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ORIGINAL PAPER

Novel IgE peptide-based vaccine prevents the increase of IgE and down-regulates elevated IgE in rodents

Z. Peng*†, Q. Liu*‡, Q. Wang*, E. Rector†, Y. Ma* and R. Warrington§†

**Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada, †Department of Immunology, University of Manitoba, Winnipeg, Manitoba, Canada, ‡Shanghai Institute of Biological Products, Shanghai, China and §Department of Internal Medicine, University of Manitoba, Winnipeg, Manitoba, Canada*

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Summary

Background Immunotherapy with anti-IgE antibodies for treatment of allergy is promising but a short half-life and extremely high cost limit its application.

Objective We sought to develop IgE vaccines that induce longer-lasting auto-antibodies to neutralize self-IgE as an alternative therapy.

Methods The vaccine was made by conjugating three synthetic peptides corresponding to human IgE receptor-binding sites to a carrier, hepatitis B surface antigen. To test the immunogenicity of the vaccine, rats were immunized with the vaccine or hepatitis B surface antigen as control. Serum IgG titres to human IgE and the IgE of other species were measured. The inhibition by rat antisera of the binding of human IgE to its receptor was assessed by ELISA, flow cytometry analysis, and passive cutaneous anaphylaxis (PCA), and its ability to recognize receptor-bound IgE was examined. The *in vivo* effect of the vaccine was evaluated in trichosanthin-sensitized mice and rats. In the preventative study, vaccination started before sensitization commenced, while in the treatment study, vaccination started after sensitization. Sensitized mice and rats receiving injections of the carrier served as controls. Trichosanthin-specific IgE was measured using PCA.

Results Sera from vaccine-immunized rats contained high titre antibodies that reacted with soluble and plate-bound but not with receptor-bound human IgE; they also reacted with mouse, rat, and dog IgE. Furthermore, the sera inhibited the binding of human IgE to its receptor in a dose-dependent manner. In preventative and treatment studies, serum trichosanthin-specific IgE levels were significantly reduced in vaccinated groups compared with controls.

Conclusion Antibodies against self-IgE can be induced by IgE peptide-based vaccines, which are effective in preventing the increase of IgE and in down-regulating IgE in sensitized animals.

Correspondence:
Dr Z. Peng, Department of Pediatrics and Child Health, University of Manitoba, 532-715 McDermot Avenue, Winnipeg, Manitoba, Canada R3E 3P4.
E-mail: zpeng@ms.umanitoba.ca

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Introduction

IgE antibodies play a major role in the development and regulation of allergic responses and are directly responsible for the allergic cascade [1]. These allergen-specific IgE antibodies bind to high-affinity receptors for IgE (FcεRI) on the surface of basophils, mast cells, eosinophils, and dendritic cells [2]. Upon re-exposure to the allergen, the allergen binds to the cell-bound IgE, triggering the release of histamine and other inflammatory mediators, causing allergic reactions [1]. Serum IgE levels have been correlated with airway hyper-responsiveness both in adults [3, 4] and in children [4], thus offering IgE as a potential target of therapy for allergy and asthma. For this purpose, a recombinant humanized monoclonal antibody (mAb) to human IgE, Xolair®, formerly named omalizumab or E25, was developed [5]. Administration of the mAb results in significant improvements clinically [6–14]. Treatment with omalizumab reduces plasma-free IgE levels and down-regulates FcεRI expression in basophils, mast cells, and dendritic cells [15, 16], resulting in a significant improvement of symptoms in asthma

especially severe asthma, allergic rhinitis, atopic dermatitis, ocular allergy, and food allergy, and the treatment is also safe and well tolerated [6–14]. However, several significant disadvantages of this agent have limited its use. Owing to the short half-life of the mAb to IgE, frequent injections (every 2–4 weeks) are required to maintain their effects, because clinical benefit rapidly disappears upon discontinuation of treatment. In addition, the therapy is extremely costly.

