Peptide-based Vaccines Derived from FcεRI Beta Subunit Can Reduce Allergic Response in Mice Model

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ABSTRACT

Current therapeutic approaches in allergic diseases especially asthma generally focus on using immunological strategies. According to the importance of FcεRI in controlling allergic response we used two extracellular regions of Fc epsilon receptor I (FcεRI) beta subunit peptides to design two peptide-based vaccines. Probably these peptides vaccines by triggering the immune response to FcεRI can reduce the allergic symptoms through blocking the IgE specific receptor.

Two extracellular parts of FcεRI beta subunit were made by peptide synthesizer and conjugated with keyhole limpet Hemocyanin. These conjugated peptides were used and evaluated as therapeutic vaccines in allergic airway inflammation mouse model. Total IgE and anti ovalbumin specific IgE were measured in mice serum and compared in vaccinated and unvaccinated allergic mice. Histamine, prostaglandin D2 (PGD2), IL-4 and IL-13 were measured in bronchoalveolar lavage (BAL) fluid of vaccinated allergic mice versus unvaccinated and histopathologic examination were performed in studied groups.

After vaccination of mice with each of the peptide vaccines the specific antibodies titer increased significantly in vaccinated groups versus unvaccinated. In histopathologic study, lavage eosinophil percentage and peribronchial inflammation in lung sections of vaccinated groups was decreased ($p<0.05$). Also the allergic components including total IgE, anti ovalbumin specific IgE, histamine, PGD2, IL-4, and IL-13 showed substantial decline in vaccinated allergic mice.

Thus targeting the extracellular regions of FcεRI beta subunit by peptide-based vaccines and induction of specific antibodies against them can reduce allergic responses in allergic mice model.

Keywords: Allergy; FcεRI beta-subunit; Peptide-based vaccine

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INTRODUCTION

The prevalence of allergic diseases has increased in the last decade. Today 23-25 percent of the world population, are suffering from one type of allergy.[1-3] Current therapeutic approaches in asthma and allergy including antihistamines, corticosteroids, ß2-adrenoceptor agonists, and phosphodiesterase inhibitors generally focusing on the control of symptoms cannot completely cure the allergic disorders.[4,5] Immunological strategies that have recently been noticed to control and treat the allergic diseases, mainly asthma, include using monoclonal antibodies against IL-4, IL-9, IL-13 and cytokine receptors;[6,7] also monoclonal antibodies against IgE, omalizumab;[4] as well as monoclonal antibodies against alpha subunit of FcεRI,[8,9] or regulatory cytokines such as IL-10, IL-12,interferon gamma,[4] IgE and cytokines domains as a vaccine;[10-13] allergen specific immunotherapy;[14,15] and small interfering RNA (SiRNA) and antisense to regulate the expression of transcription factors and important molecules in allergic response.[16,17]

Some of the above methods are in clinical trials or approved as a therapeutic approach but some others are either expensive or unsafe, which are described in details as follows. Due to the importance of IgE and its receptors in allergic response, recent studies have focused on IgE and IgE receptor (FcεRI) for treatment of allergic disorders. For example, omalizumab is a humanized monoclonal anti-IgE antibody which can bind to IgE and inhibit IgE binding to FcεRI; therefore regulates mast cell activation. It is one of the novel therapeutic approaches in the management of asthma but it is very expensive.[18] In some studies the immunogenic epitops of IgE molecule was used as a peptide-conjugated vaccine using a carrier protein such as hepatitis B surface (HBS) antigen for immunotherapy. This vaccine induces the production of IgG against the Fc region of IgE to reduce allergic responses through the inhibition of IgE binding to FcεRI.[12,13,19]

Alternatively, targeting FcεRI molecule can be another strategy to treat the allergic patients. Using monoclonal anti-human FcεRI alpha subunit antibodies is another approach for treatment of allergy.[8,9,20-22] However, there are some doubts about its safety and possibility of excessive activation of mast cells which could lead to chronic urticaria.[8,9,21-23]

