Targeting the MHC class II antigen presentation pathway: a new immunotherapeutic approach

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INTRODUCTION/BACKGROUND (MAIN TITLE

The only long-lasting treatment of allergic diseases is allergen-specific immunotherapy (SIT). Typically SIT is performed by subcutaneous administration of increasing doses of allergen during a period of 3 to 5 years. Although highly efficient [1], only about 5% of allergic patients choose SIT as therapy, mainly due to the long treatment time and the high number (30-80) of injections required to reach protection. Therefore, there is a recognized medical need in improving and shortening the current treatment schedules [2]. Vaccines are typically administrated by subcutaneous injections, however, immune responses are elicited exclusively in secondary lymphoid organs [3]. Within lymph nodes allergens are taken up by antigen presenting cells, processed, and presented t CD4+T cells through the major histocompatibility class-II complex (MHC-II) which, al low doses lead to a T_H2-biased immune response resulting in allergic symptoms [4]. Therefore, an increased allergen presentation through the MHC-II presentation pathway is a potential potent therapeutic approach to allergy as demonstrated in human cell cultures [5]. Here we show in a mouse model of allergy that engineered vaccines able to target the MHC-II presentation pathway are able to protect treated mice from anaphylactic choc if directly injected into lymph nodes even at very low concentration.

MATERIAL & METHODS

Vaccines

A modular antigen translocating vaccine for the major cat allergen Fel d 1 (MAT-Fel d 1) was engineered and produced as pure recombinant protein as described [5]. MAT vaccines are composed of a cell penetrating peptide converting extracellular to intracellular proteins, a truncated segment of the invariant chain which targets the fusion molecule to the endosomal compartments, and an allergen of choice [5].

Immunization protocols

CBA female mice (6-8 weeks old) were sensitized by 7 intraperitoneal injections of 1 μg of cat extract. Sensitization was demonstrated by challenges with Fel d 1 (10 μg i.p.) as a drastic drop of the whole body temperature measured before and 30 min after challenge [6]. To test the therapeutic potential of MAT-Fel d 1 sensitized mice were immunized by the conventional route (subcutaneous injection, s.c.) or by direct injection into the inguinal lymph nodes (i.l.) for three times with 1 μg MAT-Fel d 1 at bi-weekly intervals between the injections. All animal experiments were approved by and performed according to the guidelines from the Swiss veterinary service authorities.

Monitoring of the immune responses

IgG 1, IgG2a, and IgE antibody responses were determined by ELISA as described [6]. For the detection of cytokine production spleens of immunized mice were isolated and single-cell suspensions prepared. Erythrocytes were removed, and triplicates of 8 x 105 cells were cultured as described [6]. The cytokines secreted into the supernatants of allergen-stimulated and non stimulated cultures were determined by ELISA. Supernatants were collected after 20 h for IL-2, and after 72 h for IL-4, IL-10, and INF-γ determinations [6]. Efficacy of the immunization was demonstrated by the lack of anaphylactic choc after Fel d 1 challenge reflected by a drastic reduction of the whole body temperature drop.

RESULTS

Intralymphatic immunization induce strong humoral responses

The ration of humoral IgG2a to IgG1 responses is a parameter for the relative strength of T_H1 versus T_H2 immune responses. All ratios for i.l. injections were higher than for s.c. injections, indicating a strong deviation towards a beneficial, protective T_H1-biased response (Fig. 1).

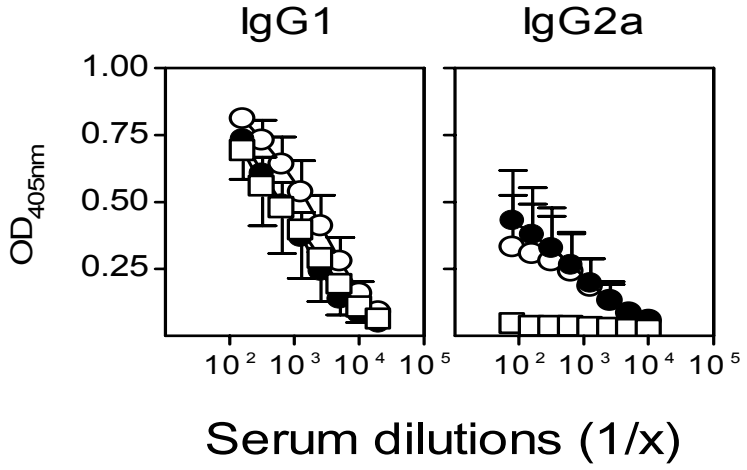

Fig.1. Cat allergen-specific IgG1 and IgG2a humoral responses to three immunizations with MAT-Fel d 1 at 0.1 µg (●), 1 µg (○) applied into lymph nodes or 10 µg (□) applied subcutaneously. The results show that MAT-Fel d 1 applied intranodally is as efficient as MAT-Fel d 1 applied subcutaneously at 100 x lower concentration in eliciting IgG1 responses. Protective IgG2a antibodies are not elicited by subcutaneous applications, in contrast to the good immunologic response obtained by intranodal immunization.

