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The influence of antigen targeting to sub-cellular compartments on the anti-allergic potential of a DNA vaccine☆

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ABSTRACT

Background: Gene vaccines offer attractive rationales for prophylactic as well as therapeutic treatments of type I allergies. DNA and mRNA vaccines have been shown to prevent from allergic sensitization and to counterbalance established allergic immune reactions. Recent advances in gene vaccine manipulation offer additional opportunities for modulation of T helper cell profiles by specific targeting of cellular compartments.
Methods: DNA vaccines encoding the major birch pollen allergen Bet v 1.0101 were equipped with different leader sequences to shuttle the antigen to lysosomes (LIMP-II), to trigger cellular secretion (hTPA), or to induce proteasomal degradation via forced ubiquitination (ubi). Mice were pre-vaccinated with these constructs and the protective efficacy was tested by subcutaneous Th2-promoting challenges, followed by allergen inhalation. IgG antibody subclass distribution and allergen-specific IgE as well as cytokine profiles from re-stimulated splenocytes and from BALFs were assessed. The cellular composition of BALFs, and lung resistance and compliance were determined.
Results: Immunization with all targeting variants protected from allergic sensitization, i.e. IgE induction, airway hyperresponsiveness, lung inflammation, and systemic and local Th2 cytokine expression. Surprisingly, protection did not clearly correlate with the induction of a systemic Th1 cytokine profile, but rather with proliferating CD4+ CD25+ FoxP3+ T regulatory cells in splenocyte cultures. Targeting the allergen to proteasomal or lysosomal degradation severely down-regulated antibody induction after vaccination, while T cell responses remained unaffected. Although secretion of antigen promoted the highest numbers of Th1 cells, this vaccine type was the least efficient in suppressing the establishment of an allergic immune response.
Conclusion: This comparative analysis highlights the modulatory effect of antigen targeting on the resulting immune response, with a special emphasis on prophylactic anti-allergy DNA vaccination. Targeting the antigen to proteasomal or lysosomal degradation reduces the availability of native allergen, thereby rendering the vaccine hypoallergenic without compromising efficacy, an important feature for a therapeutic setting.

1. Introduction

One of the unique features of gene vaccines is their potential to design optimized immunization approaches specifically tailored for a wide range of diseases including cancer [1], infectious diseases [1,2], autoimmune diseases [3] and allergic disorders [4,5]. Since their first description in the early 1990s, substantial efforts have been made to enhance the immunogenicity of gene vaccines and to instruct the proper branch of the immune defense, depending on the type of pathogen/disease. Among the numerous ways how to modulate translation of antigens, are strategies to shuttle the antigen of interest to different subcellular compartments (cytoplasm,

Abbreviations: AA, amino acid; AHR, airway hyperreactivity; APC, antigen presenting cell; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; LIMPII, 20-amino-acid C-terminal tail of lysosomal integral membrane protein-II; SIT, specific immunotherapy; Th, T helper; tPA, human tissue plasminogen activator leader peptide; Treg, T regulatory; Ubi, ubiquitin.

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endosomes/lysosomes [6–9]), to induce cellular secretion [10,11] or cell membrane insertion [12], or guiding to protein-processing machineries (proteasome [13–17], endosome/lysosome). All these modifications are part of mechanisms that play an important role in host-pathogen interactions and represent evolved strategies for optimizing immune responses.

Our working group has specialized on genetic vaccination against allergy and we, as well as others, could provide evidence that plasmid-encoded antigens trigger the induction of a Th1-balanced immune profile [18–20] that is capable of counterbalancing and protecting from allergic sensitization [21,22]. All the mentioned approaches influence the immunogenicity and balance of humoral/cellular immunity; moreover, some of them act on T-helper cell polarization (Th1, Th2, Th17, Treg). The latter aspect plays a crucial role in the development of both, protective as well as therapeutic gene vaccination approaches against type I allergy.

Here, we compared a compendium of DNA vaccine targeting strategies (Fig. 1) on major birch pollen allergen Bet v 1.0101 (Bet) specific allergy. DNA vaccines were constructed with the allergen gene linked with sequences encoding the (i) human tissue plasminogen activator leader peptide (tPA), (ii) ubiquitin (Ubi), (iii) the 20AA C-terminal tail of the lysosomal integral membrane protein-II (LIMPII), or (iv) left without modification. The tPA leader sequence encodes a strong signal peptide for protein secretion thus mediating the release of antigen into the extracellular space. Secreted antigen can be taken up and processed by APC (Fig. 1[1a]), resulting in presentation of antigenic peptides on MHC-II. Nevertheless, a small part will be presented on MHC-I molecules by a mechanism which is called cross-priming [14]. In contrast, Bet lacking a secretory signal sequence, is expressed as cytoplasmic protein (Fig. 1[1b]) and will be presented on MHC-I per default. Like with crosspriming, which ensures antigen delivery from the MHC-II pathway to MHC-I, mechanisms have evolved which enable the exchange of molecules from MHC-I to MHC-II pathways. These mechanisms include shedding of native protein from transfected cells by a still unknown mechanism, which was described as "leakage" (Fig. 1[2]), release of antigen within apoptotic vesicles (immune apoptosis), and transport of cytosolic material into the MHC-II pathway via autophagy (Fig. 1[3]) [23,24].

