze J, Takeda K *et al.* Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997; **156**:766–75.
 - 22 Swedin L, Ellis R, Kemi C *et al.* Comparison of aerosol and intranasal challenge in a mouse model of allergic airway inflammation and hyperresponsiveness. *Int Arch Allergy Immunol* 2010; **153**:249–58.
 - 23 Mascarell L, Van Overvelt L, Lombardi V *et al.* A synthetic triacylated pseudo-dipeptide molecule promotes Th1/TReg immune responses and enhances tolerance induction via the sublingual route. *Vaccine* 2007; **26**:108–18.
 - 24 Razafindratsita A, Saint-Lu N, Mascarell L *et al.* Improvement of sublingual immunotherapy efficacy with a mucoadhesive allergen formulation. *J Allergy Clin Immunol* 2007; **120**:278–85.
 - 25 Van Overvelt L, Lombardi V, Razafindratsita A *et al.* IL-10-inducing adjuvants enhance sublingual immunotherapy efficacy in a murine asthma model. *Int Arch Allergy Immunol* 2008; **145**:152–62.
 - 26 Gregory LG, Causton B, Murdoch JR *et al.* Inhaled house dust mite induces pulmonary T helper 2 cytokine production. *Clin Exp Allergy* 2009; **39**: 1597–610.
 - 27 McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 2004; **34**:497–507.
 - 28 Liu Z, Guo H, Wu Y, Yu H, Yang H, Li J. Local nasal immunotherapy: efficacy of *Dermatophagoides farinae*-chitosan vaccine in murine asthma. *Int Arch Allergy Immunol* 2009; **150**:221–8.
 - 29 Bauer L, Bohle B, Jahn-Schmid B *et al.* Modulation of the allergic immune response in BALB/c mice by subcutaneous injection of high doses of the dominant T cell epitope from the major birch pollen allergen Bet v 1. *Clin Exp Immunol* 1997; **107**:536–41.
 - 30 Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006; **61**:151–65.
 - 31 Frew AJ. Allergen immunotherapy. *J Allergy Clin Immunol* 2010; **125**: S306–13.
 - 32 Lloyd CM, Murdoch JR. Tolerizing allergic responses in the lung. *Mucosal Immunol* 2010; **3**:334–44.
 - 33 Linhart B, Bigenzahn S, Hartl A *et al.* Costimulation blockade inhibits allergic sensitization but does not affect established allergy in a murine model of grass pollen allergy. *J Immunol* 2007; **178**:3924–31.
 - 34 Bousquet J, Scheinmann P, Guinneeain MT *et al.* Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy* 1999; **54**:249–60.
 - 35 Pham-Thi N, Scheinmann P, Fadel R, Combebias A, Andre C. Assessment of sublingual immunotherapy efficacy in children with house dust mite-induced allergic asthma optimally controlled by pharmacologic treatment and mite-avoidance measures. *Pediatr Allergy Immunol* 2007; **18**:47–57.
 - 36 Mondoulet L, Dioszeghy V, Ligouis M, Dhelft V, Dupont C, Benhamou PH. Epicutaneous immunotherapy on intact skin using a new delivery system in a murine model of allergy. *Clin Exp Allergy* 2010; **40**:659–67.
 - 37 Yu HQ, Li XH, Guo H *et al.* Sublingual immunotherapy efficacy of *Dermatophagoides farinae* vaccine in a murine asthma model. *Int Arch Allergy Immunol* 2010; **152**:41–8.
 - 38 Lu M, Dawicki W, Zhang X, Huang H, Nayyar A, Gordon JR. Therapeutic induction of tolerance by IL-10-differentiated dendritic cells in a mouse model of house dust mite-asthma. *Allergy* 2011; **66**:612–20.
 - 39 Demoly P, Worm M, Fokkens W *et al.* Post-treatment efficacy of sublingual allergen immunotherapy tablets in adult patients suffering from house dust mite-related allergic rhinitis. *EAACI* 2011 (<http://www.eaaci.com>); abstract 303.
 - 40 Luger EO, Fokuhl V, Wegmann M *et al.* Induction of long-lived allergen-specific plasma cells by mucosal allergen challenge. *J Allergy Clin Immunol* 2009; **124**:819–26.
 - 41 Bohle B, Kinaciyan T, Gerstmayr M, Radakovics A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol* 2007; **120**:707–13.
 - 42 Scadding GW, Shamji MH, Jacobson MR *et al.* Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. *Clin Exp Allergy* 2010; **40**:598–606.
 - 43 Kildsgaard J, Brimnes J, Jacobi H, Lund K. Sublingual immunotherapy in sensitized mice. *Ann Allergy Asthma Immunol* 2007; **98**:366–72.
 - 44 Novak N, Haberstock J, Bieber T, Allam JP. The immune privilege of the oral mucosa. *Trends Mol Med* 2008; **14**:191–8.
 - 45 Schwarze J, Cieslewicz G, Joetham A *et al.* Antigen-specific immunoglobulin-A prevents increased airway responsiveness and lung eosinophilia after airway challenge in sensitized mice. *Am J Respir Crit Care Med* 1998; **158**:519–25.