

A potential immunotherapy approach: Mucosal immunization with an IL-13 peptide-based virus-like particle vaccine in a mouse asthma model

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Abstract

Interleukin (IL)-13 is critical in asthma pathogenesis. Previously, we have developed an IL-13 peptide-based vaccine and confirmed that subcutaneous immunization with the vaccine suppressed airway allergic inflammatory responses in a mouse asthma model. In the present study, we sought to test if mucosal immunization with the vaccine could be a potential approach, by inducing specific autoantibodies of both local IgA in the airway and systemic IgG in serum, to provide an overall suppression of redundant IL-13 effects. The results show that intranasal vaccination induces IL-13-specific IgA responses in multiple mucosal tissues and higher titers of IgG in serum than subcutaneous vaccination. This approach leads to a more effective suppression of ovalbumin-driven Th2 patterns of antibody responses and airway IL-13 and eosinophil accumulation than subcutaneous immunization, even when the induced IL-13 IgG responses were at a similar level. In conclusion, mucosal vaccination may be an innovative potential approach in the treatment of asthma.

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1. Introduction

IL-13 has been well defined as a critical mediator in the pathogenesis of allergic asthma. Its important functions include regulating the recruitment, maturation and activation of inflammatory cells, triggering subepithelial fibrosis, stimulating mucus hypersecretion as well as smooth muscle cell hyperplasia, inducing airway hyperresponsiveness, and switching B cells to IgE synthesis [1]. On the basis of these observations, blocking redundant IL-13 activity is believed to be beneficial for asthma treatment. The efficacy of administering neutralizing antibodies [2] or soluble receptors of IL-13 [3] has been demonstrated in animal models of asthma.

At present, a monoclonal antibody, CAT-354 developed by Cambridge Antibody Technology (Granta Park, Cambridge, UK), is commencing a phase I clinical trial.

However, passively delivered antagonists require repeated administrations with extremely high doses to maintain their efficacy [4], resulting in high costs for patients and possible side effects clinically, such as infusion reactions and the production of antibodies to the therapy agent [5]. The application of vaccine concepts represents a novel approach to the treatment of disorders characterized by maladaptive immuno-regulatory responses, such as allergic and autoimmune diseases [6]. Active immunization with cytokine vaccines may bypass these possible drawbacks and provide a persistent suppression effect. Vaccines targeting a variety of cytokines, such as IL-9 [7], IL-1 β [8] and IL-17 [9], have proven to be effective in animal models. A previous study conducted in our lab has shown that a subcutaneously (s.c.) administered IL-13 peptide-based vaccine suppresses

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airway responses to methacholine challenge, airway inflammation, and goblet cell hyperplasia [10]. Considering the direct effects of IL-13 being released into the airway with regards to the development and perpetuation of asthmatic responses, we speculated that by inducing both mucosal IgA and serum IgG responses to neutralize IL-13, reactions driven by the redundant cytokine would be more effectively suppressed. To test this idea, a vaccine capable of inducing strong autoantibody responses via intranasal (i.n.) administration is required. Hepatitis B virus core antigen (HBcAg) has shown to be highly immunogenic and equipped to promote high titers of antibody, and as such is broadly used as a powerful vaccine carrier for heterogeneous antigens [11]. In addition, the mucosal immunogenicity of full length HBcAg has been explored in mice, showing that i.n. administration elicits a higher serum IgG response and a superior mucosal IgA response as compared to other mucosal and parenteral administrations [12]. Further, HBcAg demonstrates an adjuvant effect when co-administrated i.n. with other antigenic proteins [13]. Altogether, HBcAg presents as an ideal candidate carrier for inducing antibodies via the mucosal route.

It has been reported that i.n. administration to mice of recombinant full length HBcAg with influenza virus M2 peptide fused to N terminus provided 90–100% protection against a lethal virus challenge [14]. Furthermore, when C terminus truncated HBcAg (amino acids 1–149) with an N terminal fused foreign peptide was administered i.n., antibody responses to both carrier and foreign peptide were induced, although the responses were of low intensity [15]. In the current study, using the same truncated HBcAg, but with a self IL-13 peptide inserted into the immunodominant epitope, we demonstrated for the first time that HBcAg can be an attractive vaccine carrier via the i.n. route, able to induce high-titer autoantibodies even without conventional adjuvant. Using an acute asthma model, we discovered that i.n. vaccination (which induced both systemic IgG and mucosal IgA autoantibody responses to IL-13) appeared to be more effective than subcutaneous (s.c.) vaccination in suppressing airway inflammation.

2. Material and methods

2.1. Preparation of the recombinant proteins

The recombinant plasmids pHbAg (expressing the carrier-modified HBcAg protein), and pHbAg-La (expressing the vaccine recombinant HBcAg with the IL-13 peptide La, ¹²LTLKELIEELSNTIQ²⁶, inserted into the immunodominant epitope) were transformed into *Escherichia coli* DH₅ α cells. The expression of HBcAg or HBcAg-La was induced with 1 mM IPTG, and analyzed by SDS-PAGE and Western-blotting using a polyclonal biotinylated goat anti-mouse IL-13 antibody purchased from R&D Systems, Inc. (Minneapolis, MN). Induced cells were harvested, resuspended in phosphate-buffered saline (PBS) (0.02 M

phosphate buffer, 0.15 M NaCl, pH 7.2), and lysed by ultrasonication. Ammonium sulfate with 40% saturation was added to the supernatants of the lysates after centrifugation. The precipitate was washed with 20% saturation ammonium sulfate in PBS twice and resuspended and dialyzed with PBS. Two milliliters of the cleared solution were loaded on a sucrose gradient of six layers from 60 to 10% (2 ml for each, except 0.5 ml for 10%), and centrifuged at 36,000 rpm for 4 h at 20 °C (Beckman Inc., rotor: SW40Ti). Twelve 1 ml fractions of the gradient were collected from top to bottom, and the presence of virus-like particles was assayed by SDS-PAGE and identified by electron microscopy technology. Fractions containing particles were purified further by a combined chromatography procedure consisting of Sepharose CL-4B (Sigma–Aldrich, St. Louis, MO) and Macro-Prep Ceramic Hydroxyapatite (Bio-Rad Laboratories Canada Ltd., Mississauga, Ontario) The main protein peaks were collected and used for immunization.

