

Original article

Novel cytokine peptide-based vaccines: an interleukin-4 vaccine suppresses airway allergic responses in mice

Background: Monoclonal antibodies or soluble receptors have been used to block over-produced endogenous cytokines. However, they have disadvantages of short half-lives, high costs, and possible adverse effects. Using interleukin (IL)-4 as a model target, we sought to develop a novel therapeutic strategy by constructing an IL-4 peptide-based vaccine for blocking IL-4 on a persistent basis, and to evaluate its efficacy in a mouse model of asthma.

Methods: A peptide was selected by antigenic prediction and structure analysis of IL-4/receptor complex. The vaccine was constructed by employing truncated hepatitis B core antigen as carrier with the peptide inserted using gene engineering methods. It was then expressed, purified and identified. Prior to intraperitoneal sensitization and intranasal challenge with ovalbumin, mice were subcutaneously immunized three times with the vaccine, or the carrier or saline as controls. Serum antibodies, inflammatory cells in bronchoalveolar lavage fluids (BALF), lung histology, and responsiveness to inhaled methacholine were analyzed.

Results: The vaccine presented as virus-like particles and reacted to polyclonal anti-IL-4 in Western blotting. Vaccinated mice produced high titers of IgG to IL-4. Serum ovalbumin-specific IgE, eosinophil accumulation in BALF, goblet cell hyperplasia, tissue inflammation and methacholine-induced respiratory responses were markedly suppressed in vaccinated mice with statistical significance, as compared with those in the control groups.

Conclusions: Administration of this novel IL-4 vaccine led to an overall decrease in the development of airway allergic inflammatory responses. The results indicate that cytokine peptide-based vaccines hold potential for treatment of asthma and, by extension, other diseases where over-expressed cytokines play a pivotal role in pathogenesis.

Y. Ma^{1,2}, K. T. Hayglass^{1,3},
A. B. Becker^{1,2,3}, A. J. Halayko^{2,4},
S. Basu^{2,4}, F. E. R. Simons^{1,3},
Z. Peng^{1,2,3}

¹Department of Pediatrics and Child Health, University of Manitoba; ²Biology of Breathing Theme, Manitoba Institute of Child Health;

³Department of Immunology, University of Manitoba; ⁴Department of Physiology, University of Manitoba, Winnipeg, MB, Canada

Key words: allergy; hepatitis B core antigen; immunotherapy; interleukin-4; vaccine.

Dr Z. Peng
Department of Pediatrics and Child Health
University of Manitoba
532-715 McDermot Avenue
Winnipeg, MB
Canada R3E 3P4

Accepted for publication 10 March 2007

Dysfunctional regulation of cytokine gene expression is associated with a variety of severe diseases. Administration of monoclonal antibodies (mAb) or soluble receptors to block specific cytokines is an effective treatment approach for some of these diseases (1, 2). However, these interventions are passively administered and have a short half-life. Frequent injections of a large dose are required to maintain their effects. For example, mAb against human IgE requires 200–400 mg or more to be injected i.v. or s.c. every 2–4 weeks to be effective (3), and soluble TNF α receptors must be administered twice a week (4). Such passively administered therapies are therefore inconvenient and costly. Furthermore, infusion reactions have been reported in 17% of patients treated with a mAb to TNF α (infliximab). Twenty-eight to 68% of treated patients develop anti-chimeric antibodies that may be associated with a loss of response to the mAb (5). New strategies to overcome these disadvantages are needed.

Interleukin-4 is important in the development of allergy and asthma: increased IL-4 levels have been found in

serum and bronchoalveolar lavage of asthmatic individuals (6, 7); genetic polymorphisms of IL-4 have been linked to the development and severity of asthma (8, 9) and airway hyperresponsiveness (AHR) and recruitment of eosinophils have increased after experimental inhalation of IL-4 (10). The crucial role of IL-4 lies in its effects on Th2 development, induction of IgE production, up-regulation of IgE receptor expression, inhibition of T cell and eosinophil apoptosis, and promotion of cell migration to inflammatory loci, as well as stimulation of mucus producing goblet cells and fibroblasts (11). Recently, IL-4 has been reported to induce IL-13-independent AHR and goblet cell hyperplasia in IL-13-deficient mice (12). Targeting IL-4 using its soluble receptors has been shown to be effective in suppressing airway allergic responses in mice (13). However, in human studies with IL-4R, IL-4 mutein, human or humanized anti-IL-4, the results have not been as promising as expected (11). Despite this, it is premature to conclude that IL-4 targeting would not be effective in asthma treatment, because the IL-4 antago-

nists used in these studies have short half-lives which, combined with other factors, are likely to contribute to the lack of efficacy (11).

In the current study, we have, for the first time, successfully designed an IL-4 peptide and hepatitis B virus core antigen (HBcAg) particle-based vaccine for the induction of autoantibodies to block IL-4 on a persistent basis. We investigated its effectiveness in suppressing airway allergic inflammatory responses in a well-established mouse model of asthma.

