

Allergen gene transfer

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DNA-based immunization induces a biased Th1 immune response, and offers a novel strategy for modulation of the Th2-associated response. Recent studies have provided evidence that antigen-induced allergic responses can be modulated in rodents immunized with plasmid DNAs encoding the sensitizing antigens. Further understanding of the regulatory mechanism involved and optimization of gene transfer/expression systems will assist greatly in proving the clinical utility of this process.

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Abbreviations

AHR	airway hyper-responsiveness
CMV	cytomegalovirus
Der p 5	<i>Dermatophagoides pteronyssinus</i> group 5 allergen
LacZ	β-galactosidase (β-gal)
OVA	ovalbumin
pDNA	plasmid DNA
Th1	T-helper type 1 cell
Th2	T-helper type 2 cell

Introduction

Allergic diseases such as allergic asthma, rhinitis and dermatitis are of great public health concern. Current treatments for allergic diseases are not satisfactory and disease prevention is not possible. Given the high prevalence of these diseases, improved and more effective therapeutic strategies are needed. The pathophysiology of allergic disease is multifactorial and involves intricate networks of interactions between cells, mediators and cytokines. Numerous clinical and experimental studies have indicated the pivotal role of T cells and mast cells/basophils, as well as cytokines, in the development of eosinophilic inflammation and IgE-associated allergic diseases [1–5]. In particular, the Th2 subset of T cells, which is distinguished functionally by its characteristic pattern of cytokine secretion (of the interleukins IL-4 and IL-5), is thought to have a key role. Cytokines released by another opposing, subset of T cells (Th1), such as interferon-γ (IFN-γ), inhibit the development and expansion of Th2 cells and the production of their cytokines [6,7]. These studies have demonstrated

the importance of cytokines in the regulation of IgE and Th2 responses, and suggest ways of targeting the IgE- and Th2-associated responses that may be effective in prevention and treatment of allergic disease.

Indeed, a phenotypic shift in antigen-specific T cells from a Th2 population to a Th1 population has been proposed to occur during allergen immunotherapy [8,9]. However, whether this shift accounts for the clinical benefit seen remains to be determined. Also, patient compliance is poor with this long-term treatment (one to three years), and adverse effects are often seen in patients receiving rush immunotherapy [10]. Several recent preclinical studies have specifically targeted the Th2-associated allergic responses. For example, IL-12, a potent IFN-γ inducer, is able to inhibit and reverse the antigen-induced airway eosinophilia and bronchial hyper-reactivity in a murine model [11]. Furthermore, a direct mucosal IFN-γ gene transfer approach also results in a significant inhibition of both antigen- and Th2-cell-induced pulmonary eosinophilia and airway hyper-reactivity [12]. Thus, the Th1- and Th2-associated phenotypes can be reciprocally regulated, providing a basis for developing more effective therapeutic approach for allergic diseases.

One promising avenue in this regard involves manipulation of the immune system utilizing 'DNA-based immunization'. This new and important therapeutic approach has been investigated in a variety of human disease models [13–16]. DNA-based immunization has been used to generate and/or modify the host immune response, with the ultimate purpose of preventing or reversing the progression of immune disorders. The basis of this approach is that injection of 'naked' plasmid DNA (pDNA) encoding specific antigen results in long-lasting cellular and humoral immune responses to that antigen [17]. Intramuscular injection of pDNA has been shown to provoke long-term immune responses characterized by the synthesis of specific IgG antibodies, and by the efficient generation of CD8⁺ cytotoxic T cells and CD4⁺ Th1 cells (for review see [18*,19]), without generating serious inflammatory reactions at the site of inoculation. In fact, clinical trials of DNA-based vaccination for infectious diseases are now underway to determine its efficacy in humans. These new and exciting developments thus show promise of safe and effective therapy for various diseases, and have provided an experimental basis for immunomodulation of the IgE- and Th2-associated allergic responses. The use of DNA-based immunization in various infectious disease models and its suggested regulatory mechanisms has been the subject of recent excellent reviews [18*,20], to which the reader is referred for more detailed information and discussion. The present review will focus on recent developments concerning

immunomodulation of antigen-induced IgE and allergic responses using DNA-based immunizations.

DNA-based immunization and modulation of the allergic response

Modulation of antigen-specific IgE synthesis

It has been shown that the immune response to inhaled allergens in non-allergic subjects is associated with Th1 responses [21,22]. In addition, data from animal experiments indicates that functionally distinct CD8⁺ T cells may have an important regulatory role in controlling IgE synthesis [23]. These immunological findings have led to a new paradigm, which proposes that immune deviation of T-cell responses to eliciting allergens may provide an approach to prophylaxis and therapy for allergic diseases. Using a DNA-based immunization approach, Hsu *et al.* [24•,25] tested whether immunization with genes for allergens was able to induce immune deviation, wherein a Th1 immune response can be established *in vivo*, thereby preventing the subsequent acquisition of Th2-skewed responses upon exposure to allergens.

