

to test SLIT protocols with the recombinant Bet v 1a (rBet v 1a) molecule formulated in mucoadhesive microparticles. We demonstrate that mice sensitized to BP exhibit strong and sustained airway hyperresponsiveness (AHR), lung inflammation and eosinophilia, associated with Bet v 1a-specific IgE and Th2 responses. Using this model, we establish the superior efficacy of vectorized rBet v 1a when compared to the corresponding soluble molecule, both in terms of dose response as well as onset of efficacy.

2. Materials and methods

2.1. Animals and reagents

Female BALB/c mice (6–8 week-old) were purchased from Charles River (L'Arbresle, France). Experimental protocols were approved by Stallergenes internal ethical committee for animal experimentation, and animal handling was performed according to international regulations. Culture medium consisted of Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% fetal calf serum, 1% L-glutamine, 200 U/mL penicillin, and 200 µg/mL streptomycin (all from Invitrogen, Carlsbad, CA). Phosphate-buffered saline (PBS) and aluminum hydroxide were purchased from Lonza (Basel, Switzerland) and Pierce (Rockford, IL), respectively. The aqueous birch pollen (BP) extract was made in ammonium bicarbonate by Stallergenes SA (Antony, France). Recombinant Bet v 1a (rBet v 1a) was produced in *Escherichia coli*, and purified as reported elsewhere [14,15]. Formulated rBet v 1a was prepared using amylopectin-based microparticles (MPA) as previously described [19].

2.2. Establishment of a mouse model of allergic sensitization to birch pollen

Mice were sensitized with 2 i.p. injections of BP extracts (using doses equivalent to 10 µg Bet v 1) on days 0 and 14 in presence of 2 mg aluminum hydroxide, then exposed from days 21 to 24 to aerosolized BP extracts (equivalent to 1 mg Bet v 1) 20 min per day, using similar doses as above. On day 25, airway hyperresponsiveness (AHR) was evaluated by unrestrained whole body plethysmography (Buxco Europe Ltd, Winchester, GB) and expressed as enhanced pause (Penh) as previously described [21]. Penh indexes were obtained as a ratio between Penh values measured after exposure to increasing doses of methacholine (i.e. 12.5, 25, 50, 100 mg/mL, Sigma, St Louis, MO) or nebulized PBS. We complemented those Penh measurements with invasive determination of compliance and resistance that directly measure pulmonary function. Briefly, mice anesthetized with pentobarbital/xylazine (50 mg/kg and 10 mg/kg, respectively by i.p., Centravet, Maisons-Alfort, France) were carefully intubated orotracheally. Animals were then placed in a plethysmograph and connected via the endotracheal cannula to a flexiVent system (Emka technologies, Falls Church, VA). Inhalation exposure in orotracheally intubated animals is focused to the lungs, with no nasal nor oral intake. Bronchial resistance and dynamic compliance were measured using the Flexivent system after exposure to increasing doses (i.e. 5, 10, 15, 20 mg/mL) of methacholine using a protocol adapted from Swedin and al. [22].

2.3. Sublingual immunotherapy in BP sensitized mice

After sensitization, mice were randomized into experimental groups exhibiting similar means of Penh values. For SLIT, sensitized mice received either placebo (PBS), BP extract (using doses

equivalent to 50 µg Bet v 1 (50 µl)), or rBet v 1a (at 5, 50, 100, 500 µg/dose (50 µl)) sublingually twice a week for 8 weeks (6 mice per group). Mice were held on their back for 1 minute to prevent immediate swallowing of the allergen. At the end of treatment, all groups were re-exposed to aerosolized BP extracts for 2 days and AHR was measured 24 h after the last aerosol. Mice were sacrificed the following day to conduct further analyses. In a second set of experiments, SLIT was performed with 5 or 50 µg of rBet v 1a, alone or formulated within MPA microparticles (50 µl). Groups of animals receiving PBS or BP extract (using doses equivalent to 50 µg Bet v 1) were used as negative and positive controls, respectively, for desensitization.

2.4. Broncho-alveolar lavage (BAL) fluid analysis and lung histology

Mice were anesthetized with pentobarbital/xylazine (50 mg/kg and 10 mg/kg respectively by i.p., Centravet, Maisons-Alfort, France), and BAL were performed with 1200 µL PBS. BAL fluids were centrifuged at 800 g for 5 min at 4 °C. Cell pellets were resuspended in PBS, spun onto glass slides by cytocentrifugation, fixed and stained with May-Grünwald Giemsa (Réactifs RAL, Martillac, France) for differential cell counting. Supernatants were stored at –80 °C for cytokine analysis. For histology studies, lungs were fixed in phosphate buffered formalin-zinc before embedding in paraffin wax. Tissue sections were stained with hematoxylin, eosin and safran (HES) for evaluation of cellular infiltrates.

2.5. Detection of birch pollen-specific antibodies

Blood was collected by cardiac puncture under anesthesia and sera stored at –20 °C. Salivation was induced by intraperitoneal injection of 50 µg pilocarpine per mouse (Sigma) 15 min before saliva collection. Specific antibody responses were evaluated by ELISA in sera and saliva samples incubated for 24 h at 37 °C in 96-well plates coated overnight with either purified rBet v 1a (10 µg/mL) or BP extract (at doses equivalent to 10 µg/mL Bet v 1). Plates were washed and incubated with biotinylated secondary antibody anti-mouse IgG1, IgG2a, IgA (BD Biosciences, CA), or anti-IgE antibodies conjugated to horseradish peroxidase (HRP) (Gentaur, Brussels, Belgium). After washing, specific antibody binding was detected with streptavidin-peroxydase/ABTS substrate tandem (Roche diagnostics, Meylan, France) or ABTS alone, and plates were analyzed at 405 nm on a multiskan Ascent spectrophotometer (Thermo Labsystems, Waltham, MA). Antibody titers were defined as the reverse of the last dilution for which the optical value was 2-fold higher than the mean values obtained in sera from control mice.