Material and methods

Selection of antigenic peptides

Antigenic peptide prediction was performed according to the occurrence of amino acid residues in experimentally known segmental epitopes (14) and the hydrophilicity and hydrophobicity analysis of amino acids (15). Priority, based on the structure analysis of IL-4/receptor complex, was given to the peptides located in the contact sites of receptor binding and/or at the terminals of the target protein to facilitate the induction of the neutralizing antibody. A peptide of 14 amino acid residues named 'A13', located in the receptor contacting site of mouse IL-4 molecule (2–15 amino acids: DKNHLREIIGILNE), was chosen to be inserted into the carrier HBcAg. NCBI BLAST searching has shown that there is no similar sequence to the IL-4 peptide in other known molecules.

Preparation and identification of recombinant vaccine

The recombinant plasmids pHbAg, expressing carrier, i.e. truncated HBcAg (1–149 amino acids), and pHbAg-A13, expressing vaccine, i.e. a recombinant protein with selected IL-4 peptide inserted between 78 and 79 amino acids of HBcAg immunodominant epitope, were constructed. The expression of recombinant protein was induced with isopropyl-beta-D-thiogalactopyranoside (IPTG) as routine procedure and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using a polyclonal biotinylated goat anti-mouse IL-4 antibody (R&D Systems Inc.). Recombinant protein was purified by a combination procedure consists of ultra-sonication lysis, 40% ammonium sulfate precipitation, 20% ammonium sulfate wash, ultracentrifugation with sucrose density gradient and size exclusion chromatography with Sepharose CL-4B (Sigma-Aldrich, St Louis, MO, USA). Lipopolysaccharide (LPS) levels of the final vaccine and carrier products were determined using E-TOXATE® test (Sigma-Aldrich) following manufacturer's instructions.

Animals

Female BALB/c mice (7–8 weeks old) were obtained from the Central Animal Care Services, University of Manitoba. The experiments were approved by the Animal Care and Use Committee at the University of Manitoba, and the investigators adhered to Canadian Council on Animal Care (CCAC) guidelines for humane treatment of animals.

Protocol for immunization, sensitization and challenge

Three groups of mice (4–6 mice per group) were injected subcutaneously three times at 2-week intervals with 100 µg of

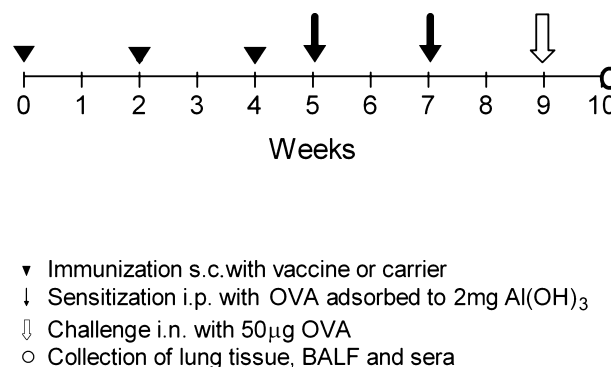


Figure 1. Immunization and sensitization/challenge protocol.

HBcAg-A13 particles, carrier or phosphate buffered saline (PBS) (0.02M sodium phosphate buffer, 0.15 M NaCl, pH 7.2), respectively, without the use of adjuvant, in a total volume of 150 µl. One week after the last immunization, mice were sensitized twice (at weeks 5 and 7) by i.p. injection of 2 µg of ovalbumin (OVA) (Sigma-Aldrich, grade IV) adsorbed to 2 mg of Al(OH)₃ adjuvant, and challenged at week 9 by a one-time application of an intranasal droplet of 50 µg OVA in a volume of 40 µl. The protocol is shown in Fig. 1.

ELISAs

Serum samples were taken on the fifth, seventh and ninth week before the succeeding OVA administration, and at the endpoint of the experiment. The levels of IL-4-specific IgG and OVA-specific IgE and IgG2a were measured by ELISAs using techniques established in our laboratory (16), and reagents purchased from PharMingen (San Diego, CA, USA), unless otherwise specified. Briefly, to measure the titers of IL-4-specific IgG, plates coated with mouse IL-4 (1 µg/ml), a gift from Professor W. Sebald (University of Würzburg, Germany), were incubated with serially twofold diluted serum samples and then with alkaline phosphatase-conjugated rabbit anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). In the measurement of OVA-specific antibodies, plates were coated with either mAb rat anti-mouse IgE at 1 µg/ml (for IgE assays) or OVA at 2 µg/ml (for IgG2a assays) and then incubated with diluted serum samples and a twofold diluted reference serum. This was followed by incubation with biotinylated OVA prepared in our laboratory for IgE assay or with biotinylated anti-IgG2a for IgG2a assay.

Bronchoalveolar lavage fluids (BALF) collection and differential cell counts

Six days after nasal challenge, mice were anesthetized and blood samples were collected by cardiac punctures. BALF were collected with 1ml of PBS, flushing three times. After centrifugation at 1000 g for 5 min, the BALF supernatant was frozen for subsequent cytokine assays and the cell pellet was resuspended for cytospin (Cytospin; Shandon, Runcorn, UK). The slides were stained with HEMA 3 (Fisher Diagnostics, Pittsburg, PA, USA). Differential cell counts were performed according to standard hematological procedures, and at least 400 cells on each preparation were counted.