They first showed that when BALB/c mice are immunized intraperitoneally (i.p.) with a house dust mite allergen Der p 5 [26] and with alum as an adjuvant, a significant level of Der p 5-specific IgE is induced in mice three weeks after immunization with recombinant Der p 5 (rDer p 5). Cytokine profilings of the splenic T cells showed a clear Th2-like phenotype with high levels of IL-4 and IL-5, but not of IFN- γ being secreted. To examine the utility of DNA-based immunization in influencing the allergic response, a full-length Der p 5 complementary DNA (cDNA) was subcloned into a eukaryotic expression vector to create a gene expression construct, pCMVD. BALB/c mice that were injected intramuscularly (i.m.) with pCMVD DNA expressed a significant level of Der p 5 at the site of injection. Immunologic data showed that there is induction of high levels of Der p 5-specific IgG2a and IgG1, but not of IgE antibodies. The cytokine phenotype of the splenic CD4⁺ T cells was Th1-like. When these pCMVD-immunized mice were sensitized i.p. with rDer p 5 three weeks after pCMVD injection, there was a 90% reduction in the level of specific IgE when compared with control vector-treated mice.

The suppression of the specific IgE response could be adoptively transferred with CD8⁺ T cells from pCMVD-treated mice. Such inhibition was antigen-specific, as the level of specific IgE to another allergen, Der p 1, remained unchanged in both control and treated groups. Hsu *et al.* [25] also investigated whether genetic immunization could downregulate the existing IgE response, thus conferring protection against a subsequent allergenic challenge. BALB/c mice were first sensitized i.p. with Der p 5 together with alum, and 2 weeks later mice received i.m. injection of either pCMVD or the control vector. Results showed that in mice receiving pCMVD after sensitization with rDer p 5, a significant reduction in specific IgE

antibody production is seen compared with those mice treated with the control vector.

Using a different model, Raz *et al.* [27•] reported similar findings demonstrating the inhibitory effect of DNA-based immunization on the synthesis of specific IgE antibody. They generated an expression gene construct, pCMV-LacZ, encoding β -galactosidase (β -gal), and injected this construct intradermally (i.d.) into BALB/c mice. Results showed that β -gal/alum-primed BALB/c mice immunized i.d. with pCMV-LacZ show a 66–75% reduction in the level of β -gal-specific IgE within 6 weeks. Also, this pDNA immunization protocol induced specific IgG2a, and IFN- γ secretion by the helper T cells in the β -gal/alum-primed mice. They also demonstrated that such a Th1 response can suppress an on-going β -gal protein-induced Th2 response in an antigen-specific manner. Interestingly, this study also demonstrated that DNA immunization can be effectively performed by scratching the skin with a tine applicator, and this method showed a similar degree of skin cell transfection as i.d. injection. Their subsequent studies [28•] also showed that the suppression of β -gal-induced IgE synthesis can be passively transferred into naive mice by both CD4⁺ and CD8⁺ T cells from pCMV-LacZ-immunized mice. Interestingly, in mice deficient of β_2 -microglobulin, pCMV-LacZ immunization also showed a reduction of subsequent β -gal-induced IgE synthesis, suggesting that in addition to regulatory CD8⁺ T cells, CD4⁺ T cells are also important in mediating IgE suppression. The differences seen in this study compared with the studies of Hsu *et al.* may be the result of different routes of pDNA injection and the nature of the antigen used.

Modulation of airway allergic responses

Since the suppression of a specific IgE response can be readily observed in mice receiving allergen-DNA-based immunization, Hsu *et al.* [24•] took this approach one step further and examined whether inoculation of pCMVD into rats can modulate allergen-induced airway hyper-responsiveness (AHR). Pulmonary resistance was measured after aerosol challenge of rats with Der p 5. In mock-immunized rats, pulmonary resistance was increased significantly to 80% over baseline 20 min after inhalation challenge with Der p 5. In contrast, no significant changes in pCMVD-immunized rats were observed. In addition, the inhibition of AHR is specific to Der p 5, because there was no significant difference in AHR between the pCMV-immunized and pCMVD-immunized rats after inhalation challenge with an irrelevant antigen, ovalbumin (OVA). Again, this suppression is persistent and can be transferred into naive rats by CD8⁺ T cells from gene-immunized rats. Significantly rats receiving pCMVD six months earlier also show a significant suppression of antigen-induced AHR when compared to the control rats. Thus, in addition to the suppression of allergen-induced IgE synthesis, the DNA-based immunization approach is able to suppress allergen-induced AHR. This finding has

recently been replicated in a murine model, in which mice were sensitized and challenged with OVA [29].

