

Targeting the MHC II presentation pathway in allergy vaccine development

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Abstract

The worldwide increase in the incidence of allergic diseases and the limited efficacy of current vaccines require the development of new efficient vaccination strategies. Based on PTD (protein transduction domain) technology, we have engineered MAT (modular antigen translocation) molecules, aimed to enhance antigen presentation through intracellular targeting of the MHC II presentation pathway. MAT vaccines consist of a cloning cassette, which fuses Tat (transactivator of transcription) peptide to a truncated Ii (invariant chain), which is able to target antigens to the nascent MHC II molecules in the *trans*-Golgi compartment. To test the efficacy of intracellular targeting, we engineered arrays of MAT-fusions and compared the effects of recombinant allergens, Tat-conjugated allergens and MAT-conjugated allergens for the ability to stimulate T-cell proliferation and cytokine production in human PBMC (peripheral blood mononuclear cell) cultures derived from allergic individuals, and to elicit protective immune responses in mice. MAT-vaccines induced a strong proliferation of PBMCs at a low concentration and induced a Th2/Treg (regulatory T-cell) cell shift in the cytokine profile, reflecting those reported in successfully desensitized allergic individuals. In allergic mouse models, we showed that MAT-vaccines are highly efficient in desensitizing mice and protect them from anaphylactic shock. The technology is applicable not only for the treatment of allergies, but also for the development of preventive vaccines in general.

Introduction

Currently, SIT (allergen-specific immunotherapy) is the only available curative treatment for allergic diseases [1]. Controlled studies have shown that SIT, by means of administration of gradually increasing doses of an allergen, can effectively treat patients with allergic rhinitis/conjunctivitis, allergic asthma and insect venom allergy. SIT is the only therapy with a persistent effect that can stop the progression from allergic rhinitis to asthma [2].

Although this makes SIT superior to pharmacological treatment with corticosteroids and antihistamines, only a minority of patients suffering from allergic rhinitis, conjunctivitis and asthma due to pollen, dust mite or animal dander are treated with SIT. This can be partly explained by the fact that SIT is a time-consuming process, requiring 30–80 doctor visits over a period of 3–5 years, resulting in a low patient compliance.

SIT modulates the specific immune response against the allergen, shifting the balance of T-lymphocyte subsets from a Th2 phenotype towards a Treg (regulatory T-cell) phenotype, with decreased production of IL (interleukin)-4, IL-5 and

IL-13, and a parallel increase in production of IL-10 by Treg [3].

Murine models suggest that the switch from Th2-dependent IgE production to Th1-dependent IgG production is favoured by immunization with high doses of allergen [4]. This effect, however, cannot be exploited in SIT, as the dose of the administered allergen is limited by the severe side effects ranging from local reactions to life-threatening systemic allergic reactions, including asthma and anaphylactic shock.

To overcome these problems, our goal was to enhance allergen presentation by MAT (modular antigen translocation) molecules in order to achieve a 'high dose effect' with low allergen doses to make SIT a more efficient, shorter and safer treatment [5].

Generally, proteins are endocytosed by antigen-presenting cells and enter the MHC II pathway of antigen presentation to CD4⁺ T-cells. In the ER (endoplasmic reticulum), MHC II molecules become associated with a type II transmembrane protein called Ii (invariant chain) [6]. The association with Ii prevents binding of peptides and nascent proteins in the ER, and targeting information in the cytoplasmic tail of Ii directs Ii–MHC II complexes to endosomal compartments where, after proteolytic degradation of Ii, the association between MHC II molecules and antigenic peptides occurs [7]. It has been shown that endogenously synthesized proteins, generally excluded from the MHC II presentation pathway, can be efficiently presented as peptide–MHC II complexes when they are expressed as fusion proteins with Ii [8].

Key words: allergy, intracellular targeting, modular antigen translocation (MAT), MHC, protein transduction domain (PTD), vaccine.