Histopathological examination

Six days after intranasal challenge, and immediately after the lavage fluids were obtained, the lungs were dissected and fixed with 10% buffered formalin, embedded in paraffin, sectioned, and stained with either hematoxylin and eosin (H&E) or periodic acid schiff (PAS), and semi-quantified as previously described (17). Briefly, lung sections stained with H&E were assigned a unit value for peribronchiolar/perivascular. The numerical scores for each view field were determined as follows: 0 = normal; 1 = few cells; 2 = a ring of inflammatory cells one cell layer deep; 3 = a ring of inflammatory cells 2–4 cells deep; 4 = a ring of inflammatory cells of >4 cells deep. In sections stained with PAS, medium-sized airways were assessed. The numerical scores for the abundance of PAS-positive goblet cells in each airway were determined as follows: 0: <5% goblet cells; 1: 5–25%; 2: 25–50%; 3: 50–75%; 4: >75%.

Airway responsiveness to methacholine

Physiological pulmonary responses to inhaled methacholine were measured 2 days after intranasal challenge in unrestrained animals using whole body plethysmography (Model PLY 3211; Buxco Electronics, Troy, NY, USA). Enhanced paused (Penh) was calculated. Penh value correlates well with airway resistance measured in ventilated mice, especially the BALB/c strain used in our studies (18). The percent increase in Penh value above the saline challenge baseline was compared between groups.

Statistical analysis

The significance of differences between experimental groups was analyzed using one way ANOVA, followed by Tukey–Kramer multiple comparison test (GraphPadPrism 3.03; GraphPad Software, Inc., San Diego, CA, USA). Values were reported as the mean $\pm$ SEM.

Results

Identification and purification of the recombinant IL-4 peptide-based vaccine

The recombinant protein HBcAg-A13 was expressed efficiently in *Escherichia coli* DH $_{5\alpha}$ cells (Fig. 2A). This recombinant protein specifically reacted with the polyclonal rabbit anti-mouse IL-4 antibody as shown in the immunoblotting (Fig. 2B). Analyzed by SDS-PAGE, the HBcAg-A13 and the HBcAg carrier protein had the identical pattern in sucrose gradient ultracentrifugation, i.e. these recombinant proteins were mostly present in the fractions from tube 6 to 8 (totally thirteen 1-ml fractions were collected from top to bottom) (Fig. 2C). This indicates that the chimeric HBcAg-A13 assembles into particles that are similar to native HBcAg. Using electron microscopes, the HBcAg-A13 were visualized as particles with a diameter of 25 nm (Fig. 2D). The LPS contamination of the carrier HBcAg and the vaccine HBcAg-A13 is reproducibly within the range of 10–20 EU/ μ g of protein, which is significantly lower than the LPS concentration required to induce a Th2 to Th1 shift (19).

Serum mIL-4-specific IgG and OVA-specific IgE and IgG2a

BABL/c mice immunized with HBcAg-A13 particles without use of adjuvant produced high (up to 256 000) and long-lasting (more than 8 weeks) titers of IgG antibodies to mouse IL-4 as measured by ELISA (Fig. 3A). These data indicate that the recombinant

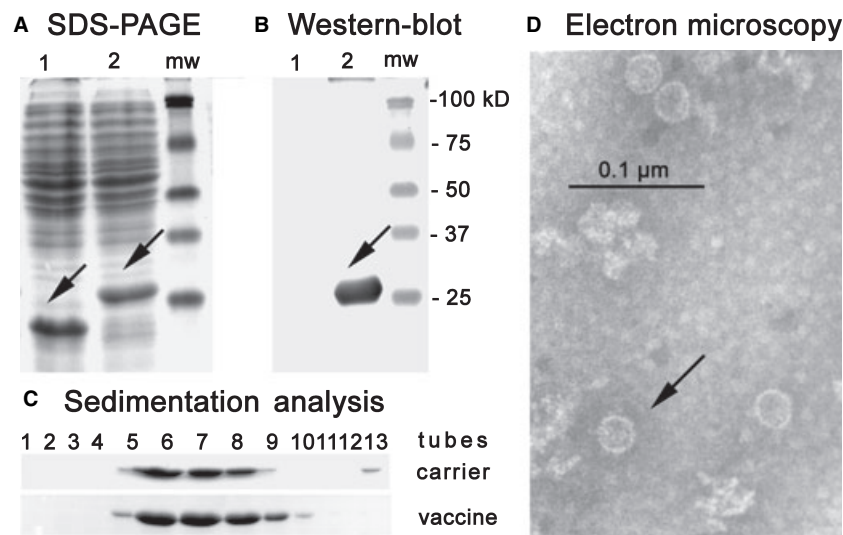


Figure 2. Identification of the recombinant protein HBcAg-A13. The expression of recombinant protein was identified using SDS-PAGE (A). The specificity of the recombinant protein was examined using immunoblotting with a polyclonal anti-IL-4 antibody (B). Arrows point to recombinant proteins. Lane 1: the carrier HBcAg; lane 2: the vaccine HBcAg-A13. Sedimentation analysis (C) indicates that recombinant chimeric HBcAg-A13 presents as HBV capsid-like particles. Thirteen 1-ml fractions collected from sucrose gradient (10–60%) centrifugation were analyzed by SDS-PAGE. The presence of HBcAg-A13 as capsid-like particles with a diameter of 25 nm was confirmed by electron microscopy (D).