2.6. Western blot experiments

Western blot analyses were carried out to determine the sensitization profile in BP sensitized-mice. BP extract and purified recombinant Bet v 1a were submitted to SDS-PAGE on 4–12% Bis-Tris acrylamide gels (Invitrogen, Cergy Pontoise, France) under non-reducing conditions, and then transferred onto nitrocellulose membranes. Membranes were pretreated with Qentix western blot signal enhancer (Pierce, Rockford, USA). IgE and IgG1 profiles were analyzed after incubation with pooled sera from BP sensitized mice (or naïve mice as a control), and subsequently with biotinylated rat anti-mouse IgE and IgG1 antibodies (Clnisciences, Montrouge, France) and streptavidin peroxydase (Sigma). The detection of Bet v 1 was confirmed using the specific 5B4 monoclonal antibody (Stallergenes, Antony, France), followed by an HRP-conjugated sheep anti mouse

(Sigma) antiserum prior to revealing with the SuperSignal West Pico substrate (Pierce). Samples were analyzed on a Chemismart 2000 CCD camera (Vilbert Lourmat, Marne la Vallée, France).

2.7. Analysis of birch pollen specific T cell responses

Lung leukocytes were recovered by incubating one lobe for 1 h in RPMI supplemented with 75 U/mL collagenase (Roche, Basel,

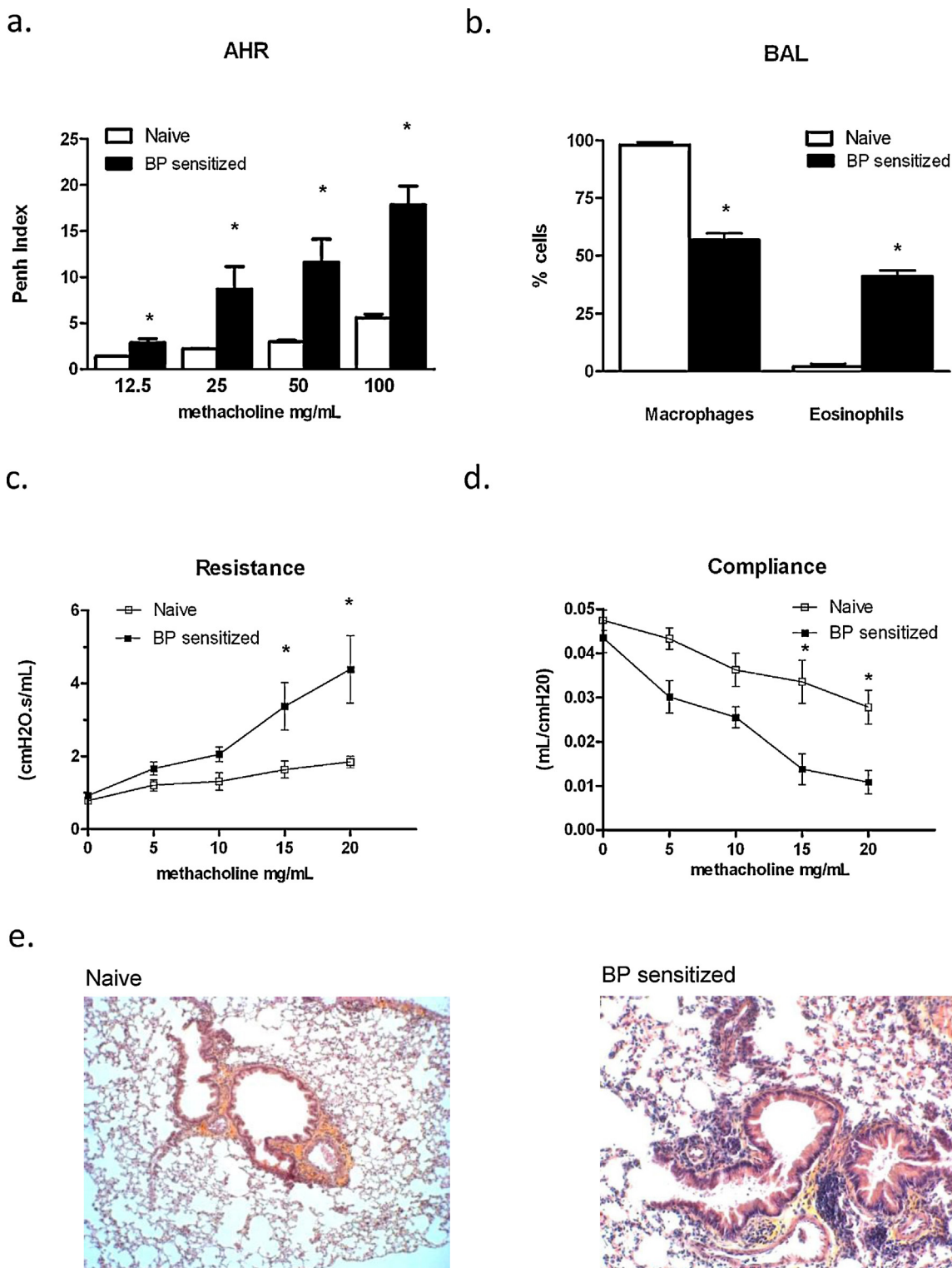


Fig. 1. BP sensitized mice exhibit AHR and lung inflammation.