Erdei, A. et al in several studies showed that some synthetic complement components such as C3a derived peptides (C3a7 and C3a9) have the ability to interact with beta subunit of FcεRI and modulate the allergic response through the inhibition of IgE-mediated triggering of mucosal-type mast cells.[24-26] According to the previous studies especially Erdei, A. et al it seems that targeting two extracellular regions of FcεRI beta subunit by induction of a specific antibody with a peptide-based vaccine can reduce allergic symptoms. Probably these peptides vaccines trigger the immune response to extracellular parts of beta-subunit of FcεRI and generated specific antibodies (IgG isotype) can reduce the allergic symptoms through binding to beta-subunit of FcεRI. This interaction may block the IgE binding site by a steric interference or inhibit the signaling pathway in mast cells to reduce allergic response in allergic mouse model (Figure 1). For this goal we designed two peptide-based vaccines that were derived from two extracellular regions of FcεRI beta subunit and evaluated them in an allergic mouse model.

MATERIALS AND METHODS

Preparation of Peptide-based Vaccine

The amino acid sequence of two extracellular region of mouse FcεRI beta subunit was obtained from Uniprot database (http://www.uniprot.org/blast.) The amino acid sequence in position 72-89 of FcεRI beta subunit with 18 aa (CSVLYVSDFDEEVLLLYK) was named peptide A and the other one in position 143-171 with 29 aa (ILNLTNNFAYMNNCKNVTED DGCFVASFTT E) was named peptide B. The sequence homology with other proteins was checked in NCBI (https://blast.ncbi.nlm.nih.gov/Blast) and Uniprot databases and then the peptides were synthesized in two forms of non-conjugated and conjugated with KLH (Selleckchem, USA). Conjugated peptide A was named "peptide vaccine A" and conjugated peptide B was named "peptide vaccine B".

Animals

Sixty female Balb/c mice (6 to 8 weeks old) were purchased from Pasteur Institute (Tehran, Iran). The mice were quarantined for at least 1 week before use. They were kept in a controlled environment regarding temperature, humidity and light, with filtered airflow,

sterilized instruments and free access to standard rodent autoclaved chow and water. All of mice were alive until the end of the study process. The animals were then sacrificed under euthanasia.

Allergic Airway Inflammation Model and Vaccination Protocol

Ovalbumin-sensitized allergic airway inflammation mouse model is referred to as "allergic model" in the rest of manuscript. For induction of allergic airway inflammation, the mice were intraperitoneally sensitized on days 0 and 14 with a mixture of 20 μg ovalbumin (OVA) , adsorbed to 1mg/mL alum adjuvant in 100 μL as the total volume. Subsequently, on days 24, 26, 28 and 30, they were exposed to 8 mL of 1% aerosolized OVA in normal saline for 30 min/day. Aerosol administration was continued on days 67, 69 and 70 under a constant pressure through an ultrasonic nebulizer (NE – UO7, Omron Co, Tokyo, Japan) in a chamber of 5500 cm^3 volume.[27, 28] The mice were divided into four groups and sensitization with allergen and vaccination with peptide vaccines were performed according to the following protocol (Figure 2).

Group1 (positive control n=15): Allergic model without any vaccination. This group received subcutaneously KLH (carrier protein) instead of vaccines on days 31, 45 and 66.

Group 2 (peptide vaccine A n=15): Allergic model vaccinated subcutaneously with 100 μg peptide vaccine A on days 31, 45 and 66.

Group 3 (peptide vaccine B n=15): Allergic model vaccinated subcutaneously with 100 μg peptide vaccine B on days 31, 45 and 66.

Group 4 (Negative control n=15): Non allergic model without any vaccination but with inhalation of sterile normal saline.

On day 70, the mice were sacrificed 15 min after the last challenge and their blood, broncho alveolar lavage fluid (BALF), and lung tissue were collected for subsequent analysis. The protocol was approved by Ethics Committee of the Tehran University of Medical Sciences (No. 412/92/34).