2.2. Mice

Female BALB/c mice (7–8 weeks old) purchased from Charles River Laboratories (Saint-Constant, Quebec, Canada) were maintained at the Central Animal Care Services, University of Manitoba. All protocols used were approved by the University Animal Ethics Committee.

2.3. Protocols

Experiments were performed according to the protocols shown in Fig. 1. No adjuvant was used in these experiments. Experiment 1: Protocol I was used for comparing immune response features of different immunization routes. Four groups of mice were used, each containing four individuals. Group A: immunized s.c. twice with 100 μ g vaccine, and then followed by one i.n. immunization with 30 μ g vaccine; Group B: immunized s.c. with 100 μ g vaccine three times; Group C: immunized i.n. with 30 μ g vaccine three times; Group D: immunized s.c. with 30 μ g vaccine three times.

Experiment 2: Antibody response levels induced i.n. with different vaccine doses were assessed (shown as protocol

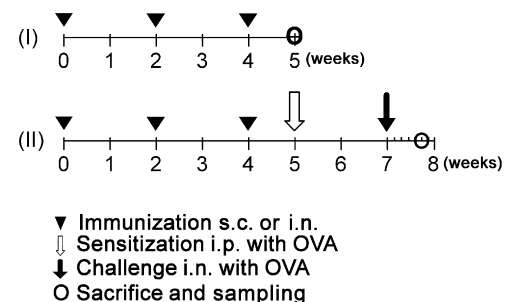


Fig. 1. Vaccine immunization and OVA sensitization/challenge protocols.

D). Twelve mice were divided evenly into three groups and administered 15, 30 or 60 μg of vaccine.

Experiment 3: Protocol II was used for assessing vaccine efficacy in an asthma model. Three groups of mice ($n=6/\text{group}$) were i.n. administered 30 μg vaccine, or carrier or PBS as controls, in a total volume of 30 μl (15 μl for each nostril). A fourth group ($n=6$) was vaccinated s.c. with 100 μg of vaccine. One week after the final immunization, mice were sensitized by an intraperitoneal injection of 2 μg ovalbumin (OVA) (Sigma–Aldrich, grade IV) absorbed to 2 mg $\text{Al}(\text{OH})_3$ adjuvant (Pierce, Rockford, IL), and followed by i.n. challenge 2 weeks later with droplet application of 50 μg OVA in 40 μl PBS.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Measurements for IL-13-specific IgA and IgG antibodies were performed successively by coating microplates with mouse IL-13 (1 $\mu\text{g}/\text{ml}$), adding serum or lavage fluid samples, incubating with alkaline phosphatase-conjugated rabbit anti-mouse IgA or IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and adding the substrate to develop the reaction. Titers were detected with two-fold serially diluted sera, and determined by the dilution multiple of serum at which the OD value was 2.1-folds higher than that of 1:10 diluted control serum from mice immunized with carrier particles.

Serum levels of HBcAg, IL-13, and OVA-specific IgG1 and IgG2a were also measured using ELISAs. Briefly, the 96 well microtiter plates were coated with 50 μl of HBcAg or IL-13 or OVA at 2 $\mu\text{g}/\text{ml}$ at 4 °C overnight. After serum samples were added and incubated at room temperature for 2 h, either biotinylated goat anti-mouse IgG1 or IgG2a (PharMingen, San Diego, CA) was used as a secondary antibody, and the reaction developed with an avidin–phosphatase system.

The concentration of IL-13 in the bronchoalveolar lavage fluid (BALF) supernatant was assessed with ELISA per-

formed using paired capture and biotinylated detection antibodies purchased from PharMingen according to supplier's instructions.

2.5. Sample collection and differential cell counts in BALF

At the endpoint of experiment, mice were anaesthetized and blood samples were obtained by cardiac puncture. Lung lavage was collected with 1 ml of PBS, flushing three times. Vagina and colon were collected and rinsed with 1 ml PBS. After centrifugation, supernatants were kept for IgA measurement. Differential cell counts in BALF were performed by counting a minimum of 400 cells on cytocentrifuged preparations (Cytospin, Shandon, Runcorn, UK) stained with HEMA 3 (Fisher Diagnostics, Pittsburg, PA), and differentiated by standard hematological procedures.

2.6. Statistical analysis

The significance of differences between experimental groups was analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test (GraphPad Prism 3.03, GraphPad Software, Inc., San Diego, CA). Values are reported as the mean $\pm$ standard errors of the mean.