(a) AHR to methacholine was measured by whole body plethysmography at day 25 and expressed as a Penh index. Results are expressed as mean values $\pm$ SEM, with $n = 6$ –8 mice per group. (b) Differential cell counts were performed after May–Grünwald Giemsa staining of BAL cytopspins. Results are expressed as mean values $\pm$ SEM, with $n = 6$ –8 mice per 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. (a, b, c, d) * $p < 0.05$ in comparison to naive mice. Data are representative of 3 independent experiments. (e) Lungs from naive and BP sensitized mice were collected for histology studies on HES-stained, wax-embedded tissue sections. Data shown are representative of 4 mice examined in each group. Data are representative of 2 independent experiments.

Switzerland). Cells were plated at 10^6 cells per well and stimulated with either 10 $\mu\text{g}/\text{mL}$ of rBet v 1a, BP extract (equivalent to 10 $\mu\text{g}/\text{mL}$ Bet v 1) or medium alone, in a final volume of 200 μL . After 72 h at 37 °C in 5% CO_2 /95% air, IL-5, IL-10 and IL-13 levels were measured in culture supernatants using a cytometric bead array (CBA) Flex kit (BD Biosciences, San Jose, CA) according to the manufacturer's instructions.

2.8. Statistical analyses

Results are presented as mean $\pm$ SEM values. Data comparing sensitized mice with controls were analyzed using non parametric Mann–Whitney tests, with results considered as statistically significant when the p -value was below 0.05. Statistical and graphical analyses were performed using the Prism 5 software (GraphPad, La Jolla, CA).

3. Results

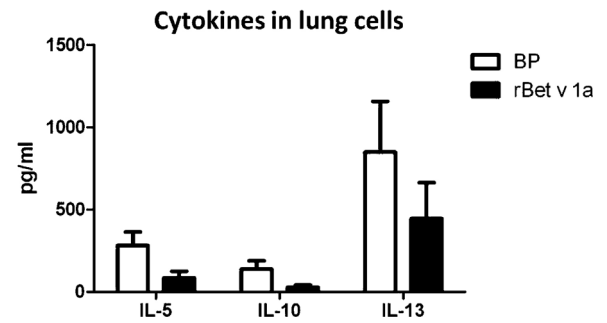
3.1. Induction of airway hyperactivity, lung inflammation and Th2 responses in BP sensitized mice

Allergic airway inflammation to BP was induced in BALB/c mice as described in Methods. Briefly, BP extract was administered via the intraperitoneal route at days 0 and 14, followed by aerosol exposure for 4 consecutive days. 24 h after the last aerosol, BP sensitized mice exhibited a strong AHR to methacholine (Fig. 1a) together with eosinophil infiltration (Fig. 1b) in BAL fluids, in contrast to naive mice. Invasive monitoring of lung function in those animals confirmed as well a significant increase in pulmonary resistance (Fig. 1c) as well as a marked decrease in dynamic compliance (Fig. 1d). In addition, histology studies revealed peribronchial cellular infiltrates in lung tissues from BP sensitized but not naive mice (Fig. 1e). T cells recovered from lung tissues of BP sensitized mice secreted IL-5, IL-10 and IL-13 following in vitro re-stimulation with either BP extract or rBet v 1a, confirming a Th2 biased profile (Fig. 2a). Strikingly, stronger T cell responses were always observed with a BP extract when compared with rBet v 1a, suggesting the induction of immune response against minor allergens beyond Bet v 1. Furthermore, as shown in Fig. 2b, sensitized mice mounted Th2 biased seric antibody responses with high titers of specific IgG1 and IgE, and to a lower level, IgG2a. Western blot analyses using BP extracts or rBet v 1a confirmed that BP sensitized mice mounted specific IgE and IgG1 antibody responses to Bet v 1a, but also against minor allergens such as Bet v 3 (24 kDa) and Bet v 6 (35 kDa), as well as undefined allergens (around 42 kDa and 60 kDa) (Fig. 2c, lanes 1 and 5 versus 2 and 6, respectively).

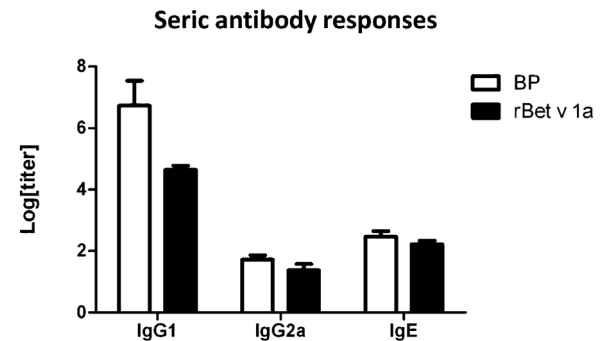
3.2. SLIT with rBet v 1a is efficacious in a dose-dependent manner in BP sensitized mice

To assess the efficacy of rBet v 1a sublingual desensitization, BP sensitized mice were randomized into either placebo (receiving PBS), or active groups (receiving 5, 50, 500 μg rBet v 1a per dose). Animals receiving BP extract (equivalent to 50 μg rBet v 1 per dose) twice a week for 8 weeks were used as positive controls for desensitization (Fig. 3a). Following completion of treatment, animals were re-exposed to a BP aerosol and AHR was measured 24 h later. As shown in Fig. 3a, the lowest dose of rBet v 1a (5 μg) induced a moderate reduction in AHR compared to placebo. A significant decrease in AHR was observed in groups receiving either higher doses of rBet v 1a, or the BP extract when compared to the PBS group. All treated groups (including the one receiving 5 μg Bet v 1 a/dose) exhibited a significant decrease in eosinophil

a.



b.



c.