## Sample Collection and Preparation for Further Examinations

Preparation of samples was done by the same method described by Farzaneh, et al.[29] In brief, the mice were anaesthetized; the blood samples were collected and their sera separated. 10 mice in each group were sacrificed for BALF sample collection.
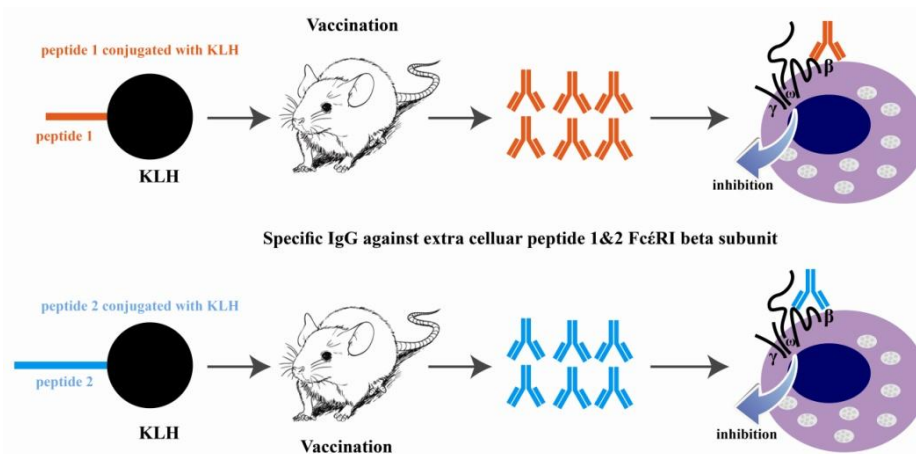
Figure 1: Supposed mechanism of FcεRI beta subunit in peptide-based vaccines.
Targeting two extracellular regions of FcεRI beta subunit by induction of a specific antibody with a peptide-based vaccine. Peptide based vaccines derived from these regions of receptor induce specific antibodies that may bind to the FcεRI beta subunit. This interaction may block the IgE binding site by a steric interference or inhibit the signaling pathway in mast cells to reduce allergic response in allergic mice model.
KLH: Keyhole Limpet Hemocyanin
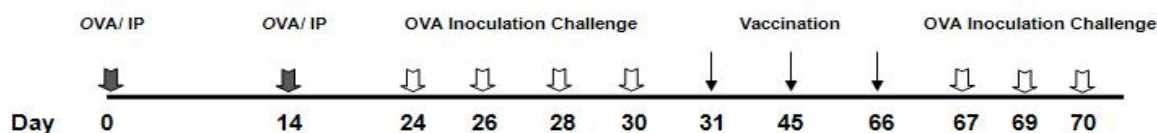
Figure 2. Induction of allergic airway inflammation and vaccination procedure
For induction of allergic model, the mice were sensitized on days 0 and 14 and challenged with ovalbumin (OVA) performed on days 24, 26, 28 and 30 and further on days 67, 69 and 70. Vaccination with peptide vaccine A and B was performed subcutaneously for groups 2 and 3 on days 31, 45 and 66.

Their tracheas were cannulated and the BALF containing cells recovered. The BALF was collected for subsequent cytokine (IL-4, IL-13), histamine and prostaglandin D2 (PGD2) analysis and cell slides preparation. The rest five mice in each group were sacrificed for collecting the lung tissue for histopathological studies. The lower lobe of their left lung was cut into three slices and fixed in 10% buffered formalin and paraffin embedded. The samples were stained with haematoxylin-eosine.

Histology and Eosinophils Count
The BALF samples were cytospined and BALF cells slides were stained with Geimsa. The cells were identified by morphologic criteria to determine the percentage of eosinophils in each sample by counting 300 cells. Histological assessment was performed in the same method described by Farzaneh et al.[29] In order to evaluate the criteria of inflammation in the lung a semi-quantitative scoring system at 100× or 400×final magnifications was used to grade the histopathologic lung changes. Peribronchial inflammatory cell infiltration including lymphocytes, macrophages and eosinophils were graded as follows:
0= lack of any infiltrate;
1+= bronchiole with scattered infiltrates;
2+= bronchiole with an infiltrate of up to 2 cells per section;
3+= bronchiole with an infiltrate of up to 5 cells per section;
4+= bronchiole with an infiltrate of more than 5 cells per section;

Evaluation of Humoral Response to Peptide Vaccines
The serum of vaccinated and unvaccinated mice were diluted (1/10, 1/100, 1/200, 1/500, 1/1000, 1/1500, 1/2000, 1/4000) in phosphate-buffered saline (PBS). The specific IgG antibodies titer against peptide A and B in serum of vaccinated mice versus control groups were measured by ELISA. Maxisorb ELISA plates

(Nunc, Denmark) were coated by peptide A and B individually.[30] To compare the amount of specific IgG to peptide A and B in each group, the optical density (OD) at 450 nm was recorded.