3. Results

3.1. Identification of recombinant fusion protein HBcAg-La

The recombinant protein HBcAg-La was expressed efficiently in *E. coli* cells (Fig. 2A). This recombinant protein specifically reacted to polyclonal rabbit anti-mouse IL-13 antibodies in Western-blotting (Fig. 2B). Analyzed by

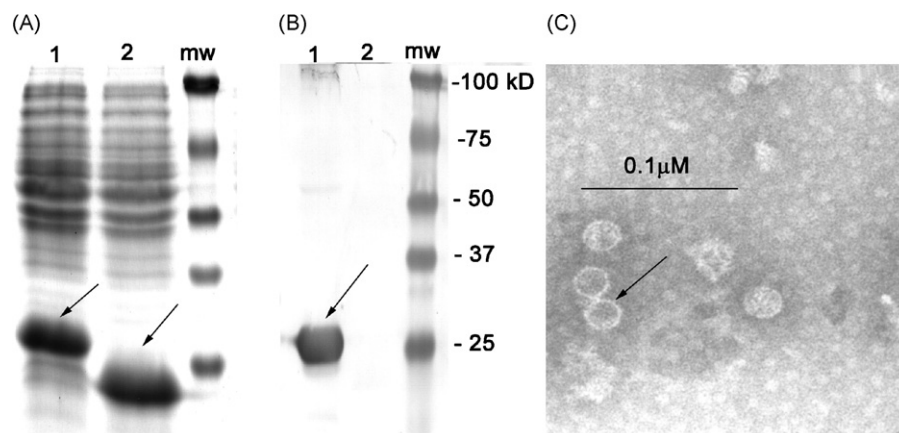


Fig. 2. Identification of the recombinant protein HBcAg-La. (A) The expression of recombinant protein HBcAg-La was identified using SDS-PAGE, and (B) the specificity of the recombinant protein was examined using Western-blotting with a polyclonal anti-IL-13 antibody. Arrows point to recombinant proteins. Lane 1: the vaccine HBcAg-La; lane 2: the carrier HBcAg. (C) HBcAg-La was presented as capsid-like particles with a diameter of 25 nm as indicated by the arrow under an electron microscope.

SDS-PAGE, the HBcAg-La and wild type HBcAg had an identical pattern on sucrose gradient centrifugation, i.e. both of the recombinant proteins presented mostly in the collected fractions 6–8. This indicated that the recombinant protein HBcAg-La assembled into particles similar to native HBcAg. The particle structure was further identified with electron microphotographic techniques (Fig. 2C). After combined chromatography procedures, purified proteins HBcAg and HBcAg-La were obtained with purity exceeding 95%.

3.2. IgA and IgG responses to IL-13

A previous study of ours has shown that our vaccine can induce high titers of IgG autoantibodies to IL-13 by s.c. administration, even without adjuvant [10]. In the present study, we investigated if i.n. immunization could also induce high titers of serum IgG, in combination with an IgA response on the mucosal surface. As shown in Fig. 3A, following two s.c. immunizations, one single i.n. treatment induced a pronounced IgA response. Furthermore, we found that three i.n. administrations induced both high IgA and IgG levels to IL-13. The resulting IgG response was comparable to that produced by three s.c. immunizations, or two s.c. followed by one i.n. vaccination, in which higher doses were used subcutaneously. When given in equal doses, i.n. administration stimulated a higher serum IgG level than s.c. immunization (Fig. 3B). These results indicate that with this vaccine, the i.n. route is more immunogenic than the s.c. route.

To further our knowledge of the immunogenicity of i.n. administration of this vaccine, we used three distinct doses to immunize the mice. IL-13-specific IgA (BALF) and IgG (serum) responses were displayed in a dose dependent manner within the dose range used. It was shown that just 15 μ g of vaccine given i.n. was able to efficiently induce both IgA and IgG responses (Fig. 3C). To investigate the distribution of anti-IL-13 IgA responses in mucosal tissues, IgA levels in the lavage samples collected from lung, vagina and colon were measured with ELISAs, using a sample dilution of 1:5. IgA in serum was also detected at a dilution of 1:50. The results show that i.n. administration with the vaccine induced IL-13-specific IgA production on the mucous surface of all the tissues as well as in serum (Fig. 4A). As expected, s.c. immunization did not induce detectable IL-13-specific IgA (Fig. 4A). During the period of the experiment on protocol II, IL-13 IgG levels induced by i.n. administration were similar to that induced by s.c. immunization (Fig. 4B). At 4 weeks after the last immunization (week 8, protocol II), levels of IgA to IL-13 slightly reduced when compared with those at 1 week after the last immunization (week 5) in the experiment on protocol I using the same vaccine dose (Fig. 4C).

3.3. Analysis of IgG₁ and IgG_{2a} responses

Carrier-specific IgG₁ and IgG_{2a} response profiles (were analyzed), with regards to different administration routes,