Active immunization using IgE vaccines to down-regulate IgE levels has the potential of being less expensive and of longer duration. Studies have demonstrated that if a self-protein is modified by inserting a foreign peptide or *is* coupled to a foreign protein, the self-component is recognized as 'foreign' by the immune system of the host and antibodies against self-epitopes are generated. Vaccination of ovalbumin-sensitized rats with rat IgE constant domain Cε2 and/or Cε3 (the location of the receptor-binding sites), coupled or fused to a heterogonous carrier protein, resulted in a profound decrease (90%) in serum IgE [17–19]. This strategy *was* also effective in the down-regulation of IgE levels in the dog [20]. However, in these studies, a large section of the IgE molecule was used to link to a carrier protein, forming a vaccine. The polyclonal antibodies induced by the vaccine may cross-react with other immunoglobulins containing the same epitopes. This is a particular concern if this strategy is to be used in humans.

In the present study, we developed an IgE peptide-based vaccine, in which small peptides (8–16 amino acids each) corresponding to human IgE receptor-binding sites are coupled to the carrier protein hepatitis B surface antigen (HBsAg). We investigated the *in vitro* effect of the antibodies induced by the vaccine in blocking the binding of human IgE to its high-affinity receptor FcεRI and the cross-reactivity of the antibodies with IgE of other species. Because the rat antibodies induced by the human IgE peptide-based vaccine cross-reacted with mouse and rat IgE, we further explored the *in vivo* effect of the vaccine in down-regulating IgE responses in rodents.

Methods

Animals

Young male Wistar rats (approximately 200 g) and female BALB/c mice (6–7 week old), used for preparation of antisera, *in vivo* studies, and passive cutaneous anaphylaxis (PCA) tests, were obtained from Shanghai Experimental Animal Centre of Chinese Academy of Sciences under the license from the home office in accordance with the Animal (Scientific Procedures) Act 1986. Male Wistar rats (approximately 500 g) used for PCA inhibition tests were obtained from the Central Animal Care Services, University of Manitoba, and handled by methods approved by the Animal Care and Use Committee at the University of Manitoba.

Preparation of a human IgE peptide-based vaccine and Novasome adjuvant

The vaccine was made by conjugating three peptides corresponding to human IgE receptor-binding sites (Table 1) to a carrier protein HBsAg. Briefly, in a preliminary experiment, several small peptides were selected from the known human IgE receptor-binding sites of IgE and from studies of synthetic human IgE peptide inhibition of basophil histamine release [17, 21–26]. These peptides were chemically synthesized by SBS Biotech Corp. (Beijing, China). Each peptide was coupled to the carrier HBsAg using the glutaraldehyde method [27] to form a vaccine conjugate. Normal mice were then immunized with each vaccine and mouse antisera were tested for their antibody titres to human IgE by ELISA. Among these vaccines, three induced high titre antibodies to human IgE. The three peptides were then selected for the preparation of the human IgE vaccine used in this study.

The characteristics of the three peptides are shown in Table 1. Coupling of all three peptides to the carrier HBsAg (a product made from donor blood in Shanghai Institute of Biological Products, China) was achieved by glutaraldehyde-mediated conjugation [27]. The final product was 50% HBsAg and 50% of peptides. Its protein concentration, as determined by the Bio-Rad protein assay, was 0.5 mg/mL.

Novasome (a non-phospholipid liposome) and CpG oligonucleotide are potential adjuvants that can also be used in humans [28, 29] and were used in the study to enhance the immunogenicity of the vaccine. Encapsulation of the vaccine and CpG oligonucleotide (TCCAT GACGTTCCTGACGTT, synthesized and purified by SBS Biotech Corp.) into Novasome was performed in accordance with the methods described previously [28].

Preparation of rat antisera to the IgE vaccine

Four rats were immunized subcutaneously at weeks 0, 3, and 6 with 40 μg of the vaccine and 25 μg of CpG oligonucleotide in Novasome. Four rats receiving 40 μg of HBsAg and 25 μg of CpG oligonucleotide in Novasome

Table 1. Sequence and location of the three peptides used in the preparation of IgE vaccine

Peptide name	Sequence	Number of amino acids	Location in Cε3 domain
A_1	^{345}PFDLFIRK^{352}	8	AB helix
C_1	^{371}NLTWSRASGKPVNHST^{386}	16	CD loop
E_1	^{407}TRDWIEGETYQC^{418}	12	EF helix

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served as controls. All rats were bled at week 8. Their sera were aliquotted and stored at −30 °C until use.