Determination of Allergic Responses in Serum and BALF
IgE and specific anti OVA- IgE were measured in the serum by ELISA kits (eBioscience, USA and Cayman Chemical company, USA; respectively). In BALF samples histamin and PGD2 were measured by ELISA kits (Cayman Chemical Company, USA) and IL-4 and IL-13 were determined by ELISA kits (eBioscience, USA)

Statistical Analysis
Data were analyzed by SPSS.20 software (IBM, USA). All values are expressed as the mean±SD. The differences were determined using the Mann-Whitney U Test for non-parametric comparison and T-Test for parametric comparison. The p-value<0.05 was considered statistically significant.

RESULTS

Purity and Homology of Peptides
The peptides and their corresponding conjugated forms were synthesized (Sellekchem, USA) according to the desired sequence with 89% purity and did not have any homology with other proteins in mice.

Vaccine Evaluation
Titration of serum in vaccinated mice according to the optimal OD for specific IgG in 1/100 dilution of serum compared with control group and the difference was statistically significant ($p<0.001$). Table 1 shows the mean of OD related to specific IgG against peptide A and B at 1:100 diluted sera.

**Histopathological and Cellular Status of Vaccinated and Unvaccinated Allergic Models**

Significant peribronchial inflammation and eosinophil infiltration were seen in allergic model ($p<0.001$); however, there was not any inflammation in negative control. A significant decrement in the score of peribronchial inflammation and also reduced percentage of eosinophils in BALF samples were seen ($p<0.001$) in both vaccinated groups compared with positive control (Table 2 and Figure 3).

Allergic Responses in Lavage and Serum in Vaccinated and Unvaccinated Allergic Models

Serum total IgE and the specific anti OVA IgE concentration in both vaccinated groups showed a significant decrease ($p<0.05$) (Figure 4: A and B). The mast cell mediators include histamine and PGD2 were significantly decreased in BALF of vaccinated mice compared with control groups ($p<0.05$) (Figure 4: C and D). Moreover, the levels of IL-4 and IL-13 in BALF of both vaccinated groups showed a significant decrease ($p<0.05$). (Figure 4: E and F)

Table1. The means of optical density (OD) related to specific IgG against peptide A and B at 1:100 diluted sera of vaccinated and unvaccinated mice. Results show humoral response to our studied peptide vaccines.

Groups	Specific IgG against peptide A (Mean of OD)	Specific IgG against peptide B (Mean of OD)
Vaccinated with peptide vaccine A	1.29±0.18	-
Vaccinated with peptide vaccine B	-	1.55±0.23
Negative control	0.046±0.025	0.047±0.031
Positive Control	0.053±0.032	0.076±0.042
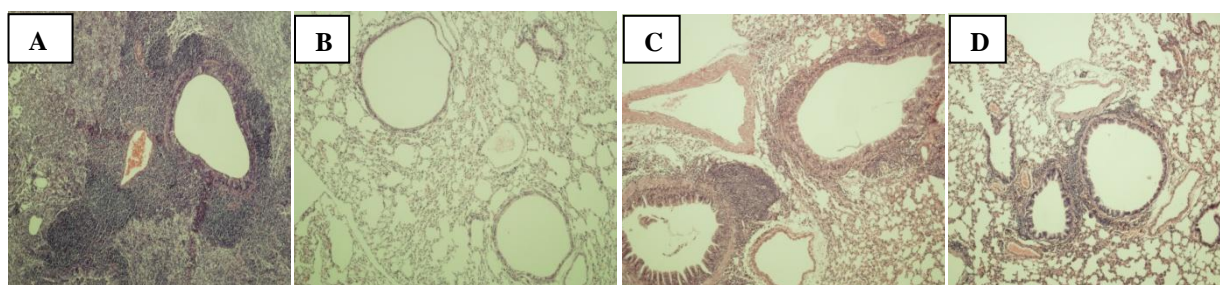
Figure 3. Peribronchial inflammation and inflammatory cells infiltration consisting of lymphocytes, macrophages and eosinophils in lung sections of vaccinated groups compared with negative and positive control by 100×final magnifications. A significant decrement in the score of peribronchial inflammation were seen in both vaccinated groups (C and D) compared with positive control.

