

DNA vaccines for the prevention and treatment of allergy

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Purpose of review

Antiallergy DNA vaccine is an attractive alternative for the prevention and treatment of allergic diseases. This review covers recent studies to enhance potency and safety of antiallergy DNA vaccines.

Recent findings

Dendritic cell-targeted allergen gene vaccination using fascin gene promoter inhibited IgE production and allergic inflammation but not airway hyperresponsiveness. Targeting allergen expression at immature dendritic cells or induction of tolerogenic dendritic cells could induce antiallergic T regulatory cells. Vaccination with DNA-encoded Ag85B and AIMP1 as adjuvants could downregulate established Th2-mediated allergic responses. Forced ubiquitination or targeting allergens to lysosomal/endosomal compartments could avoid risk of allergen sensitization. Gene gun delivery of conventional antiallergy DNA vaccine is a risk factor. Replicase-based allergy DNA vaccines showed enhanced immunogenicity and safety as compared to conventional DNA vaccines. TANK-binding kinase-1 (TBK1) is a novel key molecule in DNA vaccine-induced immunogenicity.

Summary

Dendritic cell-based approach has been actively explored to enhance immunogenicity of antiallergy DNA vaccines. Codelivery of hypoallergenic DNA vaccines with potent adjuvants via a desirable delivery mode will help to fulfill the requirements for clinical application of antiallergy DNA vaccines. Activation of TBK1 signaling pathway could be a novel strategy to enhance immunogenicity of DNA vaccines.

Keywords

adjuvants, dendritic cells, DNA vaccine, immunogenicity, replicon

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Introduction

IgE-mediated allergic diseases (type I allergies) affected 10–15% of the world population and increased prevalence of allergy is a major global problem. Effective and safe preventive and therapeutic vaccines for these diseases are unavailable. Th1-skewed antigen-specific immune responses induced by allergen gene immunization have been shown to confer protective antiallergic immune responses in a large number of rodent models of type I allergy and these publications have been extensively reviewed by Weiss *et al.* [1]. However, despite the robust responses induced by various DNA vaccines seen in rodent disease models, the potency of most DNA vaccines tested in humans is low [2[•]]. Low immunogenicity of DNA vaccines is a major hurdle for successful clinical applications of antiallergic DNA vaccines. Apart from efficacy, safety aspects of prophylactic and therapeutic antiallergic DNA vaccines are major concerns that deserve more studies before clinical trials. This review will cover recent key published findings in the field of DNA vaccine for allergy, focusing on strategies that enhance potency and safety of the vaccines. Possible novel avenues that can be

exploited to develop safe and efficacious DNA vaccines for allergy will be discussed.

Dendritic cell-based strategies to enhance immunogenicity of DNA vaccine

Dendritic cells are important antigen-presenting cells that provide the crucial link between the innate and adaptive immune responses. Various dendritic cell-based approaches to improve the efficacy of DNA vaccination by modulating the function of dendritic cells or targeting the gene expression to dendritic cells have been explored extensively [3,4[•]]. In the field of allergy, it has been demonstrated that dendritic cells are potent tools for the allergen-specific modulation of the allergic immune responses. Klostermann *et al.* [5] transfected the human monocyte-derived dendritic cells of grass pollen atopic donors with a recombinant adenoviral vector encoding the allergen Phl p 1 and showed that allergen-transfected dendritic cells preferentially induced IFN- γ and suppressed IL-4, IL-5, and IL-10 production in human autologous CD4⁺ T cells and much stronger IFN- γ production in CD8⁺ T cells. It was further demonstrated

that the Phl p 1 allergen transfected dendritic cells were able to induce the production of Phl p 1-specific IgG4 while suppressing the production of total and Phl p 1-specific IgE of autologous B cells as compared to the allergen protein pulsed dendritic cells [6[•]]. These in-vitro studies implied that dendritic cell-targeted allergen gene vaccination is a feasible strategy to induce antiallergic responses. A strategy of using the promoter of the actin-bundling protein, fascin, to preferentially restrict endogenous production of antigen to dendritic cells after DNA immunization has been shown to be a promising approach for dendritic cell-targeted allergen gene vaccination. The proof-of-concept work underlying this approach was shown by a study which demonstrated that transfection with plasmid vectors expressing a transgene driven by the promoter of the fascin gene (pFascin) allowed for antigen expression predominantly by dendritic cells and resulted in the generation of CD8⁺ cytotoxic T lymphocytes and Th1-skewed immune response, whereas transfection with the expression constructs driven by the ubiquitously expressed CMV promoter initiated a mixed Th1/Th2 response [7,8]. They had subsequently shown that protective allergen gene transfer with pFascin efficiently prevented specific IgE production [9]. However, a more recent study revealed that prophylactic vaccination of β -galactosidase (β Gal) sensitized mice with plasmids pFascin- β Gal or pCMV- β Gal inhibited pulmonary Th2 cytokine production and reduced infiltration of eosinophils but failed to attenuate airway hyperresponsiveness [10]. Therefore robust Th1/Tc1 immune responses, although highly effective in the suppression of local Th2-mediated disease, may not be a fully effective therapeutic approach for allergic asthma.