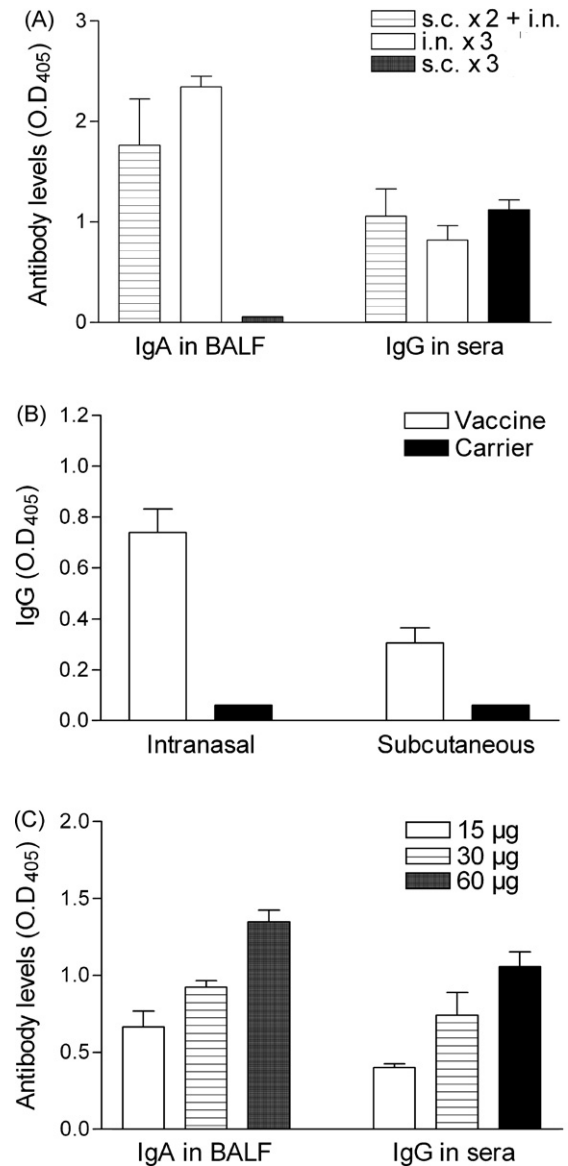


Fig. 3. Intranasal immunization induced both IL-13-specific IgA in BALF and IgG in serum and was more immunogenic than s.c. immunization. Protocol I as shown in Fig. 1 was used for immunization on mice. IL-13-specific IgA and IgG responses were measured with ELISAs. BALFs were diluted at 1:5, and sera were diluted at 1:10,000. (A) (Protocols) IgA and IgG responses with i.n. administration of 30 μ g vaccine or s.c. administration of 100 μ g vaccine. (B) Serum IgG response comparison between i.n. and s.c. vaccination groups at the same vaccine dose (30 μ g). (C) IgA and IgG antibody responses to different vaccine doses with i.n. administration.

autoantibodies to inserted IL-13 epitopes, and following OVA sensitization and challenge. Serum samples were collected 1 week after the second and last immunizations, as well as at the endpoint of the experiment on protocol II (Fig. 1). The titers of carrier- or IL-13-specific IgG₁ and IgG_{2a} in serum were analyzed using ELISAs. Calculating the ratios of IgG₁ to IgG_{2a} based on detected titers, the results show that the HBcAg-specific IgG₁ response is approximately three times that of the IgG_{2a} responses in the carrier group; the ratios of HBcAg

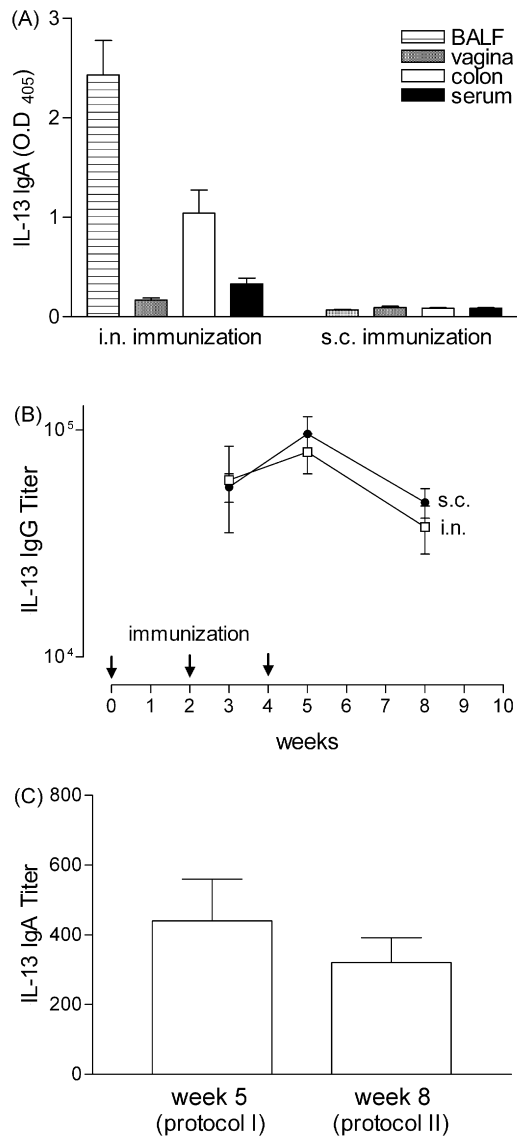


Fig. 4. Distribution and kinetic responses of IL-13-specific antibodies. (A) IL-13 IgA responses in a variety of mucosal tissues were measured with ELISAs. Samples were collected from Experiment 2 (protocol I, dose 30 μ g). Lavage fluids were diluted at 1:5 and endpoint sera were diluted at 1:50. (B) Serum IgG kinetic responses in the experiment on protocol II. (C) The IL-13-specific IgA levels in BALF at week 5 (protocol I) and week 8 (protocol II).

IgG₁/IgG_{2a} in the i.n. vaccinated group and the s.c. vaccinated group decreased significantly as compared to those in the carrier group (Fig. 5A). There were no significant differences in the HBcAg- and IL-13-specific IgG₁/IgG_{2a} ratios between the i.n. and s.c. vaccinated groups, and patterns observed were similar (Fig. 5A and B). Furthermore, the following OVA sensitization and challenge did not produce notable effects on these patterns of antigen specific responses (Fig. 5A and B).