A) Positive control: Ovalbumin-sensitized allergic airway inflammation mouse model without any vaccination. This group received subcutaneously KLH (carrier protein) instead of vaccines

B) Negative control: Non allergic model without any vaccination but with inhalation of sterile normal saline

C) Vaccinated with peptide vaccine A: Allergic model vaccinated subcutaneously with peptide vaccine A

D) Vaccinated with peptide vaccine B: Allergic model vaccinated subcutaneously with peptide vaccine B
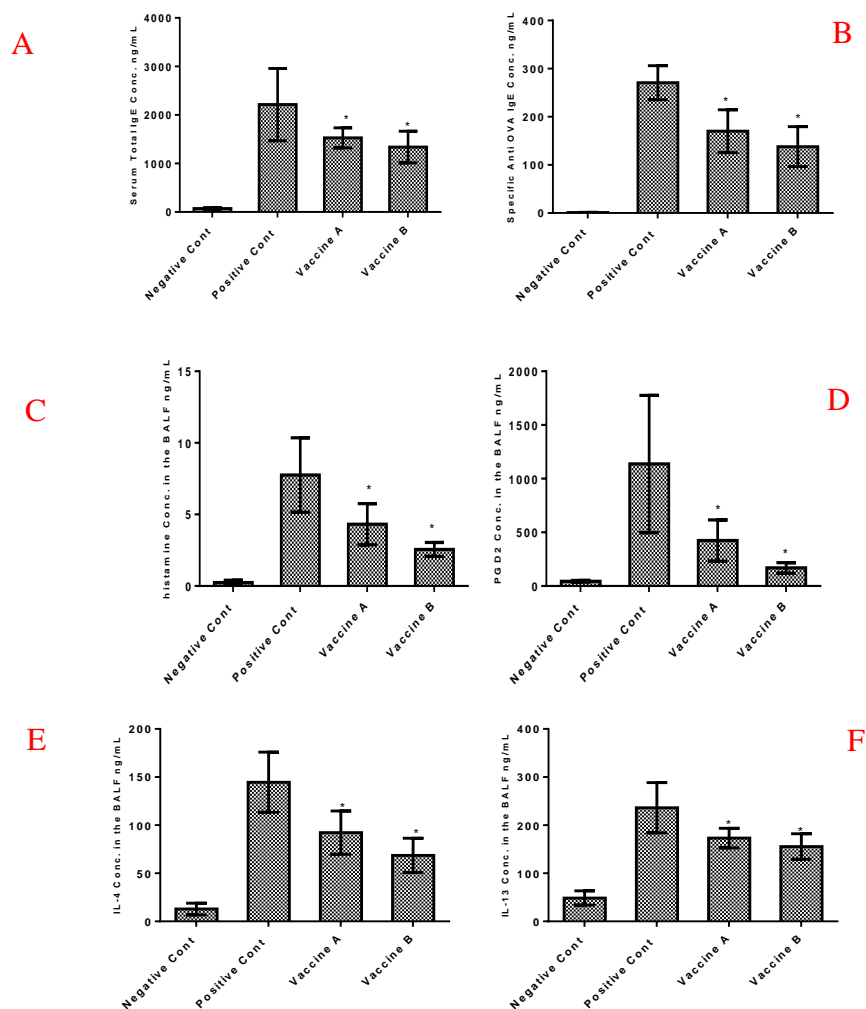
Figure 4. A) Serum total IgE concentration in studied groups. The vaccinated allergic mice showed a significant decreasing in serum total IgE production compared with positive control (*p*=0.012 for vaccine A, *p*=0.003 for vaccine B).

