



Efficacy evaluation of Der p 1 DNA vaccine for allergic asthma in an experimental mouse model

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Abstract

Der p 1 is a major allergen that reacts with IgE in more than 70% allergic sera, hence a good candidate for DNA vaccine development. Previous study demonstrated that intramuscular injection of plasmid DNA containing wild type Der p 1 gene induced specific Th1 skewed immune response. The aim of this study is to further optimize the DNA construct to achieve preventive and therapeutic efficacies. An optimized construct, containing mouse Igκ light chain leader sequence followed by codon optimized mature Der p 1 gene, was generated and used to prevent or treat Der p 1 induced allergic asthma in an experimental model. Results showed that this optimized construct was effective in the inhibition of Der p 1 specific IgE levels, attenuation of Th2 response and reduction of airway hyperresponsiveness in terms of prophylactic efficacy. It also showed therapeutic efficacy in IgE inhibition and up-regulation of IgG2a and IFN-γ. The information obtained here could facilitate the design of DNA vaccines for allergy in general.

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1. Introduction

House dust mites are the most important sources of indoor allergens responsible for the development of sensitization and allergic diseases. The prevalence of allergic asthma related to house dust mite is high and keeps increasing worldwide [1]. Der p 1 is a major house dust mite allergen from *Dermatophagoides pteronyssinus*. More than 70% of sera from allergic individuals showed IgE reactivity to Der p 1. It has been suggested that exposure and sensitization by the major mite allergens may facilitate the sensitization to other less prevalent allergens [2]. Therefore, Der p 1 is a potential candidate for DNA vaccine development.

Der p 1 protein is a cysteine protease; its full-length cDNA encodes a precursor of 320 amino acid residues, comprising of a signal peptide (18 amino acids), a proenzyme region (80 amino acids), and a mature enzyme (222 amino acids) [3]. Our previous study demonstrated that intramuscular injection of plasmid DNA containing cDNA encoding mature Der p 1 was sufficient for in vivo expression of Der p 1 and subsequent

induction of specific Th1 skewed immune response in mice [4]. The enzymatic activity of this in vivo expressed Der p 1 could be detrimental to the host [5,6], and thus was abolished by excluding its own pre proenzyme sequences. However, the resultant magnitude of the immune response was too low to achieve satisfactory protective efficacies. In 2003, Bauer et al. showed that optimization of codon usage increased immunogenicity of DNA vaccination of a major plant allergen in mice [13]. In this study, we further demonstrated the use of an efficient mouse Igκ light chain leader sequence in addition to codon usage optimization strategy in improving the preventive as well as therapeutic efficacies of DNA immunization using a Der p 1 induced experimental allergic asthma mouse model. The information obtained from this study could facilitate future designs of DNA vaccine.

2. Materials and methods

2.1. Generation of codon optimized Der p 1 construct

The codon optimized Der p 1 gene was designed based on codon preference of highly expressed human transcripts.

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Briefly, partially overlapping oligonucleotides spanning the mature part of Der p 1 coding sequence were synthesized and used to generate the codon optimized DNA fragment (hmDerp1) in a two-step PCR amplification [7]. A mouse Ig κ light chain signal sequence (m κ) was tagged in-frame to the 5' end of the hmDer p 1 by PCR and subsequently the entire DNA fragment were cloned into the pVax1 expression vector and DNA sequence confirmed. The prophylactic and therapeutic potentials of the resulting construct, pVax1-m κ -hmDerp1 (LHM), were evaluated with Der p 1 induced experimental mouse models.