To investigate if immunization with carrier or vaccine, or vaccination route itself, will produce differing effects on

the OVA-stimulated immunological responses, OVA-specific IgG₁ and IgG_{2a} were measured using ELISAs. Endpoint serum samples were collected from the experiment on protocol II (Fig. 1). The results show that OVA-stimulated Th2 biased responses as expected. The i.n. immunization with either vaccine ($n=6$) or carrier ($n=6$) slightly increased the OVA-IgG₁ responses as compared to PBS ($n=6$) but no significance was reached. Vaccine administration significantly raised the OVA-IgG_{2a} levels when compared with carrier ($p<0.05$) and PBS ($p<0.01$), and carrier also showed higher OVA-IgG_{2a} levels than PBS (Fig. 5C) ($p>0.05$). In addition, s.c. immunization slightly suppressed the OVA-IgG₁ responses and raised the OVA-IgG_{2a} responses (Fig. 5C). Calculated on the detected titers, the ratios of OVA-specific IgG₁ to IgG_{2a} in mice that received i.n. vaccination and s.c. vaccination were significantly decreased as compared to those in PBS group ($p<0.01$ and $p<0.05$, respectively). The ratio in the i.n. vaccine group was also reduced noticeably as compared to those in the i.n. carrier group, although statistical significance was not reached. Likewise, i.n. administration of carrier reduced the IgG₁/IgG_{2a} ratio as compared to PBS group, but with no statistical significance (Fig. 5D).

3.4. IL-13 level in BALF

To examine if i.n. immunization with the vaccine provided a more effective downregulation of IL-13 levels in airway as compared to s.c. vaccination, BALF was collected and analyzed with ELISA (Fig. 6). The results show that the mean level of IL-13 in the i.n. group (9.05 ± 2.30 pg/ml, $n=6$) was suppressed significantly when compared with those in either the carrier group (100.5 ± 26.38 pg/ml, $n=6$) ($p<0.05$) or the PBS group (136.6 ± 24.00 pg/ml, $n=6$) ($p<0.01$). Subcutaneous vaccination also reduced the IL-13 level (46.14 ± 16.92 pg/ml, $n=6$) when compared with that of PBS group ($p<0.05$). As well, this level in the s.c. group was noticeably higher than that found in the i.n. vaccine group, and lower than in the i.n. carrier group, although statistical significance was not reached.

3.5. Eosinophil accumulation in BALF

To assess the effects of neutralizing excessive IL-13 with i.n. immunization on the recruitment and accumulation of inflammatory cells in airway, differential BALF cell counts were performed. The percentages of each cell type (eosinophils, neutrophils, monocytes and lymphocytes) are shown in Fig. 7. Eosinophil percentage was significantly reduced in the i.n. vaccine group when compared with either the carrier group ($p<0.001$) or the PBS group ($p<0.001$), and prominently lower than the s.c. vaccine group, although statistical significance was not reached. The s.c. vaccine group presented significant suppression as compared to PBS group ($p<0.05$).

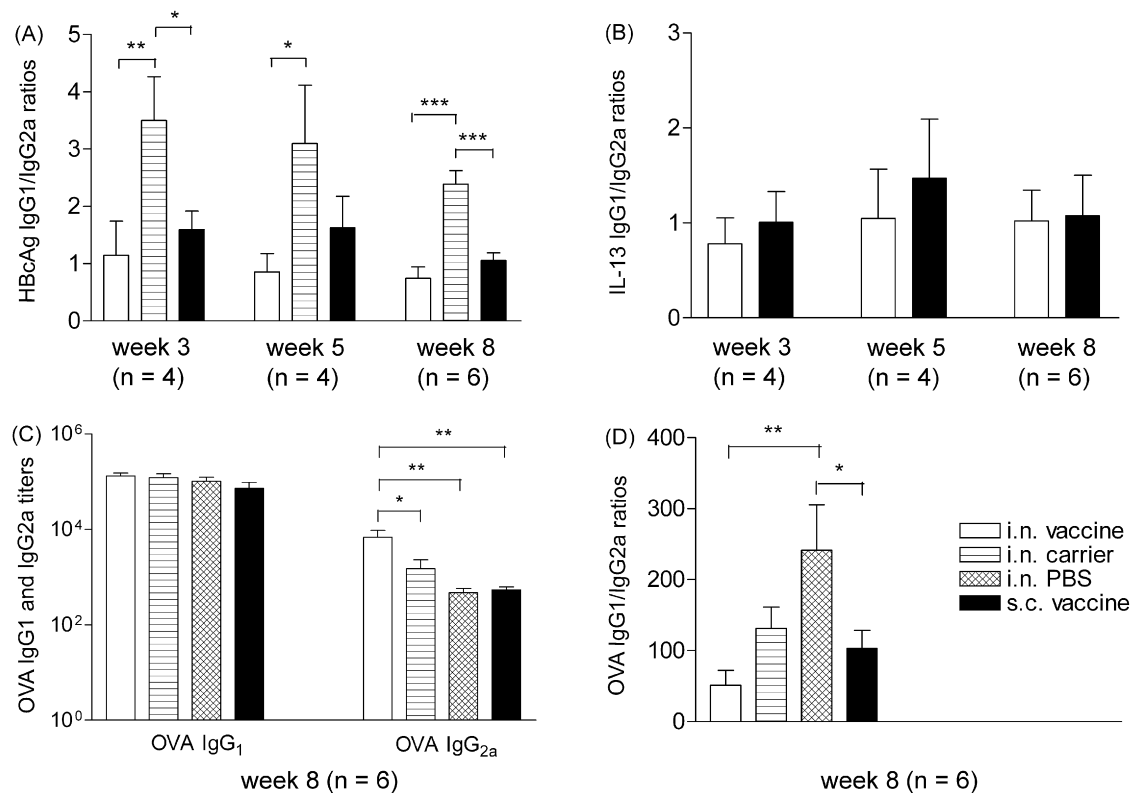


Fig. 5. Ratios and levels of specific IgG1/IgG2a in serum. (A) HBcAg IgG1/IgG2a, (B) IL-13 IgG1/IgG2a, (C) OVA-IgG1 and -IgG2a levels, and (D) OVA IgG1/IgG2a were analyzed. The ratios of IgG1/IgG2a were calculated based on the titers measured with ELISAs. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

