

# Editorial

## DNA vaccine and asthma therapy

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Allergic asthma is currently considered a chronic airway inflammatory disorder associated with the presence of activated CD<sub>4</sub><sup>+</sup> Th2-type lymphocytes, eosinophils, and mast cells. Interestingly, therapeutic strategies based on immune deviation and suppression have been shown to successfully attenuate the development of the asthma phenotype. Despite the clear rationale for immunotherapy, the usefulness of this approach has been limited by the potential for adverse effects, particularly anaphylaxis, and the relatively crude nature of the allergen extracts that are available. To overcome these problems, revised strategies have been assessed in animals and are undergoing clinical evaluation. Naturally occurring forms of allergens from plants and trees have been shown to have a reduced capacity to be bound by IgE as a result of the substitution or deletion of amino acids.<sup>1</sup> The use of hypoallergenic isoforms in immunotherapy may thereby minimize the risk of anaphylaxis. Similarly, the use of recombinant allergens should circumvent the problem of standardization of crude extracts by allowing production and purification of many of the major allergens in ways that eliminate variation between batches.<sup>2</sup>

Recently, one strategy aimed at downregulation of the allergic response is based on the development of DNA vaccines.<sup>3</sup> Plasmid vectors containing genes that encode allergens have been injected into animals, either before or after allergen challenge. This vaccine approach can markedly decrease Th2-mediated responses, enhance Th1-mediated responses, and suppress the allergic response. Virus-like particles can also induce interferon- $\gamma$ -producing CD<sub>8</sub><sup>+</sup> T cells, rather than Th2-mediated responses.<sup>4</sup> Another consideration is the induction of tolerance using mucosal DNA vaccination. Whether this approach or one directed at the airway mucosa would be efficacious for inhaled allergens still needs to be determined. All of these efforts to

downregulate the allergic response are tempered by the data that immunotherapy has shown only limited efficacy in treatment of asthma.<sup>5,6</sup> Alternative approaches have been devised to more generically decrease the Th2 response by enhancing the counteractive Th1 response. This strategy is further bolstered by the hygiene hypothesis for asthma, whereby a decrease in the normal level of Th1 responses may also contribute to allergic diseases.<sup>7</sup> A particular approach for stimulation of Th1-mediated responses has been the administration of synthetic oligodeoxynucleotides with immunostimulatory sequences either alone or in combination with allergen.<sup>8</sup> Strong immunostimulatory effects are driven by sequences containing unmethylated CpG motifs that are more highly represented in microbial than vertebrate DNA, and so are recognized as foreign by the innate immune system. These motifs appear to function as Th1-promoting adjuvants capable of switching the usual Th2 response toward a Th1 response.

Smart and coworkers<sup>9</sup> have demonstrated that a genetically modified plant-based vaccine can promote a protective immune response and attenuate experimental asthma, suggesting that plant-based vaccines may be potentially therapeutic for the protection against allergic diseases. A novel therapeutic DNA vaccine approach has been developed for the treatment of allergy and asthma.<sup>10</sup> This strategy was to use active DNA vaccination against interleukin (IL)-5 to elicit polyclonal antibodies that would neutralize IL-5 produced during recall responses to inhaled allergen and ameliorate

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disease. In mice , Th-modified IL-5 DNA vaccination induced an immune response directed against native IL-5. Moreover , Th-modified IL-5-vaccinated mice induced an immune response directed against IL-5 that reduced both pulmonary lymphocyte infiltration and eosinophilia , reduced the levels of Th2 cytokines , and inhibited the development of airway hyperresponsiveness.<sup>10</sup> These data substantiate active DNA vaccination against IL-5 as a novel therapeutic approach for the treatment of asthma and potentially other allergic diseases. In addition , modification of vaccination plasmids with IL-18 cDNA can greatly enhance the immunogenicity of antigen cDNA constructs , and generate constructs that may be clinically effective in treating patients with established allergic pulmonary disease.<sup>11</sup>