2.2. Experimental mouse models

A total of 6–8 weeks old female BALB/cJ mice were purchased from Laboratory Animals Centre (Sembawang, Singapore) and housed in the Animal Holding Unit facility in National University of Singapore under institutional guidelines for the care and use of laboratory animals. Immunization was done according to the regimens shown in Fig. 1. DNA immunization was carried out by injection of 50 μ g of LHM (in 50 μ l PBS) intramuscularly (IM) followed by in vivo electroporation using the BTX ECM830

square wave electroporator (BTX, San Diego, CA, USA) [8]. Intraperitoneal (IP) immunization was done by injecting 1 μ g of Der p 1 emulsified with 4 mg alum as adjuvant. Challenge by aerosol inhalation of Der p 1 (50 μ g/ml native Der p 1 in PBS) was carried out using an ultrasonic nebulizer (DeVilbiss Health Care, Somerset, PA). Briefly, the mice were kept unrestrained in a desiccator chamber with continued flow of aerosol for 30 min. Blood samples, taken at day 0 and every week thereafter, were centrifuged at $2000 \times g$ and sera were collected and stored at -20°C for determination of Der p 1 specific immunoglobulin responses.

2.3. Detection of Der p 1 specific mouse immunoglobulin responses

The levels of Der p 1 specific IgE, IgG1, and IgG2a were determined by ELISA. Mouse sera were incubated in duplicate with the Der p 1 coated wells for overnight at 4°C . Biotin-conjugated monoclonal rat anti-mouse IgE (R19-15), anti-mouse IgG1 (G1-1.5) and anti-mouse IgG2a (R35-92) were used for detection and followed by addition of ExtrAvidin-alkaline phosphatase. Signals were developed by

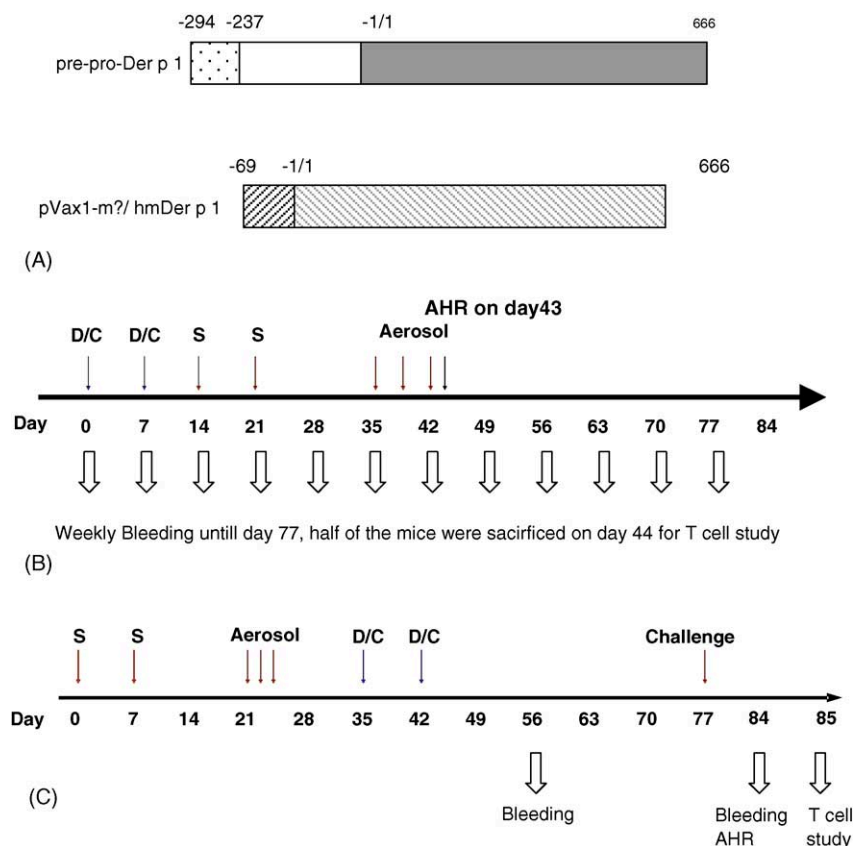


Fig. 1. Schematic representation of wild type Der p 1 gene structure and the codon optimized DNA construct (A), and immunization regimens for evaluation of preventive efficacy (B) and therapeutic efficacy (C). D, DNA immunization. C, PBS control. S, Systemic sensitization with native Der p 1/Alum intraperitoneally. Aerosol, Airway challenge with aerosolized native Der p 1. AHR, measurement of airway hyperresponsiveness. Challenge, systemic challenge with native Der p 1/Alum intraperitoneally.

addition of *p*-nitrophenylphosphate substrate. ELISA index unit was defined as the OD_{405 nm} reading corresponding to the reading of 1 ng/ml of purified mouse IgE, IgG1, or IgG2a in a sandwich ELISA with anti-mouse Igk as the capture antibody in the same plate. All the antibodies used were purchased from Pharmingen (Pharmingen, San Diego, CA).