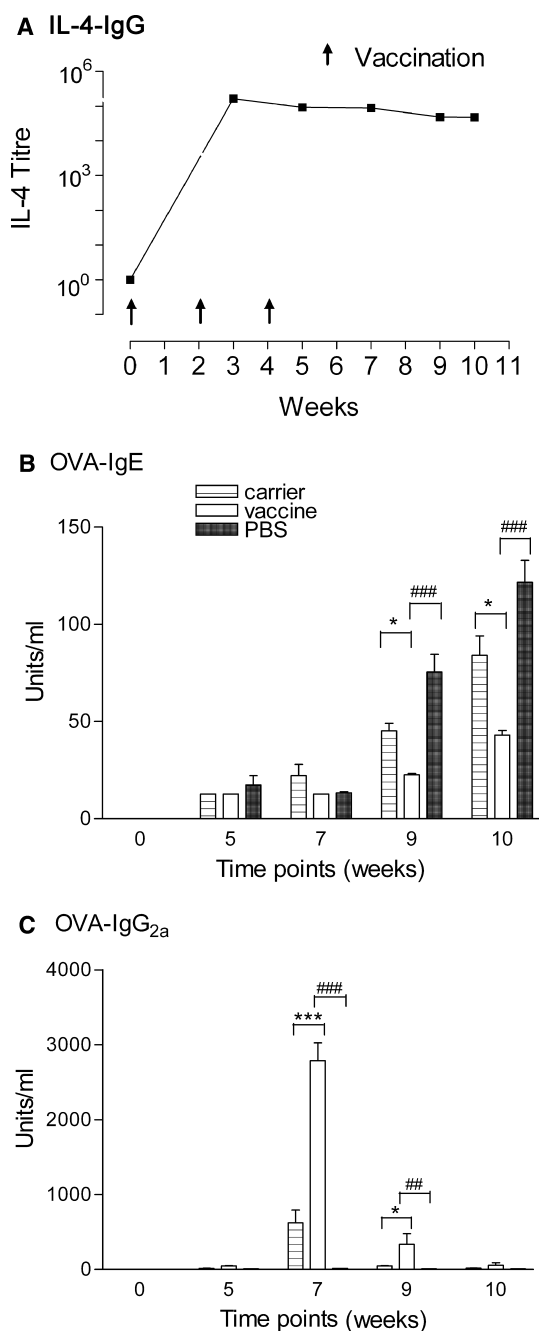


Figure 3. The vaccine induces high and long-lasting titers of IgG_{2a} to mouse IL-4 (A). The titer was determined as the reciprocal of the highest dilution of the sample in which the OD₄₀₅ value was twice of that of the corresponding control serum pooled from carrier immunized mice when its OD₄₀₅ was around 0.10. The vaccine decreases OVA-specific IgE (B) and increases OVA-specific IgG_{2a} (C) significantly. A reference serum defined as 1000 U/ml was used, and the mean value of each sample was calculated by interpolation from the dilution curve of the reference serum. Data were reported as mean ± SEM. **P* < 0.05 and ****P* < 0.001, as compared with the carrier group; #*P* < 0.01 and ###*P* < 0.001, as compared with the PBS group.

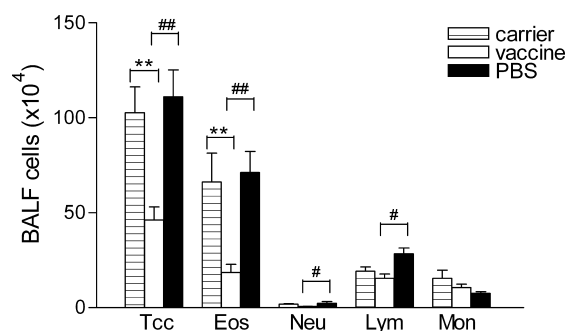


Figure 4. The vaccine inhibits inflammatory cell accumulation in bronchoalveolar lavage fluid (BALF). Tcc: total cell counts; Eos: eosinophil; Neu: neutrophil; Lym: lymphocyte; Mon.: monocyte. ***P* < 0.01, as compared with the carrier group; #*P* < 0.05 and ###*P* < 0.001, as compared with the PBS group.