Abbreviations used: ER, endoplasmic reticulum; IFN γ , interferon- γ ; Ii, invariant chain; IL, interleukin; MAT, modular antigen translocation; PBMC, peripheral blood mononuclear cell; PTD, protein transduction domain; SIT, allergen-specific immunotherapy; Tat, transactivator of transcription; Treg, regulatory T-cell.

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In an effort to generate efficient allergy vaccines, we tested the possibility of targeting the MHC II processing and presentation pathway using translocable Ii–allergen fusion constructs [6]. The vaccine is based on a gene cassette which consists of a His₆ tag for protein purification, followed by a Tat (transactivator of transcription)-derived PTD (protein transduction domain), which converts extracellular proteins into cytoplasmic proteins. This is fused to an adaptable multiple cloning site and to the first 110 amino acids of Ii, to target proteins to the endosomal/lysosomal compartment. The whole cassette is under the control of the Lac promoter/operator in the vector pQE30 (Qiagen) and allows fast efficient expression and purification of any protein of interest inserted in-frame into the multiple cloning site.

We measured the proliferation and cytokine production of mononuclear cells of allergic individuals in response to stimulation with different MAT–allergens. All MAT-induced T-cells proliferated at approx. 100-fold lower molar doses than T-cells stimulated with the respective allergens alone, whereas T-cells of control individuals did not respond to the allergen-specific stimulation at all. Supernatants of PBMCs (peripheral blood mononuclear cells), cultured in the presence of MAT-conjugated proteins, showed earlier and increased production of IFN γ (interferon- γ) and IL-10, but decreased IL-4 and IL-5 production, compared with parallel cultures with the recombinant allergen or Tat–allergen [5]. These characteristics are ideal for a vaccine against an allergy, because IFN γ is a suppressor of IL-4 synthesis, which is required for IgE production, and IL-10 production inhibits IgE production and enhances IgG4 production [1]. In fact, the major effects of conventional allergen-specific immunotherapy, as observed in PBMC cultures of treated individuals, are suppression of IL-4 production and an increase in IL-10 responses upon allergen stimulation. This is indicative of a shift from a Th2 immune response to a Treg immune response [9].

The immunogenicity of MAT–allergens was evaluated *in vivo*. Initially, mice were immunized s.c. (subcutaneously) with different doses of the major cat allergen Fel d 1, Tat–Fel d 1 or MAT–Fel d 1. In line with the *in vitro* data presented, induction of IgG1 and IgG2a with Tat-conjugate or MAT-conjugate vaccines required 100-fold lower doses, compared with the recombinant allergen alone. In contrast with the allergen alone, both Tat-conjugate and MAT-conjugate

vaccines did not induce any IgE responses (T. Kündig, unpublished work).

The immunotherapeutic potential of MAT–allergens was evaluated in mice which had been pre-sensitized with low doses of Fel d 1, to generate Fel d 1-specific IgE. If these mice were challenged with cat dander extract, they responded with a significant decrease in body temperature, a sign of anaphylaxis. We evaluated whether desensitization with Fel d 1, Tat–Fel d 1 or MAT–Fel d 1 was able to protect mice from such anaphylaxis. In fact, immunization with Tat–Fel d 1 and MAT–Fel d 1 elicited higher IgG1 and IgG2a antibody responses when compared with cat extract and allergen alone, while no boosting of the pre-existing IgE titres could be detected. The MAT–Fel d 1 vaccine was the most effective in protecting sensitized mice from anaphylaxis (T. Kündig, unpublished work).

Conclusions

Translocatable Ii–allergen fusion proteins target the allergen into the MHC II pathway, leading to enhanced CD4⁺ T-cell proliferation, increased production of IL-10, and decreased production of IL-4 and IL-5. Such enhanced CD4⁺ T-cell responses, together with a change from a Th2 response towards a Treg response, enhances production of IgG, while suppressing IgE production and augmenting the immunotherapeutic effect. These characteristics make MAT-conjugated allergens ideal candidates, not only for SIT, but also for other vaccines.

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