2.4. Non-invasive measurement of airway hyperresponsiveness

Approximately 24 h after the last challenge, conscious and unrestrained mice were analysed for airway hyperresponsiveness (AHR) to different concentration of methacholine, measured in terms of degree of airway obstruction (PenH) using

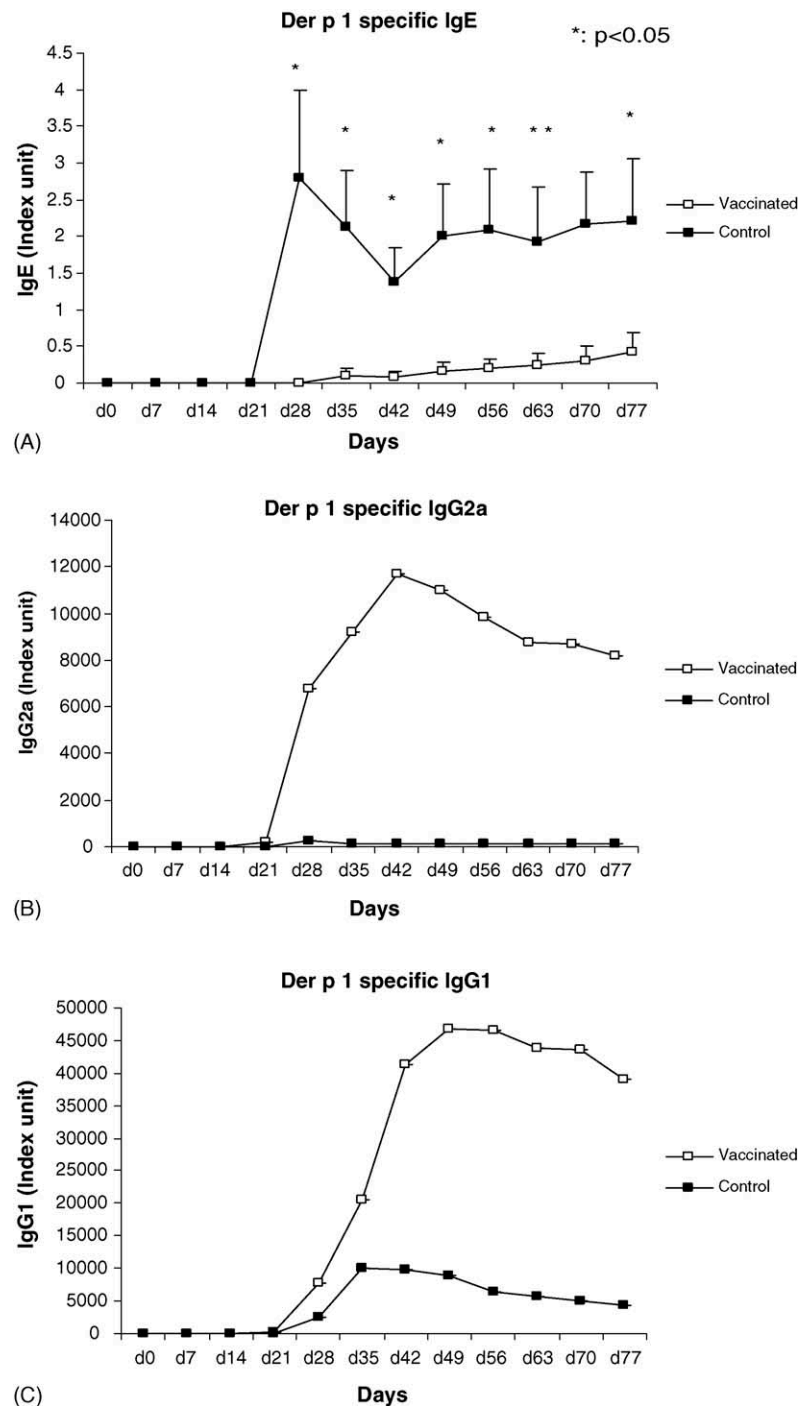


Fig. 2. Prophylactic efficacy on the inhibition of Der p 1 specific IgE. Mice were immunized with codon optimized Der p 1 DNA construct intramuscularly with electroporation on days 0 and 7. Der p 1 sensitization was done by systemic immunization intraperitoneally on day 14 and day 21 followed by three aerosolized Der p 1 challenges from days 35 to 42 (Fig. 1B). Blood samples were collected weekly and the titer of Der p 1 specific IgE, IgG1 and IgG2a in serum was determined by ELISA. Results are shown as Mean $\pm$ S.E.M. * $p < 0.05$.

the whole-body plethysmography (Buxco Electronics Inc., Troy, New York, USA) [9]. Briefly, mice were exposed to an aerosol of PBS (baseline readings) followed by cumulatively increased doses of aerosolized methacholine (5–40 mg/ml). Aerosol was generated by a portable ultrasonic nebulizer (DeVilbiss Health Care, Somerset, PA) and drawn through the chamber for 3 min with the Bias Flow Supply. The signals were recorded for the subsequent 5 min and interval between each dose was 1 min.

2.5. T cell cytokine profiling

Single-cell suspensions were prepared from spleen and red blood cell lysis was performed. Splenocytes (4×10^5

cells/well) were cultured in the presence or absence of 20 μ g/ml of Der p 1 in 96-well round-bottomed plates (Costar, Corning, NY) containing RPMI 1640 medium supplemented with 10% heat-inactivated bovine calf serum (StemCell Technologies Inc.), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin and 100 μ g/ml streptomycin (Hyclone Laboratories, Logan, UT) and 5.5×10^{-2} mM 2-mercaptoethanol (Life technology, Grand Island, NY). After 72 h, culture supernatants were collected, stored at -20°C and assayed in duplicate for the presence of the cytokines IL-4, IL-5, IL-13, IFN- γ , and IL-10. Antibodies pairs for ELISA were purchased from Pharmingen (Pharmingen, San Diego, CA) and used according to manufacturer's instructions.