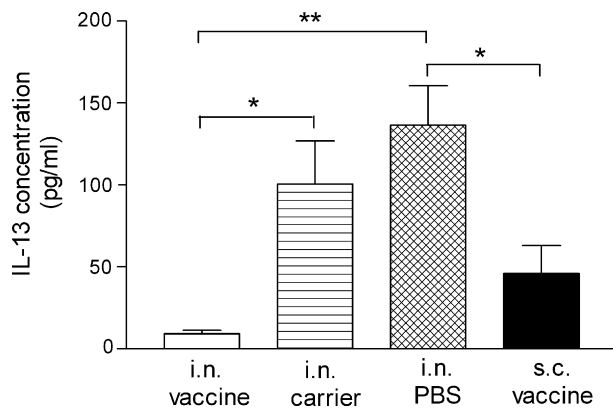


Fig. 6. IL-13 levels in BALF were reduced significantly in the i.n. vaccinated group. * $P < 0.05$; ** $P < 0.01$; $n = 6$.

4. Discussion

Targeting to IL-13 has been demonstrated as a promising approach for the treatment of asthma in a mouse model [2,3,10,16]. We provide here a proposition that, based on active immunization, producing mucosal IL-13-specific IgA in the airway may provide supplemental benefits in the suppression of asthmatic symptoms. As it is known, in the process of the development of asthmatic responses many types of immune cells in airway, such as CD4⁺ T cells, NK cells, mast cells, basophils and eosinophils, produce IL-

13. Elevated IL-13 level in airway exacerbates inflammation, as it regulates the expression of chemokines and vascular cell adhesion molecule-1, thus enhancing the recruitment of a variety of inflammatory cells [17–19]. Hence, suppression of IL-13 activity locally in the airway would reduce the accumulation of these cells and inhibit the subsequent inflammation driven cascades. Reported data reveal that IL-13 can also trigger asthmatic features via its direct effects on airway epithelial [20] and smooth muscle cells [21]. Direct administration of recombinant IL-13 leads to airway inflammation, hyperresponsiveness (AHR), and increased mucus

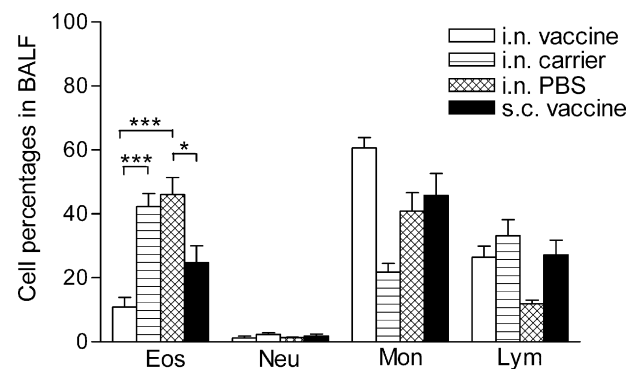


Fig. 7. Eosinophil accumulation in BALF was suppressed significantly in the i.n. vaccinated group. Eos, eosinophil; Neu, neutrophil; Mon, monocyte; Lym, lymphocyte. * $P < 0.05$; *** $P < 0.001$.

production [22,23]. These observations indicate that the accumulation and actions of IL-13 in the airway are significant contributors to asthmatic responses. Taken together, extra suppression of the effects of IL-13 locally in the airway holds a potential to be more effective than conventional systemic administration of IL-13 antagonists.

To test this idea, a vaccine consisting of a self IL-13 peptide and HBcAg particle was investigated using an i.n. immunization route. In many cases, it is not easy to induce specific IgA production, and adjuvant is required to improve immune response. It is even more complex in this study as autoantibodies are pursued, requiring immunological tolerance to be bypassed. We chose to employ HBcAg as a vaccine carrier for the self peptide due to its unique immunological and structural features [24], and its wide and successful use as a carrier for foreign antigen [11,25] and even a self peptide [26], eliminating the need for adjuvant. The highly immunogenic features of HBcAg are attributed to its well defined Th epitopes, its highly ordered repetitive epitope arrays on the particle surface, and its ability to stimulate a high frequency of naive B cells to function as primary antigen-presenting cells, showing a 10^5 -fold higher efficiency than typical dendritic cell-macrophage interactions [27]. This activation of B cells was observed to be mediated through the binding between residues 76–78 of HBcAg and a linear motif present in the FR1-CDR1 junction of the heavy and light chain of the B cell surface receptor [28]. With the purpose of improving immunogenicity, we chose to insert the self peptide into the immunodominant epitope of HBcAg, instead of replacing the original sequence.