HBcAg-A13 is highly immunogenic. At week 7, i.e. 2 weeks after the first i.p. sensitization, the OVA-IgE was still at a low level, whereas the OVA-IgG_{2a} levels were significantly elevated by immunization of the carrier and vaccine. Two weeks after the second i.p. sensitization (week 9) and 1 week after i.n. challenge (week 10), the OVA-IgE responses were boosted, while the IgG levels were decreased significantly (Fig. 3B,C). The results showed that OVA-IgE levels were significantly suppressed in the vaccine group when compared with the carrier group (*P* < 0.05) and PBS group (*P* < 0.001) (Fig. 3B); OVA-specific IgG_{2a} were increased significantly (Fig. 3C), shown in the figure as *P* < 0.001 and 0.05 at week 9 and 10, respectively, as compared with carrier, and *P* < 0.001 and 0.01, respectively, as compared with PBS group. Results showed that neutralizing IL-4 with the vaccine significantly enhanced Th1 responses while it suppressed Th2 responses.

Inflammatory cells in BALF

The absolute cell counts and the percentage of each cell type, in particular eosinophils, in BALF reflect the degree of inflammation and the differential contribution of specific cell types. As shown in Fig. 4, the numbers of increased total inflammatory cells and eosinophils were significantly reduced in the vaccine group when compared with either the carrier group or the PBS group. Total cell numbers in the vaccine group ($46.13 \pm 6.98 \times 10^4$, *n* = 6) were reduced significantly when compared with those in carrier group ($102.7 \pm 13.68 \times 10^4$, *n* = 4; *P* < 0.01) and those in PBS group ($111.01 \pm 14.29 \times 10^4$, *n* = 4; *P* < 0.01). Immunization with the vaccine significantly inhibited eosinophil recruitment ($18.49 \pm 4.31 \times 10^4$), when compared with immunization with the carrier protein ($66.21 \pm 15.29 \times 10^4$; *P* < 0.01) and the PBS group ($71.17 \pm 11.10 \times 10^4$; *P* < 0.01). Vaccination also reduced neutrophils ($0.52 \pm 0.11 \times 10^4$) and lymphocytes ($15.42 \pm 2.30 \times 10^4$) when compared

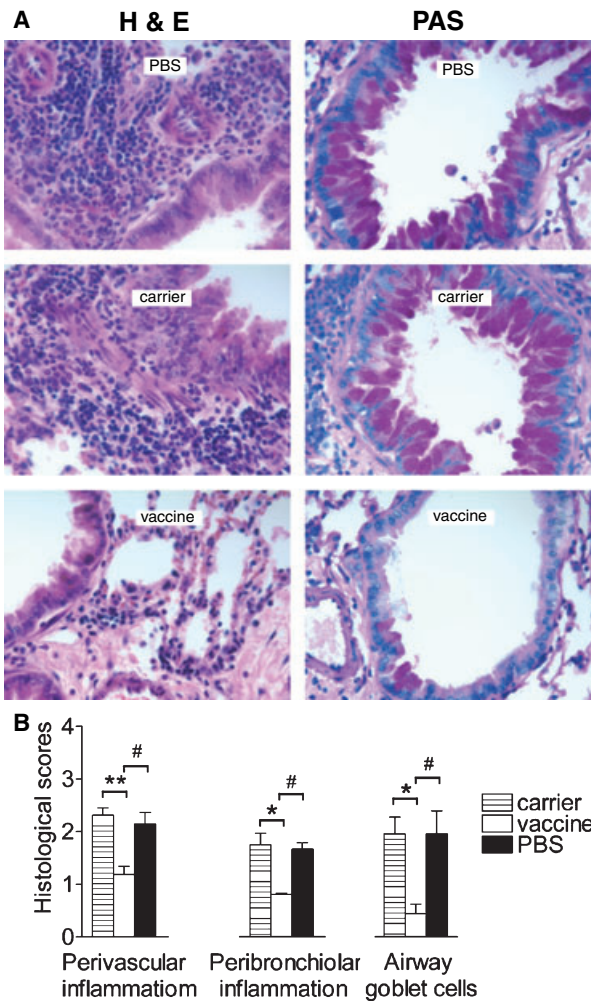


Figure 5. The vaccine decreases lung inflammation and goblet cell hyperplasia significantly. (A) Representative photomicrographs with magnification of 40 $\times$. Top: mice immunized with PBS and sensitized with OVA; middle: mice immunized with the carrier and sensitized with OVA; bottom: mice immunized with vaccine and sensitized with OVA; left: hematoxylin and eosin (H&E) staining; right: periodic acid schiff (PAS) staining. (B) Semi-quantified analysis of lung inflammation. * $P < 0.05$ and ** $P < 0.01$, as compared with the carrier group; # $P < 0.05$, as compared with the PBS group.

with those in the PBS group ($2.36 \pm 0.78 \times 10^4$; $P < 0.05$, and $28.37 \pm 3.06 \times 10^4$, $P < 0.05$, respectively).

