

SUBLINGUAL VACCINES BASED ON WILD-TYPE RECOMBINANT ALLERGENS

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SUMMARY: Sublingual immunotherapy (SLIT) represents a non invasive alternative to subcutaneous immunotherapy in order to treat type I allergies. Vaccines based on recombinant allergens expressed in a native (i.e. wild-type) configuration, formulated with *ad hoc* adjuvants designed to target Langerhans cells in the sublingual mucosa should allow to induce allergen-specific regulatory T cells. In this context, we have developed animal and human preclinical models to test the capacity of candidate vaccines to modulate selectively allergen-specific T helper lymphocyte polarization following sublingual vaccination.

INTRODUCTION

Allergen-specific sublingual immunotherapy (SLIT) is currently considered as a non invasive alternative to subcutaneous immunization (1). During SLIT, the allergen is captured within the oral mucosa by Langerhans-like dendritic cells expressing constitutively both low and high affinity IgE receptors (2). Following engagement of such receptors, sublingual dendritic cells (DCs), produce IL10 and TGF β and upregulate indoleamine dioxygenase (IDO), suggesting that such cells are prone to induce allergen-specific regulatory T cells. Such cells are heterogeneous, and include both:

- naturally occurring CD4⁺CD25⁺ T cells;
- cells induced in the periphery following antigen exposure (e.g. Tr1 cells, Th3 cells, and CD8⁺ T_R cells) (3).

In this context, we develop vaccines comprising recombinant allergens in association with adjuvants administered through the sublingual route. Such recombinant allergens are produced in a wild-type (i.e. native) conformation to preserve IgE binding, thereby facilitating capture by Fc ϵ R1⁺ Langerhans cells. In this study, we summarize our efforts to produce wild-type allergens using recombinant technologies, and to identify adjuvants eliciting regulatory T cells via the sublingual route.

METHODS

Allergens and adjuvants

Recombinant allergens are expressed either in bacteria, yeast (*Pichia pastoris*) or tobacco plants as recombi-

nant proteins, with a focus on birch (Bet v1) and house dust mites (Der p1, Der p2) allergens. Candidate adjuvants such as glucocorticoid plus 1,25-dihydroxyvitamin D3, and acid bacteria (e.g. *Lactobacillus plantarum*) have been selected because they are known IL10 inducers and have a good safety profile, allowing human administration (4-7).

Assessment of adjuvants on human immune cells

Monocyte-derived DCs were incubated with either medium, adjuvant, LPS (1 μ g/ml), LPS (1 μ g/ml) plus adjuvant. After 24 h, DCs were harvested to analyse their phenotype, and cytokine production using the cytometric bead array (CBA) technology. To assess the impact on T cell polarization, adjuvant-treated DCs were cultured with allogenic CD4⁺ naïve T cells from healthy donors in 96-well flat-bottom microtiter plates. After 7 days, cells were collected to assess changes in phenotype, intracellular cytokine production and gene expression. To the latter aim, RNA was isolated using the RNeasy Mini kit (Qiagen), reverse transcribed, and cDNAs were quantified by real-time PCR. Primers and probes were selected to assess expression of transcription factors (T-bet, GATA-3, Foxp3), as well as IL10 and TGF- β genes.

Murine model of sublingual immunization

BalbC mice were sensitized by two intraperitoneal (i.p.) injections at 14 days interval with 10 μ g OVA (ovalbumin) adsorbed on Al(OH)₃, followed by respiratory challenge with 1% w/v OVA on 5 consecutive days with an aerosol delivery system (Buxco). For tolerance induction, OVA +/- adjuvant was applied

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sublingually every week, twice a week during 2 months at a concentration of 500 µg (in a total volume of 25 µl). Control mice were sensitized with OVA followed by sham tolerization with PBS. Measurements of airway responsiveness were performed by whole body plethysmography. Airflow signals were recorded in response to aerosolized methacholine (50 mg/ml). Mice were sacrificed and spleen cells were assessed for spot forming cells (SFCs) using Elispot kits for IFN-γ, IL-5 and IL-10.