B) Serum Specific anti-ovalbumin IgE concentration in studied groups. Groups that received vaccine A and B showed a significant decrement in serum specific anti-OVA IgE production in comparison with positive control (*p*<0.001 for both groups)

C) Bronchoalveolar lavage (BAL) fluid histamine concentration in studied groups. Groups that received vaccine A and B showed a significant decrement in histamine production in comparison with positive control. (*p*=0.002, *p*<0.001, respectively).

D) BAL fluid prostaglandin D2 (PGD2) concentration in studied groups. Groups that received vaccine A and B showed a significant decrease in PGD2 production compared with positive control. (For both of them -*p*<0.001)

E) BALF IL-4 concentration in studied groups. Groups that received vaccine A and B showed significant decrease in IL4 production versus positive control. (For both of them *p*<0.001)

F) BALF IL-13 concentration in studied groups. Groups that received vaccine A and B showed significant decrease in IL13 production versus positive control. (For both of them *p*<0.001)

Positive control : Ovalbumin-sensitized allergic airway inflammation mouse model without any vaccination. This group received subcutaneously KLH (carrier protein) instead of vaccines.

Negative control: Non allergic model without any vaccination but with inhalation of sterile normal saline.

Vaccine A: Allergic model vaccinated subcutaneously with peptide vaccine A

Vaccine B: Allergic model vaccinated subcutaneously with peptide vaccine B

* shows *p*<0.05 compared with positive control.

Table 2. Peribronchial inflammation score and lavage eosinophil percentage in studied groups (Mean±SD)

Groups	Peribronchial inflammation score	Lavage eosinophil percentage
Negative Control	0.0	0.0
Positive Control	3.7±0.48	50.8±9.45
Peptide A vaccinated	2.7±.048*	30.8±5.88*
Peptide B vaccinated	2.0±0.67*	21.2±8.52*

A significant decrement in the score of peribronchial inflammation and lavage eosinophil percentage were seen in both vaccinated groups compared with positive control.

Positive control: Ovalbumin-sensitized allergic airway inflammation mouse model without any vaccination. This group received subcutaneously KLH (carrier protein) instead of vaccines.

Negative control: Non allergic model without any vaccination but with inhalation of sterile normal saline.

Peptide A Vaccinated: Allergic model vaccinated subcutaneously with peptide vaccine A

Peptide B vaccinated: Allergic model vaccinated subcutaneously with peptide vaccine B

* shows *p*<0.05 compared with positive control

DISCUSSION

According to our results peptide-based vaccines against the extracellular regions of FcεRI beta subunit can reduce allergic responses in allergic mice model.

In this research we designed two peptide base vaccines against the extracellular regions of FcεRI beta subunit and target these parts of receptor by KLH conjugated vaccines. The purity of these synthetic peptides A and B was 89% and it did not have any homology with other molecules in mice. This amount of purity is sufficient as a peptide vaccine to induce the humoral immune response.[31] After induction of allergic model according to previous studies[27,29] to make the condition of experiment similar to the real one, all of the vaccinations with peptide vaccine A and B were done after establishment of the allergic model in the mice and we used them therapeutically. Perhaps the preventative vaccination would be more effective but we used this kind of vaccination in our study because it matches more the condition in allergic diseases.

The therapeutic vaccination with these vaccines could induce specific IgG against the extracellular regions of FcεRI beta subunit. Ma Yi et al applied the same approach to induce the immune system for production of IgG against IL-13 through peptide vaccine.[11] Moreover, Peng, Z. et al provided IgE peptide-based vaccine to attenuate allergic response by induction of specific IgG production in allergic model.[19] In present study vaccination with peptide vaccine A and B created an acceptable titer of antibody that was confirmed by the results.