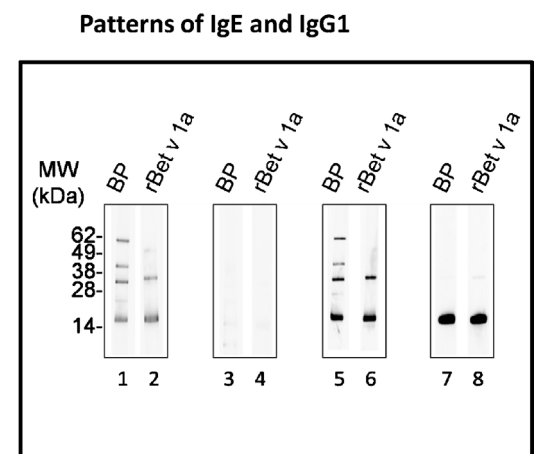


Fig. 2. Airway inflammation in BP sensitized mice is associated with BP-specific Th2 biased antibody responses.

(a) Lung cells from BP sensitized mice were restimulated in vitro with 10 $\mu\text{g}/\text{mL}$ rBet v 1a or BP extract (equivalent to 10 $\mu\text{g}/\text{mL}$ Bet v 1/dose) for 72 h. IL-5, IL-10 and IL-13 cytokine levels were measured by CBA in culture supernatants. Background levels of cytokines produced by non-activated cells were subtracted. Results are expressed as mean values $\pm$ SEM, with $n=6-8$ mice per group. (b) Seric antibody responses were assessed by ELISA using BP extract or rBet v 1a coated plates. Results are expressed as mean values $\pm$ SEM, with $n=6-8$ mice per group. Background signals detected in naive animals were used to determine antibody titers, as described in Section 2. (c) Patterns of IgE (lanes 1, 2) and IgG1 (lanes 5, 6) reactivity were assessed by western blot using BP extract or rBet v 1a and pooled sera ($n=8$) from BP sensitized mice. Western blot analysis using sera from naive mice (lanes 3, 4) or anti-Bet v 1 monoclonal antibodies (lanes 7, 8) were used as negative and positive controls, respectively. Data in a, b, and c are representative of 3 independent experiments.

BAL counts compared to the PBS group (Fig. 3b). A comparison between groups receiving either 5 μg rBet v 1a or 50 μg rBet v 1a per dose also revealed statistical differences in eosinophil BAL counts ($p < 0.05$) but not in AHR (Fig. 3a and b). This down regulation of cellular infiltrates was also confirmed in lung tissue sections (Fig. 3c). Furthermore, a reduction in Th2 cytokine secretion by lung T cells was observed in mice receiving high dose SLIT (i.e.

groups treated with 50 μ g rBet v 1a or BP) (Fig. 4a). Although not shown, we failed to detect any effect of SLIT with rBet v 1 on IFN γ or TGF β production by T cells. In accordance with our previous observations [19,20], SLIT had no significant impact on

BP specific seric IgE and IgG1 (Fig. 4b), but induced strong IgA responses detectable in saliva (Fig. 4c). No significant impact of SLIT with a 5 μ g Bet v 1a dose was detected in those experiments (data not shown).