The mucosal immunogenicity of HBcAg and its application as a vaccine carrier via the mucosal route have been rarely reported. We present here that i.n. immunization with the constructed vaccine elicited both IL-13-specific IgA and IgG responses. These results differ from those reported by Francis et al. that antibodies to carrier and foreign peptide were only induced at a low level [15]. As in ours, their experiment utilized truncated HBcAg as a carrier, and the vaccine was administrated via the i.n. route. In contrast, Lobaina et al. demonstrated that full length HBcAg strongly stimulated both IgA and IgG antibody responses to HBcAg itself by i.n. immunization [12], indicating the importance of particle incorporated RNA in immunogenicity [29]. Considering our very positive results, and that the foreign epitope was inserted at the N terminal domain of HBcAg in Francis's study [15] rather than in the immunodominant epitope as in ours, we deduced that the well-exposed and highly ordered array of antigenic epitopes inserted in the immunodominant region is a critical factor in the induction of high intensity antibody responses. In the current study, we have demonstrated that HBcAg is a potent vaccine carrier not only for foreign antigen, but also for self antigen. In our lab, several foreign and self peptides have been inserted into HBcAg, and interestingly, we found that the response intensity induced by self peptides was comparable to that elicited by foreign peptides. Moreover, the autoantibodies were maintained at a

high level for at least 6 months [10]. The explanation for this induction of high-level autoantibody is several folds. Firstly, the vaccine itself is remarkably immunogenic due to the selection of a highly immunogenic epitope, and the powerful carrier effects provided by HBcAg. Additionally, HBcAg may employ a unique mechanism to break immune tolerance above that of the provision of foreign T cell epitopes. One possibility is that the display of self polypeptides in a highly repeated ordered array on the virus-like particle surface abrogates the ability of the immune system to functionally distinguish between foreign and self [30]. However, the details need to be further elucidated. We also ascertained that the serum IgG intensity induced by i.n. immunization was higher than that of s.c. immunization when the same vaccine dose was used. At a low dose of 15 μ g vaccine without adjuvant, autoantibodies were still induced efficiently, and the response intensity increased alongside the dose. These observations indicate that both administration route and dose influence antibody response intensity, and i.n. administration of a virus-like particle vaccine is a viable approach that is more efficient when compared with s.c. immunization.

Asthma is thought to be primarily driven by biased Th2 inflammatory responses. In this report, we checked the response patterns of antibody subclass to carrier, IL-13 epitope, and OVA. Analyzing the ratio of IgG₁ to IgG_{2a}, we demonstrated that the carrier (truncated HBcAg) induced a light Th2-like response to itself. The reaction to the inserted IL-13 peptides led to a change in the responses to HBcAg; the Th2-biased responses were converted into balanced levels of Th1 and Th2 responses in the mice receiving i.n. or s.c. IL-13 vaccine. The difference of immunization route between the i.n. and s.c. group did not produce any variation in the subclass profile. Furthermore, the following OVA sensitization/challenge did not show any noticeable effect on the established antibody subclass pattern specific to carrier and IL-13 epitope, although OVA itself induced a highly biased Th2 response. This result indicated that i.n. administration of vaccine did not produce a Th2 biased response, and thus did not contribute to the development of asthma symptoms.

Consistent to a previous report, our results show that truncated HBcAg itself induced a slight Th2 type response, in contrast to the Th1 biased response produced by full length HBcAg [29]. This may be attributable to the lack of encapsidated RNA, which has a modulating effect on the immune response [29]. Similar to the reduction of HBcAg-specific IgG₁/IgG_{2a} ratios, we found that both i.n. and s.c. vaccination decreased the ratio of OVA-specific IgG₁ to IgG_{2a} substantially, indicating that the IL-13-specific response suppressed the OVA-driven Th2 biased responses.

In this report, we have demonstrated that the production of IL-13 IgA on the mucous surface provides a more significant reduction of IL-13 level in the airway than induction of systemic IgG alone. This may be beneficial for the suppression of inflammatory cell accumulation in airways, especially that of eosinophils. Airway eosinophilia is considered one of the hallmarks of allergic asthma [31]. Recently, its critical role in

experimental allergic asthma was confirmed by two studies. By using mice with a total ablation of the eosinophil lineage via the deletion of a high affinity GATA-1 binding site in the GATA-1 promoter, Humbles et al. suggested that eosinophils contributed substantially to airway remodeling [32]. Using a different approach, Lee et al. established a mouse model that is specifically devoid of eosinophils by transgenic expression of the diphtheria toxin A chain under control of the eosinophil peroxidase promoter, and reported that AHR could be completely inhibited and mucus hyperplasia partially reduced in the mouse model [33]. Taking eosinophilia in the airways as an important indicator for assessing the efficacy of IL-13-specific IgA responses, we performed differential cell counts in BALF. The results showed that i.n. immunization suppressed eosinophil accumulation in BALF more significantly than s.c. immunization. To examine if carrier administration produced effects on asthma symptoms, we compared the data between mice i.n. immunized with carrier and those given solely PBS. No significant statistical differences were observed, indicating that i.n. immunization with HBcAg alone and any contaminants presenting in the purified protein did not produce a noticeable impact on the scores of asthmatic symptoms. In other words, the statistically significant reduction of asthmatic responses observed in the i.n. vaccine group is predominantly derived from the elicited IL-13-specific IgA and IgG responses. Using an acute asthma model and based on a preventative design, we have made apparent the therapeutic potential of inducing IL-13-specific IgA responses on the mucosal surface in asthma treatment.

In summary, our data have shown evidence that HBcAg is a powerful vaccine carrier for mucosal immunization, even when the induction of autoantibodies is the intent, and that specific IgA production on the mucosal surface may provide additional benefits towards the suppression of disease development, specifically in those cases in which dysfunctions of self molecules on the mucosal surface is involved in the disease pathogenesis.