Strategies to enhance regulatory T cells

Induction of T regulatory cells is an attractive way to modulate the preestablished Th2-mediated allergic responses for therapeutic vaccination. It has been shown that CD4⁺/CD25⁺ regulatory T cells could be induced by targeting antigens at immature dendritic cells [11]. A recent study reported that Der p 2 allergen sensitized mice treated intravenously with immature dendritic cells transfected with retrovirus encoding Der p 2 allergen DNA showed inhibition of specific IgE production and suppression of established Th2 responses and allergen-induced allergic airway inflammation via induction of CD4⁺/CD25⁺/Foxp3⁺ regulatory T cells [12[•]]. The nature (i.e. naturally occurring endogenous versus transient regulatory T cells) and biodistribution of these T regulatory cells remained to be elucidated.

Another approach to induce T regulatory cells was described in another recent study that demonstrated that coimmunization with cognate flea Ag pD100 DNA and total flea protein vaccines produced an antigen-specific

expansion of CD4⁺/CD25⁺/Foxp3⁺/IL-10⁺/IFN- γ ⁺/Tbet⁺ T cells, which then inhibited flea allergy dermatitis in the mouse model. The authors proposed that coimmunization of DNA and protein vaccines induced a tolerogenic MHC class II⁺/CD40^{low} dendritic cell population that, in turn, educates naïve T cells into T regulatory cells. However, the precise underlying mechanism for the induction of tolerogenic dendritic cells remains unclear [13[•]].

Genetic adjuvants for enhanced antiallergic responses

The use of appropriate immunological adjuvants in vaccination can help to initiate and potentiate the desired immune responses, therefore representing another useful approach to enhance the immunogenicity of DNA vaccines or to modify the nature of immune responses. Use of the cytokine/chemokine genes such as IL-2, IL-4, IL-5, IL-12, IL-1 β , IL-18, IFN- γ , GM-CSF, and Flt3L alone or in combination or fused with the allergen gene to enhance protective immunity for type I allergy has been reviewed by Weiss *et al.* [1]. Here we describe two new studies on using DNA-encoded adjuvant molecules to enhance modulation of Th2-mediated allergic responses.

A recent study described a versatile DNA-based immunization approach to deliver a Th1-polarizing adjuvant to modulate established Th2 antigen-specific responses [14[•]]. Previous studies have shown that the aminoacyl-tRNA synthetase-interacting multifunctional protein 1 (AIMP1) protein could induce IL-12 secretion by macrophages and dendritic cells and hence enhanced Th1 cell polarization [15,16]. A plasmid, pAnti-CD3sFv/AIMP1, encoding AIMP1 fused to the single chain anti-CD3 (sFv), the smallest unit of the antibody recognizes the CD3 ϵ moiety of the T cell receptor, was constructed to serve as an adjuvant to polarize Th1-immune responses [14[•]]. Intramuscular injection of ovalbumin-presentation mice with pAnti-CD3sFv/AIMP1 could effectively shift the ovalbumin-specific immune response profile from Th2 towards Th1, resulting in the reduction of IgE and IL-4 production in ovalbumin-specific manner. It was further demonstrated that the use of pAnti-CD3sFv/AIMP1 expressing anti-CD3sFv and AIMP1 fusion protein is more superior to the codelivery of two separate plasmids, pAnti-CD3sFv and pAIMP1, in inhibiting ovalbumin-specific Th2 responses during antigen priming.

A microbial derivative from mycobacteria known as antigen 85B (Ag85B) has been shown to induce a strong Th1-type immune response in mice as well as in humans [17]. Mori *et al.* [18] evaluated the prophylactic and therapeutic efficacy of plasmid DNA encoding Ag85B derived from *Mycobacterium kansasii* on atopic dermatitis skin lesions induced by oxazolone application in mice.

Intraperitoneal injection of the Ag85B DNA suppressed the dermal cell infiltration accompanied with the reduced IL-4 and augmented IFN- γ , IL-10, and TGF- β mRNA expression in skin as compared to placebo-treated mice. Furthermore, Foxp3⁺ cells were increased in the Ag85B-treated mice [18].