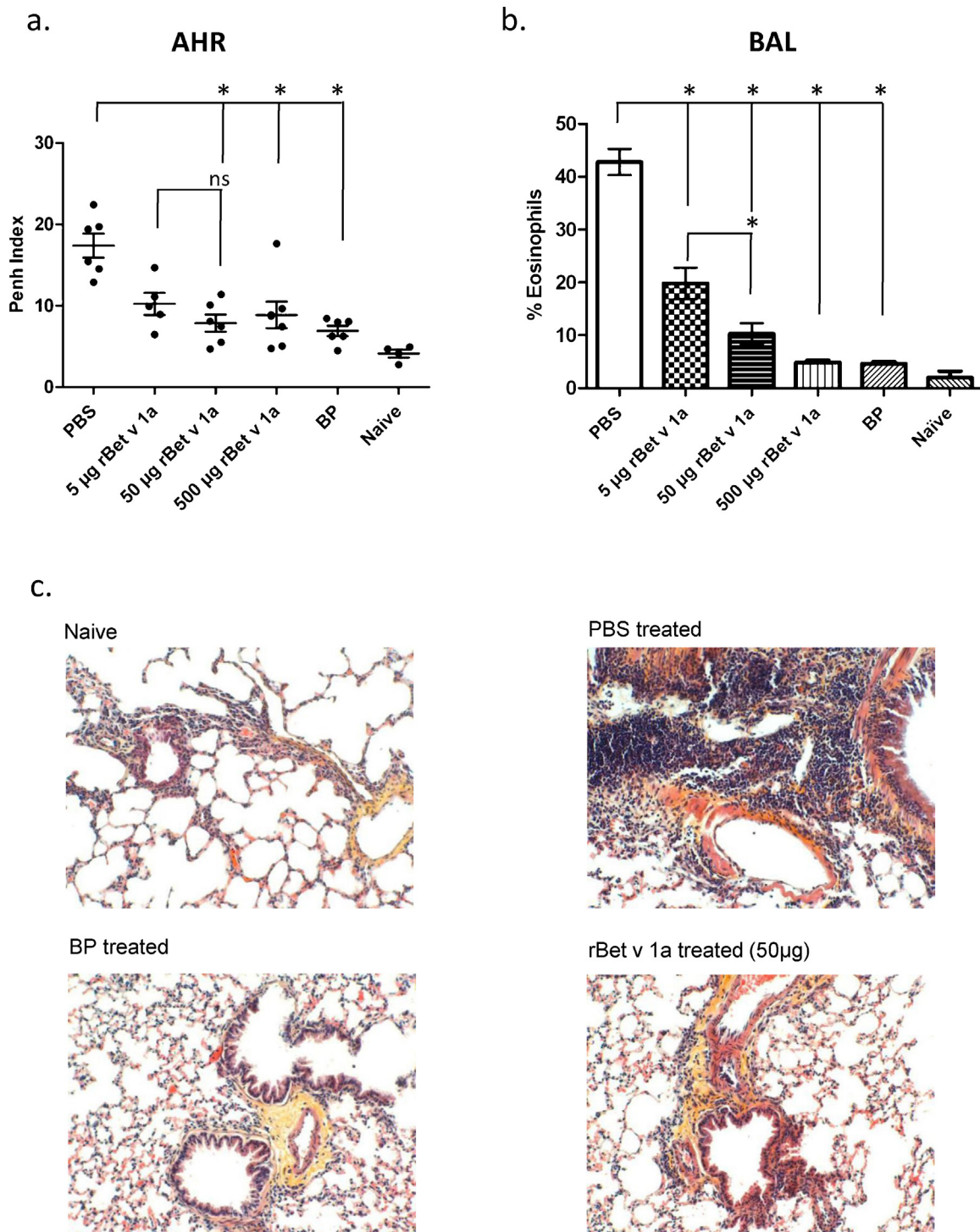


Fig. 3. SLIT with rBet v 1a is efficacious in a dose-dependent manner in BP sensitized mice.

After 8 weeks of SLIT with either PBS, rBet v 1a (5, 50, 500 μ g/dose) or BP extract (equivalent to 50 μ g rBet v 1/dose), mice were challenged with BP extract for 2 consecutive days. (a) 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 $n=6-8$ mice per group. (b) Differential cell counts were performed after May-Grünwald Giemsa staining of BAL cytopins. Results are expressed as mean values $\pm$ SEM, with $n=6-8$ mice per group. (a, b) $*p<0.05$ in comparison to naive mice or 5 μ g rBet v 1a treated mice, ns = non significant; Data are representative of 3 independent experiments. (c) Lungs from naive (upper left panel), PBS treated (upper right panel), BP treated (lower left panel) and rBet v 1a treated (lower right panel) mice were collected for histology studies using HES-stained, wax-embedded tissue sections. Data shown are representative of 4 mice examined in each group. Data are representative of 2 independent experiments.

3.3. Enhanced efficacy of sublingual rBet v 1a formulated in a mucoadhesive particulate vaccine

We subsequently investigated the efficacy of rBet v 1a formulated in a vector system compared to the corresponding soluble molecule in our model of chronic BP sensitization. To this aim, we formulated the suboptimal dose (5 µg) of rBet v 1a in an amylopectin-based particulate vector (MPA). In line with the first set of experiments reported above, when compared to the placebo group, SLIT with 5 µg soluble rBet v 1a per dose induced only a small reduction in AHR (Fig. 5a), but a clear decrease of eosinophil counts in BALs (Fig. 5b). Interestingly, treatment with particulate MPA-rBet v 1a led to a significant (and more consistent) decrease in both AHR and BAL eosinophils when compared to the placebo group (Fig. 5a and b, respectively), to a level comparable to animals receiving either 50 µg soluble rBet v 1a or BP extract. As a control, treatment with MPA alone had no significant effect in those experiments (Fig. 5a and b). Differences observed between groups receiving 5 µg rBet v 1a or 5 µg MPA-rBet v 1a did not reach statistical differences (Fig. 5a and b). Nonetheless, the trend toward enhanced efficacy observed with vectorized rBet v 1a relative to soluble rBet v 1a was associated with a superior reduction in Th2 cytokine secretion by specific T cells (Fig. 5c), with no detectable boost of specific IgA antibody levels in saliva (Fig. 5d).

Lastly, to investigate the impact on the onset of SLIT efficacy of MPA formulation, BP sensitized mice were sublingually treated with an optimal dose of rBet v 1a i.e. either 50 µg soluble rBet v 1a or equivalent doses formulated in MPA. As shown in Fig 6a and 6c, when compared to the placebo group, MPA-rBet v 1a, but not soluble rBet v 1a nor BP extract decreased significantly AHR as early as after 3 weeks. After 6 weeks of therapy, both mice receiving MPA-rBet v 1a or BP extract exhibited lower PenH values in response to methacholine (Fig. 6b and c). Taken together, these results suggest that the MPA vector formulation improves the onset of efficacy of sublingual immunotherapy with rBet v 1a.

4. Discussion

Allergen specific immunotherapy conducted via the sublingual route is being successfully used to treat patients with type I respiratory allergies [2–11]. Whereas all currently available treatments are performed with extracts, present trends in AIT include replacement of such natural extracts by purified recombinant allergens [12,13]. Although per se recombinant allergens are not expected to exhibit a superior efficacy when compared with natural extracts, the increasing availability of pure allergens for SLIT provides an opportunity to combine vector systems and adjuvants to enhance efficacy [18]. In this regard, the pertinence of using mucoadhesive particulate vectors based on carbohydrate polymers to facilitate allergen uptake by mucosal tolerogenic dendritic cells (DCs) [23–27] has been established in murine models of OVA-induced asthma [19,20].

To further document the relevance of such vector systems for SLIT, we focused on birch pollen allergy as a model. Two clinical studies previously conducted in humans with pharmaceutical grade rBet v 1a, have demonstrated the safety and efficacy of this approach for both the subcutaneous and sublingual routes [16,17]. In this context, the aim of the present study was to establish a pre-clinical model of allergic airway inflammation to birch pollen in order to test vectorized rBet v 1a-based sublingual vaccines in a therapeutic setting.