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References

- [1] Wills-karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004;202:175–90.
- [2] Kumar RK, Herbert C, Webb DC, Li L, Foster PS. Effects of anticytokine therapy in a mouse model of chronic asthma. *Am J Respir Crit Care Med* 2004;170(10):1043–8.
- [3] Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282(5397):2258–61.
- [4] 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(4):632–8.
- [5] Sandborn WJ. New concepts in anti-tumor necrosis factor therapy for inflammatory bowel disease. *Rev Gastroenterol Disord* 2005;5(1):10–8.
- [6] 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(2):123–37.
- [7] Arendse B, Van Snick J, Brombacher F. IL-9 is a susceptibility factor in *Leishmania major* infection by promoting detrimental Th2/type 2 responses. *J Immunol* 2005;174(4):2205–11.
- [8] Bertin-Maghit SM, Capini CJ, Bessis N, Chomilier J, Muller S, Abbas A, et al. Improvement of collagen-induced arthritis by active immunization against murine IL-1beta peptides designed by molecular modelling. *Vaccine* 2005;23(33):4228–35.
- [9] 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(11):2857–67.
- [10] Ma Y, Hayglass KT, Becker AB, Fan Y, Yang X, Basu S, et al. Novel recombinant interleukin-13 peptide-based vaccine reduces airway allergic inflammatory responses in mice. *Am J Respir Crit Care Med* 2007;176(5):439–45.
- [11] Pumpens P, Grens E. HBV core particles as a carrier for B cell/T cell epitopes. *Intervirology* 2001;44(2–3):98–114.
- [12] Lobaina Y, Garcia D, Abreu N, Muzio V, Aguilar JC. Mucosal immunogenicity of the hepatitis B core antigen. *Biochem Biophys Res Commun* 2003;300(3):745–50.
- [13] Lobaina Y, Palenzuela D, Pichardo D, Muzio V, Guillen G, Aguilar JC. Immunological characterization of two hepatitis B core antigen variants and their immunoenhancing effect on co-delivered hepatitis B surface antigen. *Mol Immunol* 2005;42(3):289–94.
- [14] Neirynck S, Deroo T, Saelens X, Vanlandschoot P, Jou WM, Fiers W. A universal influenza A vaccine based on the extracellular domain of the M2 protein. *Nat Med* 1999;5(10):1157–63.
- [15] Francis MJ, Hastings GZ, Brown AL, Grace KG, Rowlands DJ, Brown F, et al. Immunological properties of hepatitis B core antigen fusion proteins. *Proc Natl Acad Sci USA* 1990;87(7):2545–9.
- [16] Yang G, Li L, Volk A, Emmell E, Petley T, Giles-Komar J, et al. Therapeutic dosing with anti-interleukin-13 monoclonal antibody inhibits asthma progression in mice. *J Pharmacol Exp Ther* 2005;313(1):8–15.
- [17] Zhu Z, Ma B, Zheng T, Homer RJ, Lee CG, Charo IF, et al. IL-13-induced chemokine responses in the lung: role of CCR2 in the pathogenesis of IL-13-induced inflammation and remodeling. *J Immunol* 2002;168(6):2953–62.
- [18] Ma B, Liu W, Homer RJ, Lee PJ, Coyle AJ, Lora JM, et al. Role of CCR5 in the pathogenesis of IL-13-induced inflammation and remodeling. *J Immunol* 2006;176(8):4968–78.
- [19] Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol* 1995;154(2):799–803.
- [20] Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, et al. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med* 2002;8(8):885–9.
- [21] Shore SA. Direct effects of Th2 cytokines on airway smooth muscle. *Curr Opin Pharmacol* 2004;4(3):235–40.
- [22] Lanone S, Zheng T, Zhu Z, Liu W, Lee CG, Ma B, et al. Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling. *J Clin Invest* 2002;110(4):463–74.

- [23] Kibe A, Inoue H, Fukuyama S, Machida K, Matsumoto K, Koto H, et al. Differential regulation by glucocorticoid of interleukin-13-induced eosinophilia, hyperresponsiveness, and goblet cell hyperplasia in mouse airways. *Am J Respir Crit Care Med* 2003;167(1):50–6.
- [24] Vanlandschoot P, Cao T, Leroux-Roels G. The nucleocapsid of the hepatitis B virus: a remarkable immunogenic structure. *Antiviral Res* 2003;60(2):67–74.
- [25] Ulrich R, Nassal M, Meisel H, Kruger DH. Core particles of hepatitis B virus as carrier for foreign epitopes. *Adv Virus Res* 1998;50:141–82.
- [26] Jegerlehner A, Tissot A, Lechner F, Sebbel P, Erdmann I, Kundig T, et al. A molecular assembly system that renders antigens of choice highly repetitive for induction of protective B cell responses. *Vaccine* 2002;20(25–26):3104–12.
- [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 USA* 1997;94(26):14648–53.
- [28] Lazdina U, Cao T, Steinbergs J, Alheim M, Pumpens P, Peterson DL, et al. Molecular basis for the interaction of the hepatitis B virus core antigen with the surface immunoglobulin receptor on naive B cells. *J Virol* 2001;75(14):6367–74.
- [29] Riedl P, Stober D, Oehninger C, Melber K, Reimann J, Schirmbeck R. Priming Th1 immunity to viral core particles is facilitated by trace amounts of RNA bound to its arginine-rich domain. *J Immunol* 2002;168(10):4951–9.
- [30] Chackerian B, Lenz P, Lowy DR, Schiller JT. Determinants of autoantibody induction by conjugated papillomavirus virus-like particles. *J Immunol* 2002;169(11):6120–6.
- [31] Epstein MM. Do mouse models of allergic asthma mimic clinical disease? *Int Arch Allergy Immunol* 2004;133(1):84–100.
- [32] 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(5691):1776–9.
- [33] 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(5691):1773–6.