Histological analysis

Goblet cell metaplasia, mucus hypersecretion, and inflammation of the airway were found in OVA-sensitized and challenged mice. Representative photomicrographs are shown in Fig. 5A. Consistent with BALF cytology analyses, H&E staining of lung sections confirmed that the perivascular and peribronchial accumulation of inflammatory cells, especially eosinophils, were signifi-

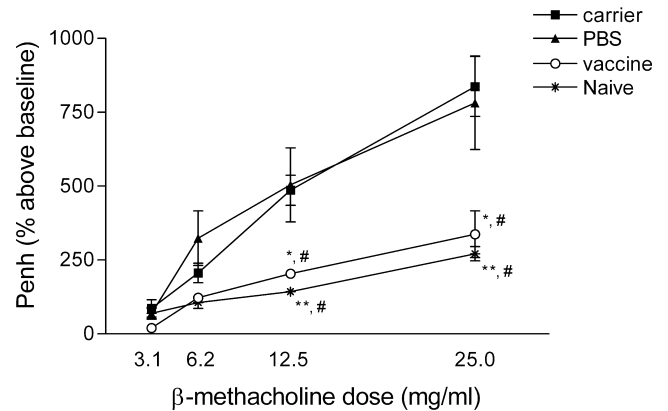


Figure 6. The vaccine decreases hyperresponsiveness to inhaled methacholine significantly. The results were expressed as enhanced pause (Penh), the percentage folds above the saline induced baseline values. * $P < 0.05$ and ** $P < 0.01$, as compared with the carrier group; # $P < 0.05$, as compared with the PBS group.

cantly suppressed in mice receiving IL-4 vaccine when compared with mice receiving carrier or PBS ($P < 0.05$) (Fig. 5B). Furthermore, the PAS-positive goblet cells were significantly reduced in vaccine group in comparison with either PBS or carrier group ($P < 0.05$) (Fig. 5B). No noticeable differences were found between carrier and PBS groups. These data indicate that immunization with the IL-4 vaccine significantly suppressed mucus hyperproduction and lung tissue inflammation induced by OVA sensitization and challenge.

Dose-dependent response to inhaled methacholine

Consistent with our experiments showing IL-4 vaccination prevented allergic airway inflammation, mice immunized with IL-4 vaccine exhibited markedly suppressed respiratory responses to inhaled methacholine (12.5 and 25 mg/ml) compared with mice receiving either carrier or PBS ($P < 0.05$) (Fig. 6).

Discussion

To overcome the current disadvantages associated with using mAb or soluble receptors to block target cytokines, strategies that use vaccines to induce autoantibodies against self-cytokines have begun to emerge (20–24). In the current study, we used a strategy of developing self-cytokine peptide-based vaccines. Autoantibodies induced by this type of vaccine are directed against only the small peptide, providing the unique advantages of inducing highly effective epitope-specific antibody response and avoiding possible cross-reactions, as compared with those consisting of a full length or a considerable part of the self-protein and foreign Th epitopes or a carrier protein. Although the immunogenicity of peptides may be weak, it

can be enhanced by using a potent heterogeneous carrier protein. Virus-like particles have shown attractive potential as a vaccine carriers for breaking B-cell tolerance (22, 25). As is well known, HBcAg particles are highly promising as a delivery system for foreign peptide-based vaccines (26). The highly immunogenic features of HBcAg are attributed to its capacity to function as a T-cell-dependent and T-cell-independent antigen, and its ability to activate naive B cells to function as antigen-presenting cells, presenting antigen with $\sim 10^5$ -fold higher efficiency than typical dendritic cell and macrophage interactions (27). The high immunogenicity of HBcAg particles is also due to unique repetitive arrays of inserted self-peptides which may assist in breaking B-cell tolerance (25), and the presence of well-defined T-cell epitopes in the primary sequence. The immune responses to HBcAg are believed to be safe, supported by a clinical trial of malaria vaccine using HBcAg as carrier (28). In the current study, we have, for the first time, successfully extended the application of HBcAg as a vaccine carrier into the area of breaking self-immune tolerance and inducing high titered autoantibodies.

Using a well-established acute inhaled allergen-challenge model, we further assessed the effectiveness of our IL-4 vaccine in preventing allergic airway inflammation, IgE elevation, mucus overproduction, and airway hyperresponsiveness. In this model, Th2 cell differentiation and subsequent Th2 cytokine production are essential for the development of asthma-like symptoms. IL-4 is a cardinal mediator that drives the differentiation of naïve Th cells into Th2 cells. Our results demonstrated that blocking IL-4 with the vaccine efficiently suppressed Th2 responses and thus down-regulated airway allergic inflammatory responses. For example, it is well known that IgE has a critical role in triggering allergic cascade, and IL-4 is the most important cytokine in mediating IgE synthesis through its ability to induce immunoglobulin isotype to switch from IgM to IgE. Our data clearly show that allergen-specific induction of IgE is markedly suppressed in mice treated with IL-4 vaccine. This is significant in improving allergic symptoms, as decreased IgE not only directly reduces allergic reactions caused by mast cell degranulation but also indirectly reduces allergic inflammation by decreasing the expression of high-affinity IgE receptor on mast cells and dendritic cells (29, 30). The effect relating to dendritic cells may be important in suppressing late-phase allergic reactions, as dendritic cells amplify allergic responses by facilitating allergen uptake and presentation up to 1000-fold (30). Thus, the effective suppression of IgE we report using our IL-4 peptide-based vaccine suggests strong potential in this regard.