IgG titres of rat antisera to human IgE, HBsAg, and the IgE of other species measured by ELISA

Measurements of rat antiserum titres of IgG to human IgE, HBsAg, and the IgE of mice, rats, and dogs were performed by modified ELISA techniques [30]. Briefly, polystyrene immuno-plates (Corning Costar Corp., Lowell, MA, USA) were coated with one of the following materials: (1) purified human PS myeloma IgE (0.2 μg/mL; a gift from Dr K. Ishizaka), (2) HBsAg (2 μg/mL), (3) mouse anti-NDP hybridoma IgE (5 μg/mL; from the hybridoma cell line 26.82 obtained from Dr D.H. Katz in the Medical Biology Institute, La Jolla, purified by Dr G. Lang in the University of Manitoba), (4) rat immuno-cytoma IgE (5 μg/mL, IR-162 immunocytoma protein obtained from Dr H. Bazin), and (5) dog polyclonal IgE (10 μg/mL, purified at our lab [31]. After blocking with 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), the plates were incubated with serial twofold dilutions of either a vaccinated rat serum or a pooled control rat serum or normal rat serum (as negative control), and then incubated with 1 : 3000 dilution of alkaline phosphatase-conjugated goat anti-rat IgG antibodies (Zymed Laboratory Inc., San Francisco, CA, USA). Colour was developed by addition of the enzyme substrate solution, *p*-nitrophenyl-phosphate. Optical density at 405 nm (OD_{405}) was read on a micro-plate reader. The titres of test samples were determined as the reciprocal of the highest dilution in which the OD_{405} was twice that of the corresponding control rat serum when its OD_{405} was 0.10.

Reaction of rat antisera to the IgE already bound to its receptor measured by ELISA

To ensure that the rat antisera will not bind to IgE that is already bound to its high-affinity receptor (FcεRIα), an FcεRIα-capture ELISA was developed. Briefly, microtitre plates coated with the recombinant human FcεRIα protein (1 μg/mL) (Heska Corp., Fort Collins, CO, USA) were incubated with PS myeloma protein (0.1 μg/mL) and then with fourfold dilutions of either sera from vaccinated rats or a mouse monoclonal anti-human IgE (from Dr A. Saxon, clone #7.12) as a positive control or the pooled HBsAg-immunized rat serum as a negative control. This was followed by incubation with 1 : 3000 alkaline phosphatase-conjugated goat anti-rat IgG antibodies (Zymed Laboratories Inc., San Francisco, CA, USA) for rat sera and alkaline phosphatase-conjugated goat anti-mouse IgG antibodies (Sigma, St Louis, MO, USA) for the mAb anti-human IgE. Colour was developed and OD_{405} was read as described before. The results were expressed as OD values.

FcεRIα capture-ELISA inhibition tests

To determine whether the rat antisera were able to block the binding of human IgE to its receptor, the FcεRI-capture ELISA was used in which microtitre plates coated with FcεRI were incubated with PS myeloma IgE (0.1 μg/mL) and then incubated with alkaline phosphatase-conjugated goat anti-human IgE antibodies (1 : 2,000, from Biosource International Inc., Camarillo, CA, USA). Serial twofold diluted standards of PS IgE were established in the same plate for plotting an IgE standard curve. In the inhibition tests, each vaccinated rat serum and a pooled HBsAg-immunized rat serum (as control) were twofold diluted from 1 : 100 to 1 : 1600. Each dilution was mixed 1 : 1 with the same volume of PS myeloma IgE (0.8 μg/mL) and incubated at room temperature for 45 min. IgE concentrations in the mixtures were then measured using the FcεRIα-capture ELISA. All mixtures were assayed in duplicate. The inhibition percentage of each rat anti-serum dilution was calculated as follows:

$$\text{Inhibition}\,(\%) = \frac{\text{IgE of control serum} - \text{IgE of antiserum}}{\text{IgE of control serum}} \times 100$$