Peptide vaccine B made from 29 amino acid sequence in position 143-171 of FcεRI beta subunit was more effective in specific IgG production. Due to IEDB data base (http://www.iedb.org/), peptide B is longer and have more antigenic and hydrophilic points compared to peptide A. In this research we targeted two extracellular region of beta subunit of FcεRI using two vaccines with hypothesis of induction of specific antibody against these parts of receptor that could lead to inhibition of allergic responses. For this goal we designed two peptide-based vaccines. Using these peptide-based vaccines, as we expected tolerance break occurred and humoral immune response against these self molecules were induced. These specific antibodies probably bind to the FcεRI via beta subunit and inhibit allergic responses. There are two possibilities for allergic response inhibition in this regard. The first through the blocking interaction of IgE with its receptor by means a steric interference and space hindering, and the second, via induction of inhibitory signaling with beta subunit directly.

Histopathologic study and evaluation of allergic inflammation response in lung tissue and lavage of vaccinated allergic mice showed a decrement in peribronchial inflammation, lavage eosinophil percentage in both vaccinated groups with peptide vaccine A and B. According to these results vaccine B was more effective in this regard. Also we noticed that the increment of serum total and specific anti Ova IgE after 3 final challenges with allergen in vaccinated mice is significantly less than positive control. It probably can be related to blocking of FcεRI and binding of this receptor to the specific antibodies derived from vaccines. In this research the vital mast cell mediators

such as histamine and PGD2 and also Th2 cytokines showed a considerable decrease in BALF of vaccinated allergic mice with both of our peptide-based vaccines. Peptide vaccine B was more effective to decrease all allergic mediators including serum total IgE and specific IgE, Th2 cytokines such as IL-4 and IL-13 and mast cell mediators. In the same approach Wang, C. Y et al designed an immunotherapeutic IgE-based peptide vaccine for allergic dogs. Their vaccine targets the binding site on IgE for the high affinity receptor FcεRI, (positions 413-435 of a loop region of C epsilon 3) and induced anti-IgE antibodies that blocked IgE-mediated histamine release and reduced total serum IgE significantly.[13] Peng and et al showed that the novel recombinant IL-13 peptide-based vaccine can reduce airway allergic inflammatory responses in OVA-sensitized allergic mice. In their research, vaccination by IL-13 peptide-based vaccine significantly suppressed inflammatory cell number, and IL-13 and IL-5 levels in BALF. In that research total and OVA-specific IgE in serum were also significantly decreased.[11] Peng and et al in another research evaluated novel IgE peptide-based vaccine made by conjugating three synthetic peptides corresponding to human IgE to Hepatitis B antigen such as a carrier protein. They reported IgE down-regulation in rodents after vaccination.[12,19] Their study showed that sera from vaccine-immunized rats containing high titer antibodies can react with human IgE; and inhibit binding of human IgE to its receptor in a dose-dependent manner.[12]

In another strategy Takai, et al targeted alpha subunit of IgE receptor by monoclonal antibodies[9] but another studies showed that these antibodies were not safe for treatment of allergic disease.[8,21]

In present study for the first time we targeted the beta subunit of FcεRI by two peptide base vaccines against this subunit and evaluated them. Considering presence of immunoreceptor tyrosine-based activation motif (ITAMs) in beta subunit of FcεRI, we targeted it in order to reduce mast cell mediator release.[32] Erdei et al used some synthetic molecules that interact with beta subunit of FcεRI and inhibit IgE-mediated triggering of mast cells. They showed that interaction between these molecules and beta subunit of FcεRI can modulate the allergic response in vitro.[24-26] We designed this study according to previous studies specifically those done by Erdei et al. In this research we evaluated our designed peptide-based vaccines in allergic mouse model and the results showed decrease in allergic response in both of

vaccinated groups with the both peptide-based vaccines derived from FcεRI beta subunit.

Results showed that induced antibodies after vaccination with both of these peptide based vaccines probably interact with external parts of beta subunit of FcεRI on the surface of mast cells and this interaction can reduce allergic response in allergic mice model. This interaction may inhibit binding of IgE to FcεRI or inhibit internal signaling of mast cells. We are further investigating in vitro the molecular mechanisms of these vaccines and the derived polyclonal specific antibody against them and will report the results in the near future and assume that probably making a monoclonal antibody against these peptides would provide similar results.

Present study showed that targeting the extracellular regions of FcεRI beta subunit by induction of a specific antibody with a peptide based vaccine can reduce allergic response in allergic mice model.

ACKNOWLEDGEMENTS

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