Interleukin-4 is a key mediator in inducing airway and tissue eosinophilia which is a cardinal feature of allergic asthma. Eosinophils are thought to contribute substantially to airway remodeling (31, 32), including acute responses such as goblet cell hyperplasia (33). We performed differential cell counts in BALF and histological

examination of lung tissue to detect the effects of our IL-4 peptide-based vaccine on airway inflammation and mucus hyperplasia. As expected, vaccination significantly reduced the accumulation of eosinophils in both the BALF and lung tissue, and we observed a dramatic suppression of allergen-induced mucous production, as indicated by reduced goblet cell number. Our studies also investigated the effects of IL-4 vaccination on the development of hyperresponsiveness to inhaled methacholine, a hallmark feature of asthma. The vaccine had profound effects in preventing allergen-induced increases in responses to inhaled bronchial spasmogen, which are consistent with previous studies that reported administration of recombinant IL-4 induced methacholine hyperresponsiveness in association with the recruitment of inflammatory cells within hours of administration (34). Collectively, our studies show for the first time that vaccination against self-IL-4 significantly suppresses allergen-driven airway asthmatic responses. Although passive administration of soluble IL-4 receptors has failed to display significant evidence of efficacy in human asthma (11), the strategy described here provides a novel approach characterized by a persistent blockade of IL-4 action, which is difficult to achieve when passive administration is used. This approach may lead to a significant improvement of allergic symptoms clinically, because IL-4 is an important mediator in the pathogenesis of allergy and asthma.

Based on theoretic analysis of cytokine biology, Zagury et al. (35) have suggested that the antibodies against cytokines neutralize local increased cytokine activity in pathological tissues, but have little effect during normal physiological conditions. This assessment is chiefly based on the fact that normal tissues exhibit negligible lymph turnover flow, and cytokines work over a short distance between membranes through the immune synapse; therefore, circulating cytokine antibodies do not impair homeostatically regulated local cytokine communication in normal tissues. This is strongly supported by documented animal experiments and human clinical trials (20, 21), as well as the observation in the present study where mice immunized with IL-4 vaccine behaved normally and had no visible pathological changes in any major organs (data not shown). The safety of targeting IL-4 has also been reviewed by Steinke (11), and no manifestation of any major concerns is found, such as defense against parasitic diseases, maintaining the state of immune privilege, stimulating B-cell proliferation and producing Th2 response, when IL-4 signaling was inhibited or blocked with IL-4 mutein, soluble receptor, and mAb. This is supported by the absence of adverse effects in phase I or II clinical trials in which various antagonists were used to down-regulate IL-4 (11). Another concern could be the emergence of type III hypersensitivity reactions. We make the point that, in this current study, the autoantibodies only specifically target the 14 amino acid peptide of IL-4 and that PubMed BLAST searching indicates there is no known similar sequence in other

molecules. This should reduce the risk of inducing epitope spreading that may begin with molecular mimicry and the formation of immune complex.

In summary, we demonstrated that the administration of cytokine peptide-based virus-like particle vaccines

might be an effective therapeutic approach to treat allergic asthma and, by extension, other diseases where over-expressed cytokines play a pivotal role in pathogenesis.