From an epidemiological point of view , *Dermatophagoides pteronyssinus* group species ( Der p 1 ) , a protein synthesized by *Dermatophagoides pteronyssinus* mites , is one of the top candidates for an allergy vaccine : house dust mites are major indoor triggers of atopic allergy ; the majority of allergic individuals are sensitized to one or more species that are common in their environment ; *Dermatophagoides pteronyssinus* is one of the three most common species of mites in temperate regions ; the majority of mite sensitive patients respond to group I and group II allergens , and Der p 1 is one of the prevalent antigens in this mixture.<sup>12,13</sup> It has been reported that plasmid immunization with DNA carrying the complete Der p 1 cDNA sequence , pcDNA3-pre-pro-Der p 1 , can elicit antibodies with native Der p 1 specificity. At least two immunizations were required to detect the response. A booster injection given 2 – 3 weeks after primary immunization resulted in circulating anti-Der p 1 antibodies for over 2 months.<sup>14</sup> More recently , Jarman and coworkers<sup>15</sup> have demonstrated that mice exhibiting Th2-mediated airway inflammation induced following sensitization and challenge with Der p 1 , were treated with plasma DNA ( pDNA ) vaccines. In pDNA-vaccinated mice , infiltration of inflammatory cells , goblet cell hyperplasia and mucus production were reduced and subepithelial fibrosis attenuated. The reduction in eosinophil numbers correlated with a fall in levels of the profibrotic mediator transforming growth factor- $\beta$ 1 in bronchoalveolar lavage fluid and lung tissue. Protection , conferred irrespective of the specificity of the pDNA construct , did not correlate with a sustained increase in systemic interferon- $\gamma$

production but in a reduction in levels of the Th2 pro-inflammatory cytokines. Notably , there was a reduction in levels of IL-5 and IL-13 produced by systemic Der p 1 reactive CD<sub>4</sub><sup>+</sup> Th2 cells on *in vitro* stimulation as well as in IL-4 and IL-5 levels in bronchoalveolar lavage fluid. These data suggest that suppression of CD<sub>4</sub><sup>+</sup> Th2-mediated inflammation and eosinophilia were sufficient to attenuate progression towards airway remodeling.

In the current issue of the journal , Li and coworkers<sup>16</sup> have investigated whether DNA vaccine encoding Der p 2 could generate immunologic protection in recombinant Der p 2 allergen-induced allergic airway inflammation mice model , and demonstrated that DNA vaccine encoding Der p 2 allergen generates immunologic protection in recombinant Der p 2 allergen-induced allergic airway inflammation mice model with regulating the immune response towards a Th1-type reaction. DNA vaccine encoding mite dust major allergen Der p 2 might be a novel therapeutic approach against allergic asthma.

Since major allergen of birch pollen , Bet v 1a , representing one of the most frequent plant allergens , a DNA vaccine containing the entire coding region of Bet v 1a has been also studied.<sup>17</sup> Preimmunization with this DNA vaccine induced high levels of IgG<sub>1</sub> and nearly equal amounts of IgG<sub>2a</sub> antibody titers indicating a substantial Th1-type component of the response. This reaction profile did not change after a sensitization with recombinant allergen. Furthermore , DNA vaccination prevented IgE production by protein sensitization and induced functional protection against an allergic immune reaction as indicated by the inhibition of IgE-mediated basophil cell release.<sup>17</sup> The results demonstrate the allergen-specific protective and therapeutic efficacy of a DNA vaccine encoding the clinically highly relevant allergen Bet v 1a indicating the suitability of this concept for the treatment of allergic diseases.

It should be noted that although preclinical results are promising , the outcomes of clinical trials of DNA vaccine for allergic asthma are still pending. Some experimental models suggest that interferons or IL-12 ( or other Th1 components ) may mediate the observed efficacy. Because previous trials of these agents have not yet been effective in treating asthma ,<sup>18,19</sup> these cytokines and related targets will need to be monitored in future studies. As

mentioned above , at least some aspects of the vaccine approach must also be tempered by studies indicating that the Th1 response may augment the allergic and inflammatory response and that some elements of a heightened Th1 response may be characteristic of asthma.<sup>20</sup> Another concern is that immunostimulatory agents have the potential for causing autoimmune disease after long-term administration.

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( Received February 4 , 2005 )

Edited by WANG Mou-yue