Hypoallergenic DNA vaccines for allergy

Safety consideration of vaccines is of utmost importance. Although it is well established that allergen gene vaccination induced predominantly Th1-skewed responses and inhibited IgE production. However, we have shown that mice immunized intramuscularly with a plasmid DNA construct encoding a major mite allergen, Der p 2 linked with a strong signal peptide (p52), induced Th1/Th2 mixed phenotype and produced a substantial amount of circulating intact Der p 2 protein. Furthermore, mice adoptively transferred with dendritic cells primed by p52 plasmid DNA construct were sensitized to produce high levels of Der p 2-specific IgE. This study clearly demonstrated the potential risk of Th2 sensitization by allergen gene vaccination [19]. This problem could be overcome by linking allergen gene sequences to the targeting sequences of the lysosomal membrane protein-1 (LAMP-1) to target the newly synthesized allergen *in vivo* to the vacuolar lysosomal/endosomal compartments containing MHC class II molecules. This approach also significantly enhanced the priming of Th1-skewed cellular immune responses probably by improving the efficiency of antigen processing and presentation [19,20].

Another approach to reduce risk of Th2 sensitization by conformationally intact allergens expressed *in vivo* after allergen gene vaccination is by linking the allergen genes to ubiquitin. The forced ubiquitination of allergens channel the allergens expressed *in vivo* into the polyubiquitination pathway, thereby skewing the allergen-specific immune response toward a Th1 type with minimal antibody production. This approach was first used by Bauer *et al.* [21] to show that a hypoallergenic DNA vaccine encoding the major birch pollen allergen Bet v 1 covalently linked to ubiquitin could effectively induce anti-allergic T cell responses without detectable antibody responses and confer protective antiallergic reactions in both prophylactic and therapeutic settings in mouse model for type I allergy.

Route/mode of DNA vaccine delivery

The route/mode of immunization for allergy DNA vaccine is of important consideration as the route of immunization may tilt the balance from protective to harmful immunity by allergen gene vaccination. An earlier study by Slater *et al.* [22] revealed that mice injected subcutaneously at the base of the tail with a plasmid

encoding a latex allergen Hev b 5 showed widespread tissue expression of Hev b 5 transcript. These data reflect the widespread tissue transfection or transcript expression by migratory surveillance cells, which represents a serious risk factor of allergen gene vaccination. A recent study evaluated the potential of gene gun immunization to prevent type I allergic reactions by comparing the model antigen β Gal with two clinically relevant allergens Bet v 1 and Phl p 5. It was found that gene gun immunization induced a Th2-biased immune reaction, which did not prevent mice from production of IgE after subsequent sensitization. Plasmid DNA vaccines encoding Bet v 1 and Phl p 5 allergens induced more pronounced Th2-bias immune responses as compared to model antigen β Gal, suggesting that the nature of antigens has profound influence in the Th2 effects. In contrast to needle injection of plasmid DNA, which triggers a clearly Th1-biased and allergy-preventing immune response, gene gun immunization with all three plasmid-encoded antigens failed to confer protective effects against allergic inflammation and disease in the lungs. Thus gene gun immunization is an undesirable delivery route for the conventional plasmid-based antiallergy DNA vaccines [23^{*}].

Replicon-based DNA vaccine for allergy

Plasmid-based DNA vaccines have been suffered from the poor immunogenicity, especially in humans, and the requirement of a large amount of plasmid DNA per injection that represents a major hurdle for clinical application [2^{*}]. Gene gun delivery method requires much lower doses of plasmid DNA but it is undesirable for allergy DNA vaccine unless the mixed Th1/Th2 sensitization can be controlled [23^{*}]. Alternative approach such as replicon-based DNA vaccines may provide solutions to such problems. Replicase-based vectors for self-replication of delivered genes within the cells were developed primarily to enhance the innate immune responses by activating the innate antiviral pathways [24,25]. The genome of the alphaviruses such as Sindbis virus contain a single-stranded positive-sense of RNA that serves as the template for the synthesis of the negative-strand RNA by its own RNA replicase translated from the positive-strand genome. The negative-strand RNA therefore serves as a template for the additional positive-strand genomic and internally initiated subgenomic RNA synthesis. By replacing the viral genome coded for nonstructural and structural genes with the antigen gene of interest, the replicon-based DNA vaccine can be delivered with at least 100-fold less of plasmid DNA and enhanced efficacy as compared to the conventional plasmid-based DNA vaccines. The studies using a model antigen β Gal [26] and a clinically relevant allergen Phl p 5, the major timothy grass pollen allergen, [27] showed the induction of antigen specific Th1-biased immune responses