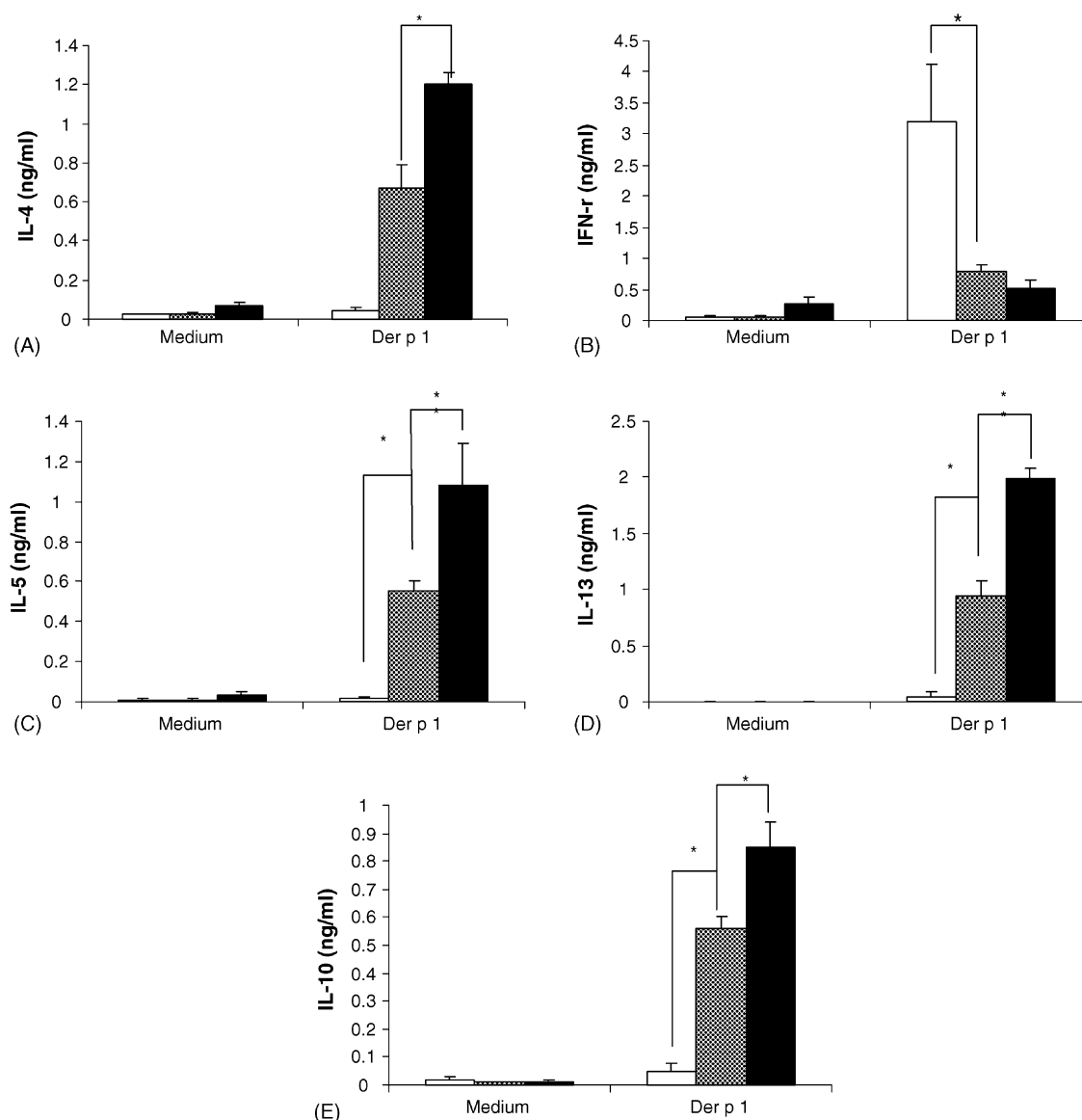


Fig. 3. Prophylactic efficacy on the Der p 1 specific T cell cytokine profiles. Mice were immunized with DNA vaccine and challenged with Der p 1 the same way as described in Fig. 1B (grey bar). Mice that were sensitized with Der p 1 protein alone (black bar), or received DNA vaccination alone (open bar) were included as controls. Two days after the last airway allergen challenge, mice were sacrificed and the splenocytes were cultured in RPMI with or without Der p 1. The concentration of IL-4 (A), IL-5 (C), IL-10 (E), IL-13 (D), and IFN- γ (B) were measured from the culture supernatants with ELISA. * $p < 0.05$.

3. Results and discussion

3.1. Prophylactic evaluation of DNA immunization

Our previous result has shown that the Der p 1 gene without its pre, proenzyme sequence could be expressed in vivo resulting in induction of Der p 1 specific immune responses after intramuscular DNA immunization [4]. However, the induced specific immune responses were insufficient to suppress the development of asthma in the mouse model. Here we aimed to improve prophylactic and therapeutic efficacies of Der p 1 gene vaccination by optimization of codon usage of Der p 1 cDNA sequence to that of highly expressed human genes (Sequence information available upon request). The humanized mature Der p 1 gene was tagged with a strong leader sequence from mouse Ig κ chain, and inserted into pVax1 expression vector (Fig. 1A) The resulting construct, pVax1-m κ -hmDerp1 (LHM), was shown to induce 10-fold higher antibody titers in immunized mice as compared to pCI-neo-Der p 51/Der p 1(1–222), a DNA construct in pCI neo expression vector containing native Der p 1 with a Der p 5 leader sequence. (Data not shown [4].) For the prophylactic efficacy assessment, mice were pre-immunized with LHM construct and challenged intraperitoneally with Der p 1 protein in alum according to the prophylactic regimen shown in Fig. 1B. Der p 1 specific IgE production in DNA vaccinated mice was significantly down-regulated (Fig. 2A); whereas both IgG2a and IgG1 levels were greatly boosted in these mice after intraperitoneal Der p 1/alum challenge (Fig. 2B). These data indicated that Der p 1 gene immunization induced Th1 skewed immune responses, which probably mediated the attenuation of Th2 responses upon subsequent allergen challenge.