Flow cytometric analyses for inhibition of IgE-FcεRI binding

Inhibition *by* the rat anti-sera of the binding of human IgE to its receptor was also evaluated using flow cytometric analyses [32] with NIH 3T3 cells bearing human IgE receptors. These cells are transfected with $\alpha\beta\gamma_2$ chains of human FcεRI and stably express high and homogenous FcεRI (provided by Dr J.-P. Kinet) [33]. Briefly, a 5×10^6/mL single-cell suspension of the cells, cultured in complete Dulbecco's modified Eagle's medium (Biosource International Inc.) containing 10% heat-killed newborn calf serum and 0.5 mg/mL geneticin (Gibco, Grand Island, NY, USA), was prepared on ice. Meanwhile, each rat antiserum and a pooled control rat serum were diluted and mixed with the same volume of 0.5 μg/mL PS IgE for 60 min. Then, the cells were incubated with 0.1 mL of the mixtures for 45 min on ice. After washing, 0.1 mL of 1 : 50 fluorescent isothiocyanate-conjugated goat anti-human IgE (Biosource International Inc.) was added and incubated for 45 min. After washes, the cells were fixed with 0.5 mL fixation buffer and protected from light and flow cytometric analysis was performed on a FACScan (EPICS Altra Cell Sorter, Beckman Coulter, Inc., Fullerton, CA, USA). Data were analysed with software EXPO32 MultiComp and expressed using the mean fluorescence intensity linear scale. The inhibiting percentage was calculated as described above.

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PCA and PCA inhibition tests

The PCA test is a gold standard for the detection of IgE. It detects only free IgE, i.e. the IgE whose receptor-binding sites are not blocked by anti-IgE antibodies or cell-bound receptors. Thus, the PCA test is considered as the method of choice to measure biologically active IgE. Mouse and rat trichosanthin-specific IgE levels obtained from the *in vivo* studies that are described later were measured using this method. Briefly, test mouse and rat sera were twofold diluted (1 : 8 to 1 : 32). Then, 50 μL of each dilution was injected intradermally in duplicate into the shaved skin of a recipient rat. Fifty microlitres of PBS was injected as a blank control. After 24 h (*to* exclude IgG4-induced mast cell degranulation), the recipient rat was challenged intravenously with 2 mg of trichosanthin in 2% Evan's blue dye. Thirty minutes later, the rat was euthanized. Each blue skin area was cut into strips and the strips were immersed in 3 mL of extract solution (acetone: 0.5% sodium sulphate = 7 : 3) in a sealed tube for more than 24 h. After centrifuging, the supernatants were added into a 96-well microplate and OD_{610} was read. The results were expressed using OD_{610}.

Because the rat antisera obtained from IgE vaccine-immunized rats cross-reacted with mouse IgE (Table 2 of the results), PCA inhibition tests were performed to examine the ability of rat antisera to inhibit the binding of mouse IgE to FcεRI. Mouse anti-dinitrophenyl (anti-DNP) IgE was diluted (1 : 3200) and mixed with an equal volume of each serially twofold diluted vaccinated rat serum or the pooled control rat serum for 30 min at room temperature. Then, 50 μL of each mixture, or 50 μL of PBS as a negative control, was injected intradermally into a shaved rat's back skin. After 24 h, the rat was challenged intravenously with 1 mL of DNP_{33}-BSA (1.1 mg/mL, Dr A. Sehon, University of Manitoba) containing 2% Evan's blue dye. The remaining steps and calculation of inhibition percentages were the same as described above.

In vivo studies of the effect of the vaccine in trichosanthin-sensitized mice and rats

Because the rat antisera cross-reacted with mouse and rat IgE as shown in Table 2, *in vivo* studies of the effect of the vaccine on the down-regulation of IgE responses in mice and rats sensitized to trichosanthin allergen were performed in both preventive and treatment studies. Trichosanthin is a plant-derived ribosome-inactivated protein, purified from a Chinese medical herb (purchased from Shanghai Jin Shan Pharmaceutical Company, Shanghai, China) [34]. The protocol of immunization and sensitization is shown in Fig. 1.