characterized by significant IFN- γ production from antigen specific T cells. Upon subsequent sensitization with protein allergens, the replicon-based DNA vaccines suppressed the allergen-specific IgE production [26,27], the eosinophils, and IL-5 in bronchoalveolar lavage fluid and goblet cell metaplasia [27]. The self-replicating double-stranded RNA intermediates mimic viral infection and may trigger apoptosis of the cells that take up the replicon-based DNA constructs [28,29], hence reducing the potential risks and concerns of genomic integration. Thus the advantages of replicon-based DNA vaccines include: firstly, enhanced immunogenicity of vaccine by breaking tolerance through activation of innate antiviral pathways; secondly, induced highly effective antigen-specific Th1-biased responses with nanogram quantity of vaccines; and thirdly, increased safety by removing the transfected cells probably through apoptosis.

The full elucidation of the underlying mechanisms of the promising replicon-based allergy vaccines will further facilitate the development of the vaccines for clinical evaluation. It is well known the double-stranded and the single-stranded RNA present in the extracellular or vesicular compartments are recognized by the transmembrane Toll-like receptor (TLR) 3 and TLR7/TLR8, respectively. In addition, the cytoplasmic viral RNAs can be differentially recognized by RNA sensors such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) (see review [30]). These cytoplasmic RNA sensors signal through the TNF-receptor-associated factor (TRAF) 3 signaling pathways. TRAF3 recruits and activates TRAF family member-associated NF- κ B activator (TANK)-binding kinase 1 (TBK1) and inducible I κ B kinase that phosphorylate transcription factors interferon-regulated factor (IRF) 3 and IRF7 and mount the antiviral type I IFNs production. TRAF3 signaling pathways also activate NF- κ B and downstream proinflammatory cytokine production. Whether the single-stranded RNA or the double-stranded RNA intermediate generated in the cytoplasm by replicon-based DNA vaccines were recognized by the RIG-I and MDA5 and activated the antiviral innate immunity remain to be determined. It is conceivable that the RNA intermediates generated from replicon-based DNA vaccines could serve as the intrinsic molecular adjuvants to enhance the potency of antigen-specific responses.

TANK-binding kinase-1 plays critical roles in immunogenicity of DNA vaccine

Ishii *et al.* [31**] showed that the production of antigen-specific IgG2a and IFN- γ secretion from both CD4⁺ and CD8⁺ cells were dependent on the type I IFN signaling as the IFN- α R-deficient mice produced lower humoral and cellular immune responses after DNA immunization

with electroporation. Using *Tbk1*^{-/-} mice in *Tnf*^{-/-} background, the upstream kinase TBK1 was required for successful induction of antigen-specific B cells and T cells immune responses by DNA vaccine. Analysis with dendritic cell transfer experiments suggested that TBK1 signaling in bone marrow-derived dendritic cells were sufficient for DNA vaccine-induced humoral immune responses. Further analysis with bone marrow transfer experiments showed that TBK1 signaling in nonhematopoietic cells were required for proliferation of antigen-specific CD8⁺ cells, whereas TBK1 signaling in bone marrow-derived, most probably hematopoietic cells, were required for proliferation of antigen-specific CD4⁺ cells [31**]. Analogous with the cytoplasmic RNA sensors, the DNA-dependent activator of IFN-regulatory factors (DAI), also called DLM-1 or ZBP1, has been identified as a cytoplasmic double-stranded DNA sensor. Coimmunoprecipitation experiments suggested that the DAI was associated either directly or indirectly with TBK1 and IRF3 after DNA binding that mediated the IRF3 activation and type I IFN production [32**]. These studies indicate that TBK1 is a key molecule in plasmid-based DNA vaccine induced immunogenicity and cytoplasmic DNA sensors like DAI could be the crucial regulators for robust innate immunity [33*].

Conclusion

Strategic and selective dendritic cell subsets polarization to induce the desired Th1 and T regulatory cells for antiallergic responses may hold the key for prophylactic and therapeutic vaccination against allergy. It is crucial to delineate the different subsets of dendritic cells or other antigen-presenting cells involved in the priming stage of DNA vaccines. The recent characterization of cytoplasmic RNA and DNA sensors responsible for nucleic acid recognition provides some clues about the molecular mechanism for DNA vaccines primarily targeting at the innate immunity, and this will open new avenues for novel design of DNA vaccines. The take home message is that more mechanistic studies are necessary for the rational design of allergy vaccines.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 83–84).

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