These results are corroborated by an increased production of the cytokines IL-1, INF-γ, and IL-10, the cytokines needed for a successful immunotherapy [5], in cell cultures obtained from spleens of i.l-immunized animals compared to the levels detected in culture supernatants of the same cells from s.c-immunized mice (Fig. 2).

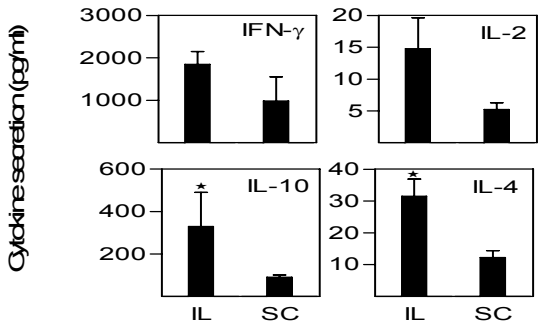

Fig. 2: Cytokine secretion from cell cultures of mice immunized with MAT-Fel d 1 either i.l. or s.c. after *in vitro* re-stimulation with Fel d 1.

Enhanced protection against anaphylaxis
Mice sensitized to cat extract have high levels of serum IgE and IgG1, but not detectable IgG2a antibody responses. Upon immunotherapy with MAT-Fel d 1 applied intranodally the mice developed robust protective IgG2a responses (Fig. 1), whereas mice immunized through the s.c. route failed to develop IgG2a. Three weeks after completed immunotherapy, mice were challenged with a high dose of cat extract and monitored for anaphylactic signs (Fig. 3). MAT-Fel d 1 treated mice immunized through the i.l. route showed a marked improvement of the allergic status reflected by a drastic reduction of the whole body temperature decrease. In contrast, mice treated s.c. were not protected from anaphylactic choc as demonstrated by a moderate change of the whole body temperature.

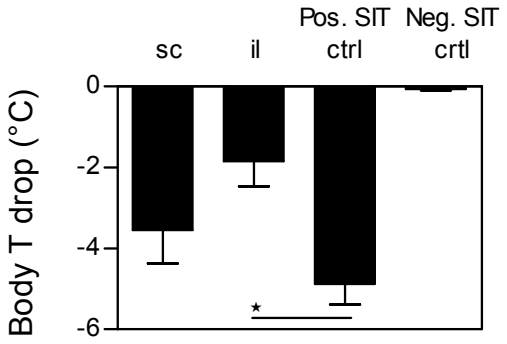

Fig. 3. Protective effect of intranodally applied MAT-Fel d 1 vaccine. As positive control Fel d 1-sensitized mice were used, as negative control naïve mice were challenged with placebo. Immunization through the i.l. route confer a statistically significant protection of the mice to a whole body temperature drop induced by allergen challenge, whereas s.c. treated mice are much less protected.

CONCLUSIONS & OUTLOOK

Allergen specific immunotherapy of Fel d 1-sensitized mice with MAT-Fel d 1 vaccine through intranodal injections showed an enhanced production of INF-γ, IL-2, and IL-10 compared to mice immunized subcutaneously. These cytokines are required to elicit protective IgG2a antibody responses which were observed only in MAT-Fel d 1 mice treated by i.l. injections. Moreover, the dose of allergen required to induce a protective humoral response was 100 time less than those required to induce a humoral response through the s.c. route of application (Fig. 1). According to the *in vitro* results, mice mounting a strong IgG2a response were protected against challenge-induced anaphylaxis (Fig. 3). In conclusion, direct allergen administration into lymph nodes increase SIT efficacy and MAT-vaccines allow a drastic reduction of the dose required to elicit strong protective responses, thus diminishing the risk to treatment-induced anaphylaxis. This work served as a basis for planning of a Phase I/IIA human clinical trial based on the MAT-Fel d 1 vaccine. The protocols for the clinical study have now been accepted by SwissMedics and we have obtained the permission to start the Phase I/IIA clinical study under the restriction that we have to use cGMP produced vaccines. The start of the study is planned for the second half of 2008.

Acknowledgments
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