Ubiquitination shuttles the antigen into the polyubiquitination pathway (Fig. 1[1c]), thereby specifically promoting the presentation of antigenic peptides on MHC-I [17]. In contrast, LIMPII peptide attachment facilitates the antigenic transport from the cytosol to lysosomes (Fig. 1[1d]), thus allowing MHC-II presentation [6].

Our data indicate that targeting has a substantial effect on the strength of humoral immunity, and all targeting variants demonstrated a Th1-bias. Furthermore, targeting proved to be a valuable approach to develop new rationales for optimized anti-allergic gene vaccines.

2. Materials and methods

2.1.1. Mice, treatment schedules and serology

Female, 6–10 week-old BALB/c mice were obtained from Charles River Laboratories (Sulzfeld, Germany). All animal experiments were conducted according to local guidelines approved by the Austrian Ministry of Science and in accordance with EU Directive 2010/63/EU.

To assess humoral/cellular profiles after vaccination, BALB/c mice ($n = 5$) were immunized intradermally (i.d.) with plasmid DNA encoding Bet, Ubi-Bet, tPA-Bet or Bet-LIMPII on days 0, 7 and 14. 100 μg plasmid DNA in 200 μl sterile PBS were i.d. injected at 6–8 spots on the back of isoflurane-anesthetized animals. On day 49, blood samples were taken and splenocytes were prepared (Fig. 2A). To analyze the protective efficacy of the vaccine on alum-induced allergic sensitization (Fig. 3A), vaccinations on days 0, 6, and 13 were performed as described above. On days 27, 41 and 48, mice

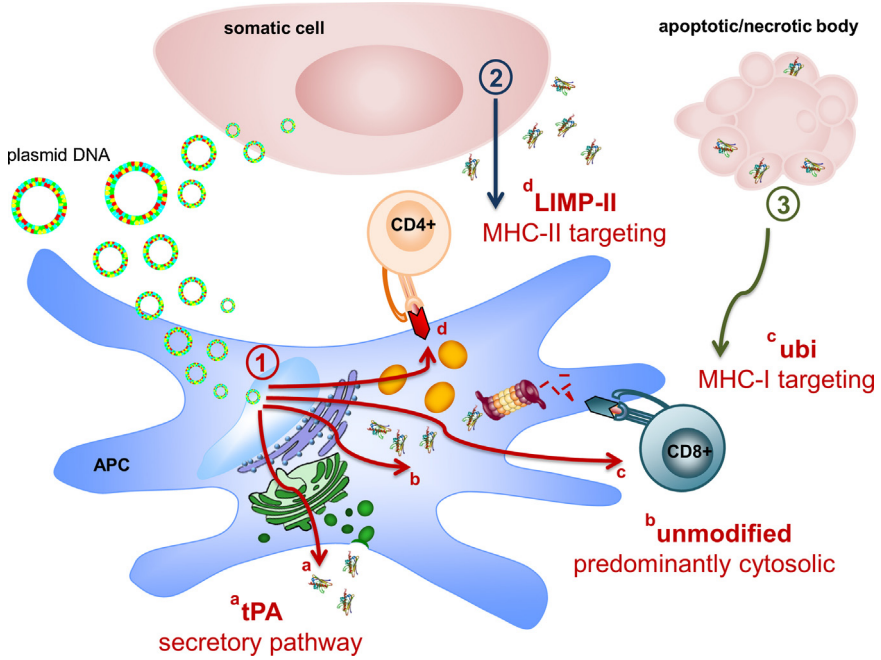

Fig. 1. DNA vaccine-targeting strategies. After entering the nucleus (1), the plasmid DNA is transcribed and differentially processed, depending on the respective modification. (a) The 5 'attached tPA signal sequence leads to shuttling of the vaccine-derived Bet to the exterior of transfected cells via the Golgi apparatus, while (b) the unmodified genetic information is translated into the cytosol, leading to vaccine-derived endogenous peptide presentation on MHC-I molecules. (c) Ubiquitin attachment feeds the translated protein into the polyubiquitination pathway thereby specifically targeting peptides to MHC-I. (d) In contrast, LIMPII peptide attachment promotes the antigenic transport to lysosomes that facilitate peptide presentation on MHC-II. Along with direct transfection of both, resident immunocompetent as well as somatic cells, (2) the engulfment of secreted vaccine-derived antigens, that have been shed from transfected cells, enforce peptide processing within the endocytic pathway, or, (3) MHC-I cross-presentation of cell-associated exogenous antigens, e.g. by engulfment of transfected and apoptotic cells, are potential modes of neoantigen presentation to the immune system.