Pulmonary function variables commonly used in humans (e.g. Force Expiratory Volume in 1 second (FEV₁), Forced Vital Capacity (FVC), Tiffeneau index) are not measured in mice. More generally, no correlation can be made between the severity of AHR in mice

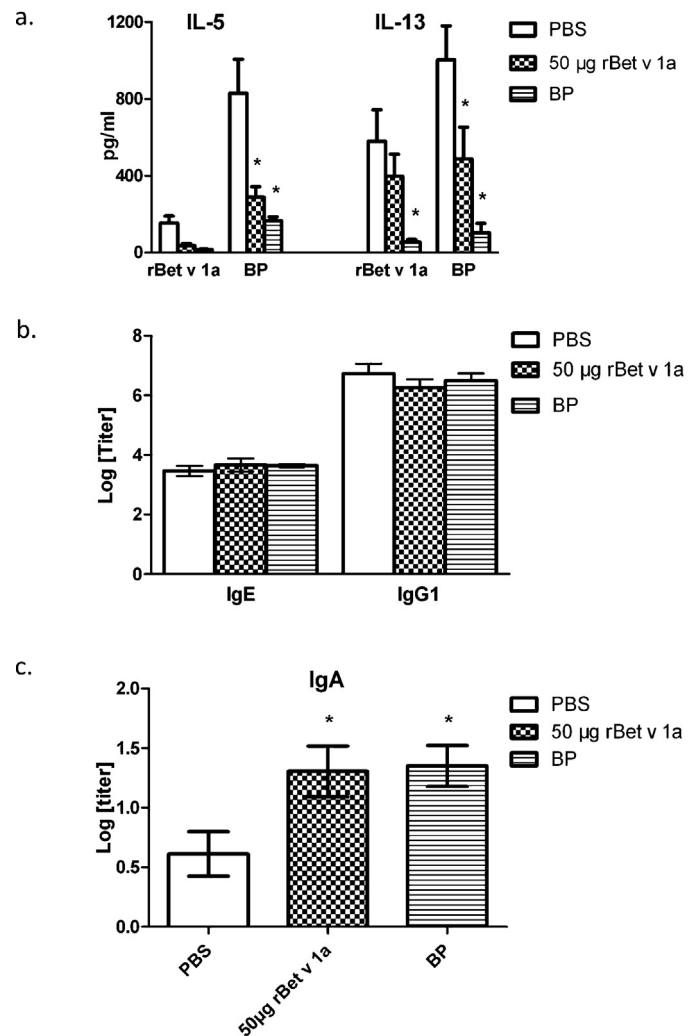


Fig. 4. SLIT with rBet v 1a reduces Th2 responses while increasing BP specific IgA antibodies in saliva.

(a) Lung cells from mice treated with either PBS, rBet v 1a (50 µg/dose) or BP extract were restimulated in vitro with 10 µg/mL rBet v 1a or BP extract (at doses equivalent to 10 µg/mL Bet v 1) for 72 h. IL-5 and IL-13 cytokine levels were measured by CBA in culture supernatants. Background levels of cytokines produced by non-activated cells were subtracted. Results are expressed as mean values $\pm$ SEM, with $n=6-8$ mice per group. (b) Blood was collected from mice treated with either PBS, rBet v 1a (50 µg/dose) or BP extract (equivalent to 50 µg Bet v 1/dose), as well as from naive animals. Seric anti-BP IgG1 and IgE were assessed in sera by ELISA as described in Methods. (c) Saliva was collected after induction with pilocarpine as described in Section 2. Anti-BP IgA responses were assessed by ELISA as described in Section 2. (b, c) Titers were defined as the reverse of the last dilution for which the optical value was 2-fold higher to the one of naive mice. Results are expressed as mean values $\pm$ SEM, with $n=6-8$ mice per group. (a, b, c) * $p < 0.05$ in comparison to PBS treated group. Data are representative of 2 independent experiments.

and human asthma in relationship with the doses of methacholine administered [28]. Also, extrapolation of doses of active ingredient (i.e. rBet v 1a) efficacious in humans cannot be made from observations obtained in animals based on body weight or surface area as discussed by Reagan-shaw et al. [29]. Herein, we combined non invasive and invasive measurements commonly used in murine models [19–21,25,26,30,31], such as Penh measurements by whole body plethysmography, as well as compliance and resistance determinations that directly measure pulmonary function (protocol adapted from Swedin et al. [22]).

Data obtained in BP-sensitized mice confirmed that animals exhibit a chronic airway inflammation associated with AHR and eosinophil infiltrates in BAL. Sensitized animals also mounted