Table 2. Serum IgG titres to human IgE and the IgE of dogs, rats, and mice

Test serum	Anti-human IgE	Anti-dog IgE	Anti-mouse IgE	Anti-rat IgE
Rat #1	800 000	32 000	8000	2000
Rat #2	200 000	64 000	8000	1000
Rat #3	200 000	32 000	8000	500
Rat #4	200 000	32 000	8000	1000

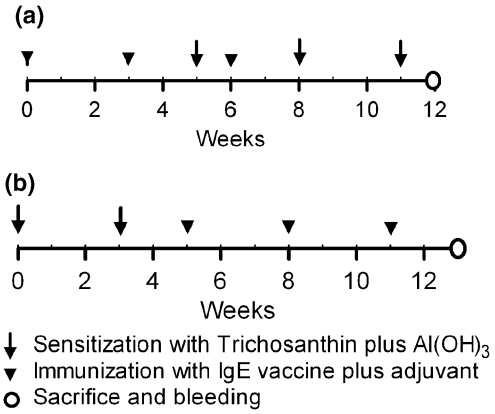

Fig. 1. Sensitization and vaccination protocols. (a) Preventive study, (b) Treatment study.

In the preventive study, BALB/C mice and Wistar rats (four each group) were subcutaneously immunized with the vaccine in Novasome adjuvant at week 0, 3, and 6 (20 μg/mouse and 100 μg/rat for the first immunization and 10 μg/mouse and 50 μg/rat for subsequent immunizations). Mice and rats receiving the carrier protein, HBsAg, served as controls. Both vaccinated and control mice and rats were sensitized by intraperitoneal injections of trichosanthin in alum at weeks 5, 8 and 11 (5 μg of trichosanthin in 1 mg of alum/mouse and 400 μg of trichosanthin in 10 mg of alum/rat for the first sensitization and 2.5 μg/mouse and 200 μg/rat for subsequent sensitizations). One week after the final sensitization, mice and rats were killed and serum samples were collected and stored at −30 °C for measurement of trichosanthin-specific IgE using PCA tests.

In the treatment study, mice and rats (four per group) were sensitized twice with trichosanthin at the same dose as in the preventive study. Two weeks after the second sensitization (week 5), the mice and rats were immunized with the vaccine three times as in the preventive study. Two weeks after the final immunization, the mice and rats were killed and serum samples were collected and stored.

Results

Rat antisera react with human IgE and the IgE in other species

All four rats immunized with the IgE vaccine developed high titres (up to 800 000) of IgG antibodies to human IgE (Table 2). This antibody also cross-reacted to a lesser extent with dog IgE and with a much lower titre to mouse

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and rat IgE (Table 2). All four rat sera had similar high titres to the carrier protein HBsAg (data not shown). Because of the cross-reactivity to mouse and rats IgE, the *in vivo* effect of this vaccine can be tested in mice and rats.

Rat antisera do not recognize the IgE that are already bound to its receptor

As shown in Fig. 2, all four vaccinated rat sera, as well as the negative control rat serum, do not recognize the IgE molecules that had already bound to the FcεRI coated on the plates. Only the mAb to human IgE (not to the receptor-binding sites of the IgE) strongly reacted with the receptor-bound IgE. This result provided evidence that the epitopes to which these antisera were binding were not available when the IgE had bound to the IgE receptor, suggesting that the rat antisera do not react with the cell-bound IgE, and thus cannot trigger histamine release from mast cells.

Rat antisera inhibit the binding of IgE to its receptor FcεRI

To evaluate whether the rat antisera could inhibit the binding of IgE to its receptor, three methods were used: FcεRI-capture ELISA, flow cytometry analysis, and PCA tests. In these inhibition tests, the IgE that had been pre-incubated with the antisera from rats immunized with the IgE vaccine was not able to bind to its receptor, FcεRI, which was either coated on the plates (in ELISA) or expressed on the cell surface (flow cytometry analysis) or expressed on the rat mast cell surface.

In the ELISA inhibition test, all four rat antisera were able, in a dose-dependent manner, to inhibit the binding of human myeloma IgE to its receptor, FcεRI, that was coated onto the plates (Fig. 3a). The inhibitory effect was also seen in the flow cytometric analysis in which the rat antisera successfully inhibited the binding of human IgE to FcεRI that was expressed on the surface of the cells (Fig. 3b). The inhibition percentages were up to 44% in the ELISA and up to 58% in the flow cytometric analysis. These experiments were repeated twice with similar results.