Implication for potential prophylaxis and treatment of allergic diseases

Taken together, the results generated so far have provided evidence that DNA-based immunization is able to induce a protective Th1 response against the undesirable Th2-like allergic reaction. In addition, this Th1 response is capable of downregulating an on-going Th2 response. These preclinical studies thus indicate the potential efficacy of the DNA-based immunization approach in modulating the IgE- and Th2-associated allergic responses. DNA-based immunization has been used successfully in models involving a latex allergen, Hev b 5 [30]. Given its persistent and effective effects in these animal models, it is conceivable that DNA-based immunotherapy can effectively reduce both the doses and duration of immunotherapy treatment. Also, the Th1-skewed response and the suppression of specific IgE synthesis is antigen specific. The antigen specificity is an advantage, because of the concern that a universal Th1-skewed response poses the potential danger of inducing other forms of immune disorders.

However, despite the recent success of DNA-based immunization in altering the IgE- and Th2-associated immune response in various models, the molecular basis and the immunoregulatory mechanism are far from clear, and the regulatory T-cell population responsible for mediating the suppression remains controversial. In addition, one enduring mystery is the identity of the antigen presenting cells [31,32] that so efficiently present the antigen and activate CD8⁺ T cells as well as CD4⁺ Th1 cells, the common features seen in pDNA-immunized hosts. It is also apparent that pDNAs containing antigen cDNAs with or without leader sequences for secretion are equally efficient in generating the immune responses [14,33]. Thus, further understanding of the regulatory mechanism of DNA-based immunization will undoubtedly facilitate its clinical utility.

Furthermore, a number of issues concerning allergen DNA-based immunization strategy have to be addressed. First, multiple allergenic components are involved in the development of allergic responses and, on the basis of the preclinical studies, the protective immune response is highly antigen specific [24••,27••]. It may, therefore be, necessary to include a cocktail of allergen genes to confer full protection; alternatively, immunization with a gene expression library [34] may be a useful approach. Second, further work is required to address the safety issues and to evaluate the dose-response relationship. It has been reported that, depending on the nature of antigen, the strain of mice used and the route of pDNA injections

[13,35,36•,37••], a Th2 response can be generated following pDNA immunization. It is, therefore, imperative that each experimental system be carefully examined in sufficient detail to avoid a potential anaphylactic response. It is also important to optimize gene transfer and gene expression by using the improved vector constructs and DNA delivery systems that are currently available. For example, vectors with a short palindromic DNA sequence containing CpG motifs [38••] could potentially be more effective in inducing potent Th1-associated responses. In addition, co-injection of modulatory cytokine genes [15,16] and/or genes encoding T-cell co-stimulatory factors [39•] to enhance the protective immune response should be explored. Although of limited efficacy, the use of humanized monoclonal anti-IgE antibodies, which have been tested for asthma therapy in two recent small clinical trials [40,41], may perhaps be combined with the DNA-based immunization approach to improve its clinical efficacy.

Conclusions

'Proof of the concept' has been provided of the utility of DNA-based immunization in modulating IgE- and Th2-associated allergic responses, and this approach may offer a novel avenue for the immunoprophylaxis of allergic disorders. More important, it may offer a more effective strategy for the treatment of human allergic diseases. However, the etiology and pathophysiology of allergic disease are multifactorial and involve intricate regulatory networks. Rigorous studies of DNA-based immunization in respect of its regulatory mechanism and its safety will be crucial in its ultimate application in human atopic diseases; specifically, we need a careful assessment of multiple protocols and disease parameters in order to improve its applicability. The extraordinary progress of DNA-based immunization in various disease models has facilitated its use in the field of allergic disease and it is now being investigated in many laboratories. Novel gene constructs and methods of delivery should be actively pursued. At the functional level, we need to develop a deeper understanding of its immunologic mechanism and the regulatory elements. This information is needed to define the immunologic parameters involved in the modulation of allergic disease. The heterogeneity of experimental outcomes that are under different experimental strategies should be compared. It is of particular importance to conduct comprehensive research to gain further understanding of its regulatory mechanism and to improve the forms of gene construct/delivery. These approaches will greatly facilitate the effort to design better, safer, and more effective DNA-based immunization strategies for allergic diseases.

Note from the publisher

We were saddened to hear of the death of Professor Kue-Hsiung Hsieh. He will be greatly missed by his colleagues.

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