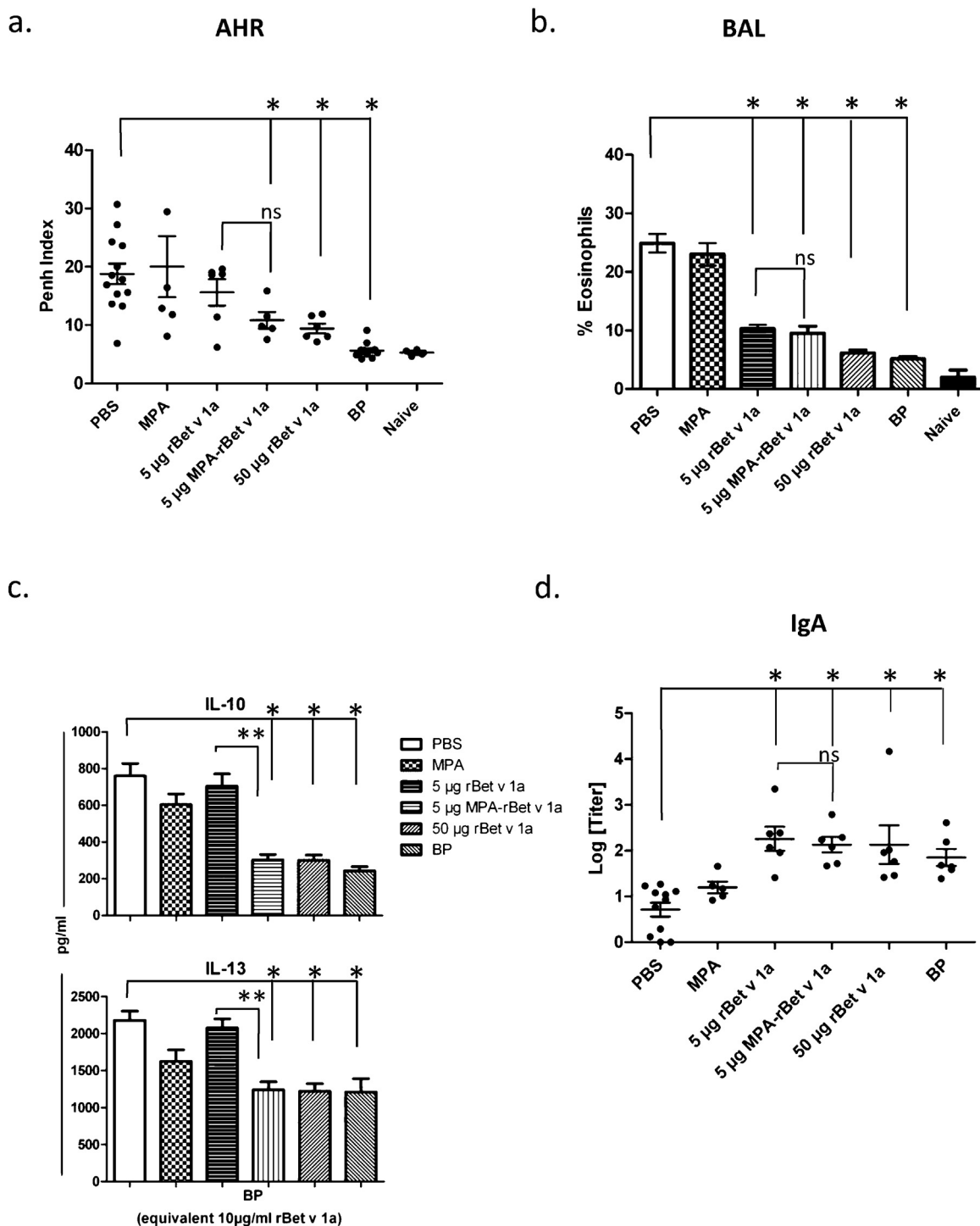


Fig. 5. SLIT with rBet v 1a formulated in mucoadhesive particles.

After 8 weeks of SLIT with either PBS, rBet v 1a (5 or 50 µg/dose), BP extract (equivalent to 50 µg Bet v 1/dose), MPA alone or 5 µg MPA-rBet v 1a, mice were challenged with BP extract for 2 consecutive days. (a) 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 $n=6-8$ mice per group. (b) Differential cell counts were performed after May–Grünwald Giemsa staining of BAL cytopins. Results are expressed as mean values $\pm$ SEM, with $n=6-8$ mice per group. (c) Lung cells were restimulated *in vitro* with BP extract using a dose equivalent to 10 µg/mL Bet v 1 for 72 h. IL-10 and IL-13 cytokine levels were measured by CBA in culture supernatants. Background levels of cytokines produced by non-activated cells were subtracted. Results are expressed as mean values $\pm$ SEM, with $n=6-8$ mice per group. (d) Saliva was collected after induction with pilocarpine as described in Section 2. Anti-BP IgA responses were assessed by ELISA as described in Section 2. Titers were defined as the reverse of the last dilution for which the optical value was 2-fold higher to the one of naive mice. Results are expressed as mean values $\pm$ SEM, with $n=5-11$ mice per group. (a, b, c, d) * $p < 0.05$ or ** $p < 0.01$ in comparison to PBS or 5 µg rBet v 1a treated group, ns = non significant. Data are representative of 2 independent experiments.

BP-specific Th2-biased T cell and antibody responses. Importantly, mice sensitized to BP extracts developed high IgE/IgG1 responses directed to both Bet v 1 and minor birch allergens. Consistent with this observation, T cells from BP sensitized mice exhibited a higher

Th2 response to the BP extract than to rBet v 1a, suggesting the involvement of T cells specific for other allergens beyond Bet v 1. Our results are in agreement with a previous study from Fernvik et al. demonstrating the induction of Th2 responses and bronchial