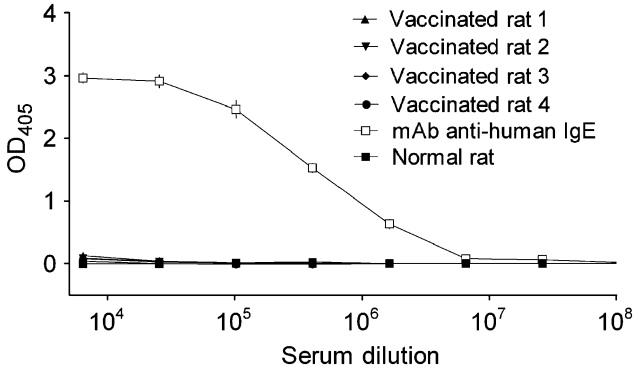

Fig. 2. Reaction of rat antisera to the IgE already bound to its receptor (FcεRIα) measured by ELISA. In the ELISA, microplates coated with human FcεRIα were incubated with myeloma human IgE and then the dilutions of rat antiserum to IgE vaccine or the mAb to human IgE or the control serum from rats immunized with HBsAg carrier alone, followed by incubation with enzyme-conjugated anti-rat IgG antibody.

Because our experiments showed that the rat antisera also bound to mouse IgE (Table 2), an *in vivo* test was performed to examine the antisera's ability to inhibit the binding of mouse IgE to normal rat skin mast cells. In the PCA inhibition test, a mouse monoclonal IgE to DNP was mixed with each rat antiserum before injection into rat skin. All four rat antisera were capable of inhibiting the binding of mouse IgE to its receptor in a dose-dependent manner (Fig. 3c). The inhibition percentages were up to 85%, which was higher than those in the ELISA and flow cytometric analysis. This may be due to the higher serum concentrations used in the PCA test and the higher sensitivity of the PCA test than those *in vitro* assays.

It can be seen from these data that all four sera from rats immunized with the IgE vaccine were able to inhibit the binding of IgE to its receptor and that rat #4 had the highest inhibition and rat #2 had the lowest inhibition in all three methods used.

In vivo studies of the effect of the vaccine in the down-regulation of IgE responses

In various serum dilutions, trichosanthin-specific IgE levels were significantly lower in the vaccinated group than those in controls, in both mice and rats. Figure 4 shows the comparison of serum trichosanthin-specific IgE levels (1 : 16 dilution for mice and 1 : 8 dilution for rats) between the vaccinated and control groups measured by PCA. Regardless of the time of vaccination, either before or after sensitization commenced, the trichosanthin-specific IgE antibody responses were greatly reduced in both mice and rats injected with the IgE vaccine, with statistical significance. In the preventive study, the mean level of IgE in the vaccinated group was reduced to 32% of the mean level in the control group in mice and to 12% in rats, while in the treatment study, the mean level of IgE in the vaccinated group was reduced to 21% of that in the control group in mice and to as low as 8% in rats. These data indicate that the IgE peptide-based vaccine is able to prevent the increase of IgE levels and to down-regulate the IgE levels that are already elevated, especially the latter, suggesting that administration of the IgE vaccine is a potential therapeutic approach in the treatment of allergy and asthma.

Discussion

Studies have shown that the Cε3 domain is the main region for the interaction of the IgE molecule with its

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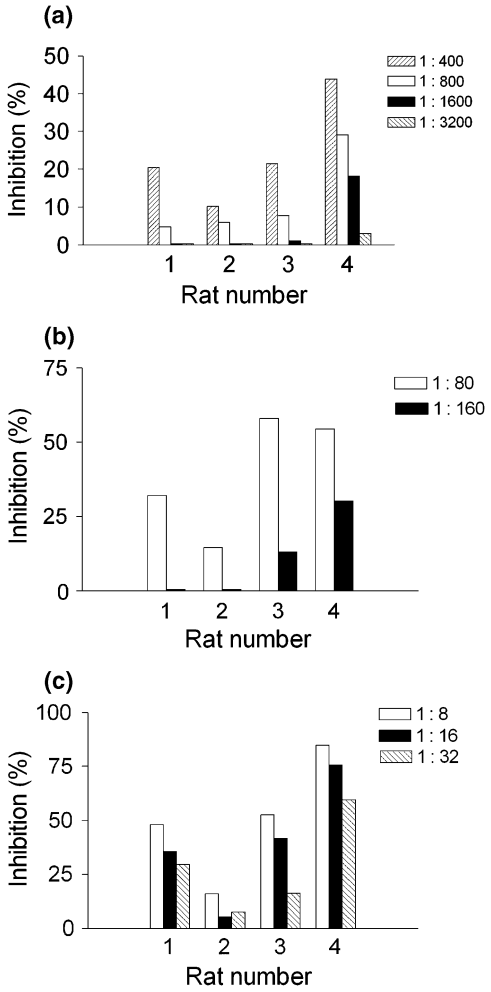