Allergen specific Th2 skewed immune response is critical for the development of allergic asthma [10]. Studies have shown that Th2 cytokines also have direct effects on the pathogenesis of this disease [11]. To confirm the suppression

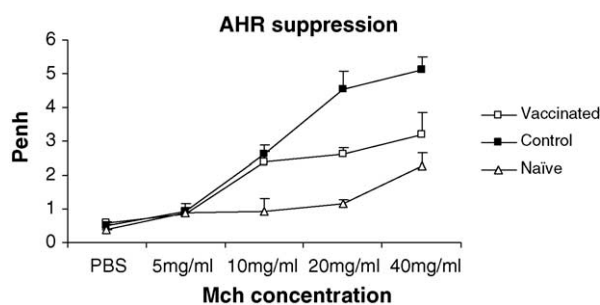


Fig. 4. Prophylactic efficacy on the reduction of airway hyperresponsiveness (AHR). Mice were immunized and challenged the same way as described in Fig. 1B. AHR was measured with whole-body plethysmography 24 h after the last aerosolized allergen challenge. AHR was successfully established in Der p 1 sensitized and challenged mice (control group, filled square). DNA immunization before the Der p 1 sensitization reduced the Penh values significantly (vaccinated group, open square). The differences between vaccinated group and control group are statistically significant at Mch doses 20 and 40 mg/ml. * $p < 0.05$.

of Th2 response by DNA vaccination, we further examined the cytokine profile of spleen T cells upon in vitro stimulation with Der p 1. Mice that were immunized and challenged as shown in prophylactic regimen (Fig. 1B) were sacrificed on day 44 and in vitro culture of splenocytes was carried out with or without Der p 1. As shown in Fig. 3, the levels of Th2 cytokines IL-4, IL-5, IL-10, IL-13 were significantly decreased in DNA vaccinated mice, while the Th1 cytokine IFN- γ levels were boosted. The ratios of IL-4 to IFN- γ were also significantly decreased from 2.5 in control mice to 1