References

1. Liossis SN, Tsokos GC. Monoclonal antibodies and fusion proteins in medicine. *J Allergy Clin Immunol* 2005;**116**:721–729; Quiz 730.
2. Prete M, Perosa F, Favoino E, Dammacco F. Biological therapy with monoclonal antibodies: a novel treatment approach to autoimmune disease. *Clin Exp Med* 2005;**5**:141–160.
3. Holgate ST, Chuchalin AG, Hebert J, Lotvall J, Persson GB, Chung KF et al. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (omalizumab) in severe allergic asthma. *Clin Exp Allergy* 2004;**34**:632–638.
4. Tying S, Gottlieb A, Papp K, Gordon K, Leonardi C, Wang A et al. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet* 2006;**367**:29–35.
5. Sandborn WJ. New concepts in anti-tumor necrosis factor therapy for inflammatory bowel disease. *Rev Gastroenterol Disord* 2005;**5**:10–18.
6. Humbert M, Durham SR, Ying S, Kimmitt P, Barkans J, Assoufi B et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against “intrinsic” asthma being a distinct immunopathologic entity. *Am J Respir Crit Care Med* 1996;**154**:1497–1504.
7. Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G et al. Expression of IL-4 and IL-5 mRNA and protein product by CD4⁺ and CD8⁺ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1997;**158**:3539–3544.
8. Kabesch M, Schedel M, Carr D, Woitsch B, Fritsch C, Weiland SK et al. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. *J Allergy Clin Immunol* 2006;**117**:269–274.
9. Hytonen AM, Lowhagen O, Arvidsson M, Balder B, Bjork AL, Lindgren S et al. Haplotypes of the interleukin-4 receptor alpha chain gene associate with susceptibility to and severity of atopic asthma. *Clin Exp Allergy* 2004;**34**:1570–1575.
10. Shi HZ, Deng JM, Xu H, Nong ZX, Xiao CQ, Liu ZM et al. Effect of inhaled interleukin-4 on airway hyperreactivity in asthmatics. *Am J Respir Crit Care Med* 1998;**157**:1818–1821.
11. Steinke JW. Anti-interleukin-4 therapy. *Immunol Allergy Clin North Am* 2004;**24**:599–614 vi.
12. Perkins C, Wills-Karp M, Finkelman FD. IL-4 induces IL-13-independent allergic airway inflammation. *J Allergy Clin Immunol* 2006;**118**:410–419.
13. Henderson WR Jr, Chi EY, Maliszewski CR. Soluble IL-4 receptor inhibits airway inflammation following allergen challenge in a mouse model of asthma. *J Immunol* 2000;**164**:1086–1095.
14. Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett* 1990;**276**:172–174.
15. Hopp TP, Woods KR. A computer program for predicting protein antigenic determinants. *Mol Immunol* 1983;**20**:483–489.
16. Wang H, Mao X, Simons FER, Peng Z. Induction of IgE responses using a recombinant mosquito salivary allergen rAed 2 without adjuvant in mice. *Int Arch Allergy Immunol* 1999;**120**:135–140.
17. Ford JG, Rennick D, Donaldson DD, Venkayya R, McArthur C, Hansell E et al. IL-13 and IFN-gamma: interactions in lung inflammation. *J Immunol* 2001;**167**:1769–1777.
18. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG et al. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;**156**:766–775.
19. Eisenbarth SC, PIgG2aott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;**196**:1645–1651.
20. Gringeri A, Musicco M, Hermans P, Bentwich Z, Cusini M, Bergamasco A et al. Active anti-interferon-alpha immunization: a European-Israeli, randomized, double-blind, placebo-controlled clinical trial in 242 HIV-1-infected patients (the EURIS study). *J Acquir Immune Defic Syndr Hum Retrovirol* 1999;**20**:358–370.
21. Gonzalez G, Crombet T, Catala M, Mirabal V, Hernandez JC, Gonzalez Y et al. A novel cancer vaccine composed of human-recombinant epidermal growth factor linked to a carrier protein: report of a pilot clinical trial. *Ann Oncol* 1998;**9**:431–435.
22. Rohn TA, Jennings GT, Hernandez M, Grest P, Beck M, Zou Y et al. Vaccination against IL-17 suppresses autoimmune arthritis and encephalomyelitis. *Eur J Immunol* 2006;**36**:2857–2867.
23. Uyttenhove C, Arendse B, Stroobant V, Brombacher F, Van Snick J. Development of an anti-IL-12 p40 auto-vaccine: protection in experimental autoimmune encephalomyelitis at the expense of increased sensitivity to infection. *Eur J Immunol* 2004;**34**:3572–3581.
24. Uyttenhove C, Van Snick J. Development of an anti-IL-17A auto-vaccine that prevents experimental autoimmune encephalomyelitis. *Eur J Immunol* 2006;**36**:2868–2874.
25. Chackerian B, Lenz P, Lowy DR, Schiller JT. Determinants of autoantibody induction by conjugated papillomavirus virus-like particles. *J Immunol* 2002;**169**:6120–6126.
26. Pumpens P, Grens E. HBV core particles as a carrier for B cell/T cell epitopes. *Intervirology* 2001;**44**:98–114.
27. Milich DR, Chen M, Schodel F, Peterson DL, Jones JE, Hughes JL. Role of B cells in antigen presentation of the hepatitis B core. *Proc Natl Acad Sci U S A* 1997;**94**:14648–14653.
28. Oliveira GA, Wetzel K, Calvo-Calle JM, Nussenzweig R, Schmidt A, Birkett A et al. Safety and enhanced immunogenicity of a hepatitis B core particle *Plasmodium falciparum* malaria vaccine formulated in adjuvant Montanide ISA 720 in a phase I trial. *Infect Immun* 2005;**73**:3587–3597.

29. Beck LA, Marcotte GV, MacGlashan D, Togias A, Saini S. Omalizumab-induced reductions in mast cell Fcε receptor 1 expression and function. *J Allergy Clin Immunol* 2004;**114**:527–530.
30. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell Fcε receptor 1 expression. *J Allergy Clin Immunol* 2003;**112**:1147–1154.
31. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004;**305**:1776–1779.
32. Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR et al. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004;**305**:1773–1776.
33. Dabbagh K, Takeyama K, Lee HM, Ueki IF, Lausier JA, Nadel JA. IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo. *J Immunol* 1999;**162**:6233–6237.
34. Venkayya R, Lam M, Willkom M, Grunig G, Corry DB, Erle DJ. The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. *Am J Respir Cell Mol Biol* 2002;**26**:202–208.
35. Zagury D, Le Buanec H, Bizzini B, Burny A, Lewis G, Gallo RC. Active versus passive anti-cytokine antibody therapy against cytokine-associated chronic diseases. *Cytokine Growth Factor Rev* 2003;**14**:123–137.