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Efficacy of sublingual vectorized recombinant Bet v 1a in a mouse model of birch pollen allergic asthma

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ABSTRACT

Background: Second generation sublingual allergy vaccines based upon recombinant allergens combined with vector systems are being developed as an alternative to conventional allergen extracts. Herein, we evaluated the efficacy of a recombinant form of the major allergen Bet v 1a (rBet v 1a) formulated as a mucoadhesive particle in a preclinical model of birch pollen (BP) respiratory allergy.
Materials and methods: BALB/c mice were sensitized to BP extracts by intraperitoneal injections followed by aerosol exposures. Sensitized mice underwent sublingual immunotherapy (SLIT) twice a week for eight weeks with either a BP extract or rBet v 1a formulated in amylopectin-based microparticles (MPA). SLIT efficacy was assessed using whole body plethysmography, lung histology and cell counts in broncho-alveolar lavages (BAL) as read outs. BP and/or rBet v 1a-specific T cell and antibody responses were monitored in lung and serum, respectively. IgA levels were measured in saliva.
Results: Mice sensitized to BP exhibit chronic airway hyperresponsiveness (AHR), lung inflammation (documented by compliance and resistance measurements), eosinophil infiltrates in BAL, as well as Bet v 1-specific Th2 biased responses. Both SLIT with soluble rBet v 1a (50 μg/dose) and BP extract (equivalent to 50 μg rBet v 1 per dose) lead to a significant reduction in AHR, lung eosinophilia and Th2 responses. A sub-optimal dose of 5 μg of rBet v 1a displays a similar level of efficacy with a significant decrease of Th2 responses when formulated with MPA microparticles. In addition, allergen vectorization with mucoadhesive particles allows a faster reduction in AHR in sensitized animals.
Conclusion: We demonstrate in a murine model of chronic BP respiratory allergy the efficacy of SLIT with vectorized rBet v 1a. Thus, combining recombinant allergens with mucoadhesive vector systems paves the ground for improved second generation sublingual allergy vaccines.

1. Introduction

Allergen immunotherapy (AIT) is a well established approach to treat IgE mediated type I respiratory allergies. Whereas subcutaneous immunotherapy (SCIT) has been successfully used for more than 100 years [1], sublingual immunotherapy is now raising considerable interest as a valid non invasive alternative [2–11]. Current SLIT vaccines are based on aqueous biological extracts which require standardization based upon potency measurement to ensure batch-to-batch consistency. In addition, second-generation SLIT vaccines based on well-characterized recombinant allergens are under development, most particularly for allergies involving a limited number of relevant allergens, such as birch pollen (BP) allergy, for which more than 90% of patients are sensitized to the major BP allergen termed Bet v 1 [12,13]. Pharmaceutical-grade recombinant major allergen Bet v 1a [14,15] has been tested in humans via both the subcutaneous and sublingual routes, with encouraging results obtained, both in terms of safety and efficacy [16,17]. One advantage of recombinant allergens relative to natural extracts is that they can be combined with vector systems to improve targeting of the immune system [18]. Specifically, the value of mucoadhesive particles in enhancing SLIT-induced tolerance has been suggested in previous preclinical models of OVA induced asthma, [19,20]. However, the relevance of this approach remains to be established with clinically relevant allergens.

In this context, we report herein the development of a murine model of chronic allergic asthma to birch pollen (BP) in order

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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 tohorseradish 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 (Clinisciences, 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

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