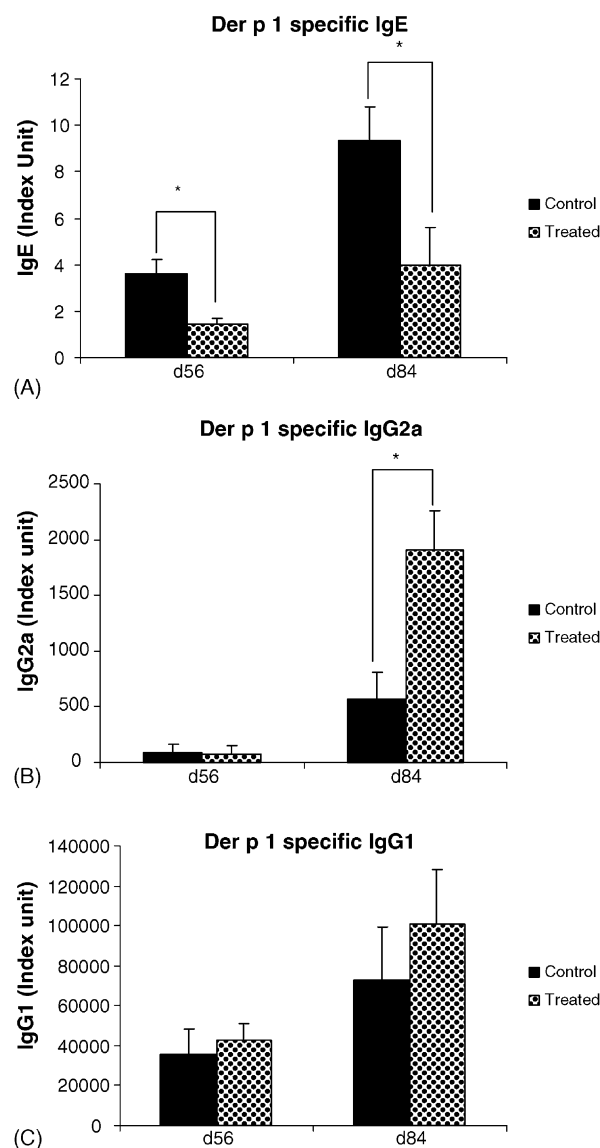


Fig. 5. Therapeutic efficacy on the attenuation of Der p 1 specific IgE. Mice were systemically sensitized by intraperitoneal injection of native Der p 1 intraperitoneally with alum, followed by aerosol allergen challenge on days 21, 22, 23. On days 35 and 42 mice were either treated with DNA immunization (treated, grey bar) or PBS (control, black bar). On day 77, mice were challenged with Der p 1 intraperitoneally again (Fig. 1C). Blood were collected on days 56 and 84 and titers of Der p 1 specific IgE (A), IgG2a (B) and IgG1 (C) were determined by ELISA. Results are shown as Mean $\pm$ S.E.M. * $p < 0.05$.

in DNA vaccinated mice, which confirmed the significant suppression of Th2 responses concurrent with a shift to Th1 response. Furthermore, the airway hyperresponsiveness, which is a hallmark of the asthma symptom, was significantly attenuated in the LHM vaccinated mice (Fig. 4).

3.2. Therapeutic evaluation of DNA immunization

To evaluate the therapeutic effects of DNA vaccination with LHM construct, experimental asthmatic mouse model was established and treated with LHM immunization regimen as shown in Fig. 1C. The Der p 1 specific IgE levels were compared on day 56 and day 84, 2 weeks after the second DNA immunization and 1 week after the final Der p 1 challenge, respectively. At both time points, the Der p 1 specific IgE levels in the LHM treated mice were significantly lower than that in the control group (Fig. 5A). On the other hand, Der p 1 specific IgG2a levels, but not the IgG1 levels, were significantly higher in the vaccinated group on day 84 (Fig. 5B and C). T cell cytokines profiling showed that there was an elevation of IFN- γ levels in DNA treated mice, but there was no significant difference in Th2 cytokines levels (data not shown). The data on airway hyperresponsiveness were inconclusive. Recently, Jarman and Lamb [12] reported significant therapeutic effect of a plasmid DNA encoding T cell epitope of Der p 1 spanning residues 111–139, on the reduction of Th2 cytokines levels and suppression of airway eosinophilia. Vaccine design using T cell epitopes has also been exploited by other groups [14]. However, due to the concern about the complexity and polymorphic nature of human MHC genes, full length Der p 1 gene was used in our vaccine design to achieve a broader spectrum of protective immunity for clinical use. It is also worth noting that unlike the allergy model used by Jarman and Lamb, a strong immunomodulating adjuvant, alum, was used in our study, to establish a strong Th2 biased immune response, which could be more difficult to reverse.

In conclusion, we have demonstrated that codon usage optimization of mature Der p 1 and the use of an efficient mouse Ig κ light chain leader sequence in the DNA vaccine construct could effectively inhibit Der p 1 specific IgE production, down-regulate Th2 cytokines and subsequently attenuate airway hyperresponsiveness in the prophylactic approach. Evaluation of the therapeutic effects of this construct revealed that it was effective in IgE inhibition, IgG2a and IFN- γ up-regulation, but its effect on airway hyperresponsiveness was inconclusive. Further studies using allergy model induced by sensitization protocol without adjuvant in combination with DNA prime-protein boost strategy will be explored.

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