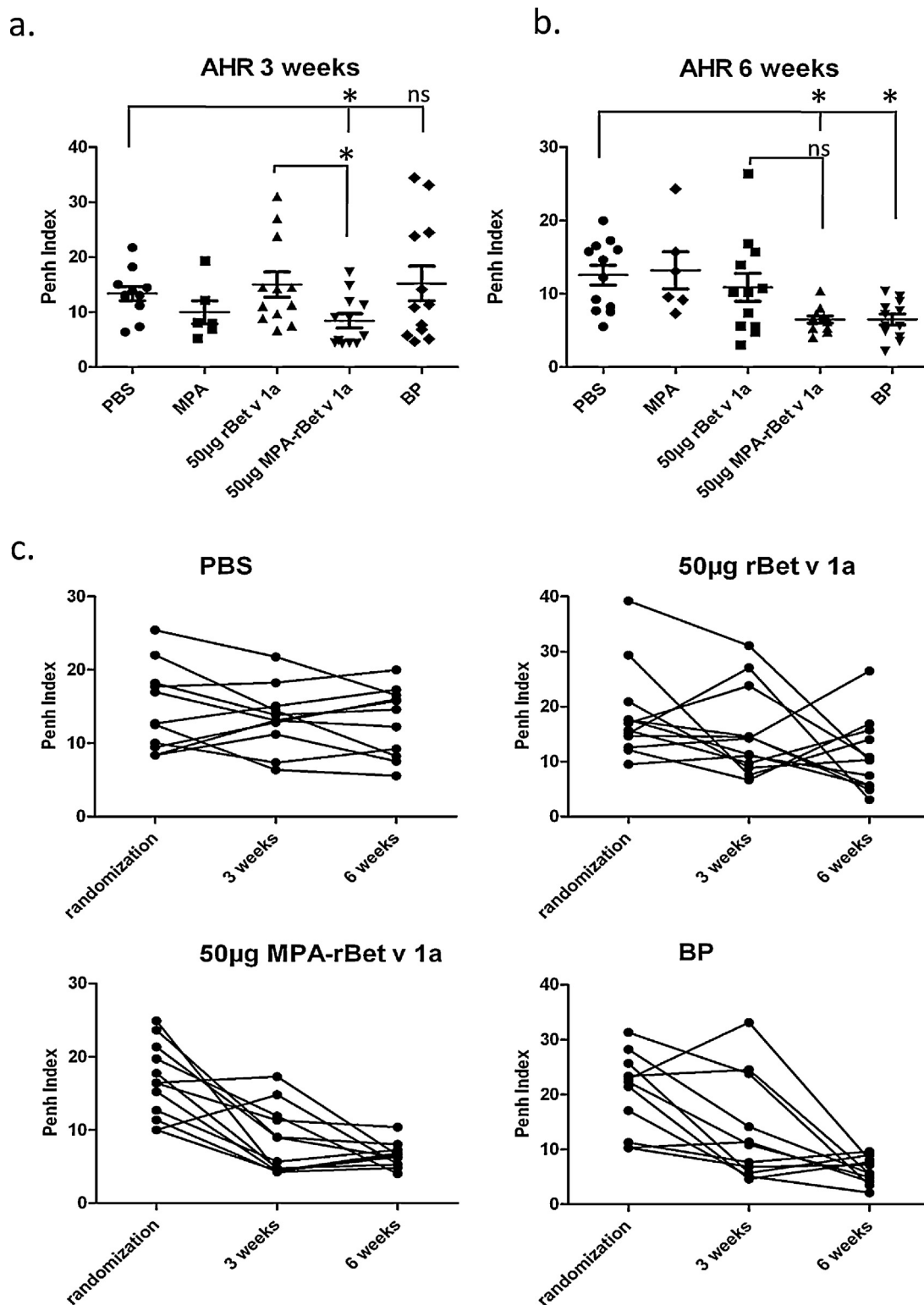


Fig. 6. SLIT with rBet v 1a formulated in mucoadhesive particles exhibit faster onset of efficacy.

(a) After 3 or (b) 6 weeks of SLIT in BP sensitized mice using either PBS, 50 µg rBet v 1a, 50 µg MPA-rBet v 1a or BP extract (equivalent to 50 µg Bet v 1/dose), animals were challenged with BP extract for 2 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 $n = 6$ –12 mice per group. * $p < 0.05$ in comparison to PBS or 50 µg rBet v 1a treated group, ns = non significant. Results are shown as cumulative data of 2 independent experiments. (c) Line-diagram representing mice's AHR at 0–6 weeks.

hyperresponsiveness in animals following chronic exposure to BP extracts [32].

We also confirmed in our model of BP allergy that SLIT was efficacious to treat sensitized animals. Specifically, sublingual

immunotherapy with various amounts of rBet v 1a (ranging from 5 to 500 µg per dose) led to the identification of the 50 µg dose as the one efficacious to achieve a reduction in AHR, lung eosinophilia and specific Th2 responses. These results are in agreement with

previous studies conducted in Bet v 1-sensitized mice demonstrating the efficacy of mucosal (e.g. intranasal, oral) desensitization with various forms of this allergen, although the contribution of minor birch allergens could not be assessed in those models [33–37]. Noteworthy, even if the context of major and minor allergens refer to patterns of IgE sensitization in humans, we observed herein that mice sensitized to BP exhibit specific IgE and IgG1 antibodies against Bet v 1, but also Bet v 3 and Bet v 6 allergens. Thus, notwithstanding the limitations associated with any animal model, we believe that overall conditions of exposure and sensitization to the allergen(s) in this model are comparable to the ones observed for allergic patients.

Herein, SLIT efficacy was further observed with a sub-optimal dose (5 µg) of rBet v 1a, when formulated within MPA particles, with most particularly a decrease in Th2 responses. Importantly, an earlier onset of tolerance induction was obtained via the sublingual route when administering such a vectorized form of rBet v 1a, as opposed to soluble rBet v 1a or BP extract. These results further corroborate the interest of particulate vectors to enhance the efficacy of SLIT vaccines, likely by improving the duration of allergen contact with the sublingual/oral mucosa.

In humans, SLIT consists into the administration of high doses of natural allergen extracts (pollens, mites or animal dander) as drops or tablets under the tongue, prior to swallowing. The immunological mechanism underlying SLIT efficacy likely imply the induction of allergen-specific IFN γ /IL10 producing CD4⁺ regulatory T cells. We previously demonstrated that the latter correlates with the uptake of the allergen by tolerogenic oral DCs located at the mucosal surface within minutes after initiating SLIT [25,26]. We also demonstrated that maltodextrin nanoparticles and microparticles made from chitosan facilitate allergen uptake by tolerogenic sublingual/oral DCs [19,20]. Such an improved targeting of the allergen to oral phagocytic APCs was directly correlated with a stronger priming of IFN γ /IL10-producing CD4⁺ regulatory T cells in draining cervical lymph nodes, thus leading to a superior allergen specific tolerance induction [25,26]. Interestingly, others have also demonstrated the value of chitosan particules as an allergen formulation to treat HDM allergic mice via the intranasal route [38].

In conclusion, using a clinically relevant allergy model, we confirmed that vectorization with a mucoadhesive particulate delivery system allows to reduce the dose of allergen when used sublingually. We also demonstrated that vectorized Bet v 1a has a more rapid onset of action. Thus, such vectorized recombinant allergens might represent valid options to desensitize patients with type I respiratory allergies via the sublingual and other mucosal routes.

Acknowledgement

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