RESULTS

Production of recombinant allergens

Various expression systems were compared to express recombinant allergens in the most native configuration, including *E coli*, yeast (*Pichia pastoris*) and tobacco plants. Results (summarized in table 1) suggest that it is possible to produce with good yields recombinant Der p1, Der p2 and Bet v1 molecules. All such molecules were found to bind patient's IgE, as well as specific murine monoclonal antibodies in a comparable manner to purified natural allergens, suggesting that recombinant allergens were correctly folded (table 1 and ref. 8).

Screening of candidate T reg adjuvants on human immune cells

Because DCs are controlling T-cell activity, we evaluated candidate adjuvants known to induce IL10 for their capacity to modulate DCs maturation. Collectively, our results suggest that a combination of Vit D3 plus Dexamethasone inhibits DC maturation (assessed following LPS stimulation). In contrast stimulation with *Lactobacillus plantarum* induced DC maturation. The impact of such adjuvants on DC phenotype and cytokine production, as well as their capacity to drive subsequent T cell polarization, are summarized in table 2.

Evaluation of candidate Treg adjuvants in a murine model of sublingual immunization

Sensitization with ovalbumin (as described in material and methods) was shown to induce OVA-specific IgG1 and IgE, but little IgG2a antibodies in serum. Spleen cells from OVA-sensitized mice displayed a strong capacity to produce IL5 in response to OVA (not shown). In addition to a clearcut Th2 immune response, OVA-sensitized mice exhibited airway hyper responsiveness to OVA in presence of metacholine when compared to PBS-treated mice. In such sensi-

Cellular system	Allergen expressed	Comments
E coli	Bet v1, Der p2	Both allergens were expressed successfully in a native configuration and shown to bind patient's IgEs
Yeast (<i>P. pastoris</i>)	Der p2	Der p2 expressed in yeast was shown to bind IgEs in a comparable manner to natural purified Der p2.
Tobacco plant	Der p1, Der p2	Der p2 with a decreased capacity to bind IgE was produced in plants. Various forms of Der p1 (+/- propeptide, +/- enzymatic activity, +/- N-glycosylation site) were expressed in tobacco plants.

Table 1: Expression systems for pharmaceutical-grade recombinant allergens.

Biological activity	Adjuvants	
	Vit D3 +Dexamethasone	<i>L. plantarum</i>
Human dendritic cell maturation	↘ (inhibition of LPS induced expression of CD80, CD83, CD86)	↗ (increase in CD40, CD80, CD83, CD86, MHC class II)
Cytokine production by human DCs	↘ of LPS induction of IL12 or IL6	↗ IL12, IL6, IL1β
Human T cell polarization	↗ IL10, ↘ IFNγ, ↘ IL5	↗ IL10, ↗ IFNγ, ↗ T Bet
Activity in a murine model of sublingual immunotherapy	Improvement in respiratory function. ↗ in TGFβ and ↘ in IL5 OVA-specific production in splenocytes.	Improvement in respiratory function. ↗ in TGFβ and ↘ in IL5 OVA-specific production in splenocytes.

Table 2: Effect of selected adjuvants on human and murine immune cells.

zed mice with ovalbumin, both *Lactobacillus plantarum* and Dexamethasone + Vit D3 coadministered with OVA through the sublingual route were shown to elicit a significant improvement in their respiratory function (table 2).

CONCLUSION

Sublingual vaccines based on recombinant allergens are being developed to treat house dust mite and tree pollen allergies. These vaccines associate recombinant allergens in a native configuration, together with candidate T Reg adjuvants. On the basis of qualitative and quantitative *in vitro* assays based on human immune cells, a variety of candidate adjuvants are being screened. Such adjuvants act either on DCs and/or T lymphocytes to stimulate IL10 and TGF β production. Candidates vaccines based on r Bet v1a in association with a candidate adjuvant will be tested in birch allergic humans through the sublingual route (8). In those exploratory clinical studies, tools such as soluble HLA tetramers-peptides will be essential to assess Betv1-specific T cell responses as a read-out of vaccine efficacy.

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