Fig. 3. Inhibition of the binding of IgE to its receptor by sera from rats immunized with a human IgE vaccine, compared with the control sera from rats receiving HBsAg carrier alone. (a) FcεRIα-capture ELISA inhibition tests in which human FcεRIα was coated onto the microplates, (b) Flow cytometric analysis in which cells expressing FcεRI were used, (c) passive cutaneous anaphylaxis (PCA) inhibition test in which rat mast cells were sensitized with mouse dinitrophenyl-specific IgE.

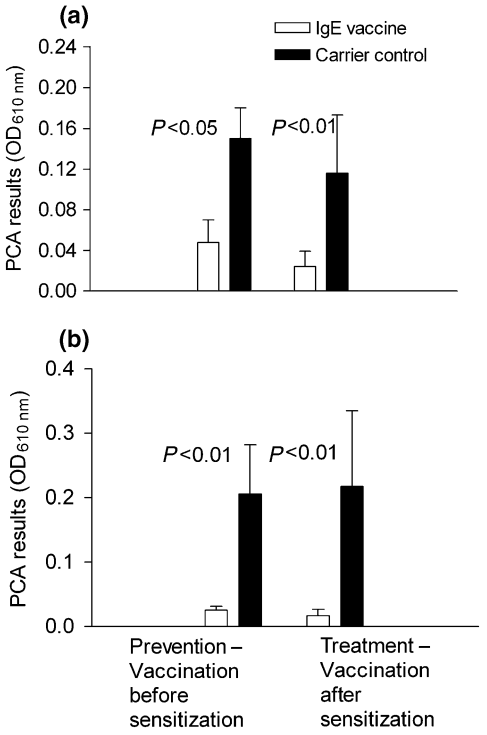

Fig. 4. The *in vivo* effect of administration of an IgE vaccine on the down-regulation of serum IgE levels. Sensitized mice (a) and rats (b) were immunized with the IgE vaccine as described in Fig. 1. Sensitized mice/rats receiving injections of the HBsAg carrier protein served as controls. Serum Trichosanthin-specific IgE levels were measured using passive cutaneous anaphylaxis tests (PCA).

receptor FcεRI [17, 21–26]. The crystal structure of human IgE Fc–FcεRI complex has revealed that several loops and helixes in the Cε3 domain act as binding sites or influence the binding for IgE–FcεRI interaction [25, 26]. The three peptides used in the vaccine in the present study are all from the Cε3 region. The Cε3 domain contains 110 amino acids with a molecular weight of 14 kDa. Polyclonal antibodies induced by the vaccine containing *the whole* Cε3 domain may cross-react with other immunoglobulins that have the same epitopes as Cε3. Small peptide-based vaccines have several advantages to limit any possible cross-reactivity. Some linear peptides ranging between 6 and 20 consecutive amino acid residues are fully antigenic. Although their immunogenicity may be weak, it can be increased by using a carrier protein and adjuvants. In the present study, the three peptides we selected were small, containing 8–16 amino acids. One of the peptides A1 has been previously shown to inhibit passive sensitization of basophils [22]. To increase the antigenicity, selection of the appropriate carrier protein is very important. Virus-like particles can induce potent B cell responses even in the absence of adjuvants [35]. These large particles are highly antigenic because they improve the presentation of the epitopes to cells of the immune system. Immunization with the self-peptides displayed on the surface of a virus-like particle has been proven to produce high titre antibodies to the self-antigen [35, 36]. In addition, the HBsAg protein itself is an approved human vaccine component with an approximate 20-year license and safety record in the market. Therefore, HBsAg was chosen as the carrier protein for this study.

The IgE peptide-based vaccine in this study has unique advantages compared with the IgE vaccines reported previously. Wang et al. [37] prepared an IgE vaccine by chemically coupling a 23 amino acid-peptide derived from Cε3 domain to a carrier keyhole limpet haemocyanin (KLH). This vaccine requires the strong adjuvant CFA/IFA to elicit anti-IgE responses. The presently reported vaccine has a stronger immunogenicity than the vaccine using KLH as a carrier due to the virus-like particle HBsAg that is used as a carrier in the vaccine. Recently, data from our

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laboratory have shown that administration of the virus-like particle vaccine induced high and long-lasting immune responses to the target self-protein even without the use of any adjuvant [38, 39], although an adjuvant had been used in the present report. Self-peptides (derived from TNF, IL-4, IL-13, or CCR5) coupled to a virus-like particle such as papillomavirus [36, 40, 41] or HBcAg [35, 38, 39, 42, 43] have been reported to break down B cell tolerance, inducing auto-antibodies to the target self-protein, which leads to improvement of the disease symptoms in mice.

In another study, IgE vaccines were made by coupling or fusing a significantly large portion of rat IgE (whole Cε2 and/or Cε3) to a heterogonous carrier protein (either opossum Cε2 and Cε4 or the glutathione-*S*-transferase protein), forming a chimeric IgE molecule [18] or a fusion protein [19]. Although these vaccines induced anti-self-IgE responses, the polyclonal antibodies to IgE induced by the vaccine are against all epitopes in the target Cε2 and/or Cε3, which may cross-react with other immunoglobulins containing the same epitopes. Our peptide-based vaccine minimizes possible cross-reactivity because the peptide sequences used are short (8–16 amino acids) and are IgE specific.

Taken together, these facts imply that our IgE peptide-based vaccine may be a better vaccine approach in the treatment of allergy and asthma.

In the *in vitro* tests, the vaccine-induced rat antisera contained high titre antibodies that reacted with soluble IgE as shown in the inhibition tests (Fig. 3) and plate-bound human IgE (Table 2) but not with receptor-bound human IgE (Fig. 2), indicating the vaccine-induced antibodies will not trigger histamine release from mast cells. In the preventive and treatment studies, even in the rodents whose IgE cross-reacts with the human IgE, administration of the vaccine induced a profound decrease of serum IgE levels to approximately 8–32% of the controls, suggesting that this human IgE vaccine would be even more effective in humans than in rodents. Using similar techniques, we recently developed mouse IL-4 and IL-13 peptide-based vaccines and found them to be effective in the down-regulation of the target cytokine, resulting in a decrease of airway allergic inflammatory responses in mice [38, 39].

IgE antibodies are responsible for the allergic cascade. Decreased levels of serum free IgE not only directly reduce the symptoms caused by mast cell degranulation but also indirectly reduce allergic inflammation by leading to a decrease in high-affinity IgE receptor (FcεRI) expression on the surface of mast cells and dendritic cells [15, 16]. The latter effect may be more important than the direct action on IgE levels.

However, there are concerns regarding the consequences of inhibiting IgE antibodies on a long-term basis using vaccines. Some studies indicate that IgE may be important in the immune defence system against parasitic diseases [44]. However, other studies have found that anti-IgE treatment decreases worm burden and egg production in parasite-infected mice [45, 46]. Studies of patients with parasitic infections reveal an inverse correlation between serum IgE concentration and *the* severity of re-infection or the number of parasite larvae [47–49]. Most importantly, studies with Xolair therapy show that there is no increase in parasitic or other infections [8, 50, 51]. With the use of self-protein vaccines, antibody titres to cytokines last about 4 months and are reversible; they can be adjusted by the frequency of immunization, because the immunogenicity of cytokine vaccines is less than that of microbe vaccines [52, 53]. Therefore, theoretically, cytokine vaccines should be safe and effective, as has already been documented by studies in humans [52, 53]. This encourages the use of IgE vaccines, especially the presently reported IgE peptide-based vaccine, as a new avenue for the treatment of allergy and asthma.

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