

RESEARCH ARTICLE

Comparison of Ovalbumin Sensitized Mice Model for Allergy: A Preliminary Study

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Abstract

Allergy diseases tend to increase with variety of clinical manifestations. To date, pharmacotherapy do not treat the underlying disease. Many researches concerned to study new approach of allergy therapy which need allergy mouse model for evaluation. This study was aimed to compare the effectiveness of two treatment methods by using ovalbumin for allergy sensitization. This study was conducted in PT.Bimana Indomedical animal facility Bogor on June until September 2016. Six mice were divided into 2 groups. First group was sensitized by ovalbumin 100ug subcutaneous (sc) day-0, 50 ug intra peritoneal (ip) day-14 and 100 ug intranasal (in) day-21. Second group was sensitized by ovalbumin 10 ug ip day-0 and day-7, 10 ug in day-21, 22, and 23. Body weight, hematology parameters (red blood cells, white blood cells, hemoglobin and hematocrit) and total IgE concentration of day-0 (pre-treatment) and day 24 (post-treatment) were measured. The results showed that there was an increase of mice body weight of both treatment group, while hematology changes of pre and post treatment were not significantly different (paired t-test). Whereas the increase in total IgE pre and post treatment was significantly different between the two groups (paired t-test). In conclusion, the second allergy sensitization method is better than the first allergy sensitization method.

Key words: allergy; mice; ovalbumin; total IgE.

Perbandingan Mencit yang Disensitisasi dengan Ovalbumin untuk Model Alergi: Suatu Studi Pendahuluan

Abstrak

Penyakit alergi cenderung meningkat dengan berbagai manifestasi klinis. Sampai saat ini farmakoterapi tidak mengatasi penyakit yang mendasarinya, sehingga banyak penelitian yang difokuskan untuk mempelajari pendekatan baru terapi alergi yang memerlukan model hewan coba alergi untuk evaluasi. Penelitian ini bertujuan untuk membandingkan efektifitas dua metode perlakuan dengan menggunakan ovalbumin untuk sensitisasi alergi. Penelitian ini dilakukan di fasilitas hewan coba PT. Bimana Indomedical Bogor pada bulan Juni sampai September 2016. Enam mencit dibagi menjadi 2 kelompok. Kelompok pertama disensitisasi dengan ovalbumin 100ug subkutan (sc) hari ke-0, 50 ug intra peritoneal (ip) hari ke-14 dan 100 ug intranasal (in) hari ke-21. Kelompok kedua disensitisasi dengan ovalbumin 10 ug ip hari ke-0 dan hari ke-7, 10 ug intra nasal pada hari ke-21, 22, dan 23. Berat badan, hematologi (eritrosit, leukosit, hemoglobin dan hematokrit) dan konsentrasi IgE total hari ke-0 (sebelum perlakuan) dan hari ke-24 (setelah perlakuan) diukur. Hasil penelitian menunjukkan terdapat peningkatan berat badan mencit dari kedua kelompok setelah perlakuan, namun tidak disertai dengan perubahan hematologi yang berbeda bermakna (uji t berpasangan). Sedangkan peningkatan IgE total sebelum dan setelah perlakuan kelompok kedua berbeda bermakna (uji t berpasangan) dibandingkan kelompok pertama. Penggunaan ovalbumin total per mencit kelompok pertama lebih tinggi dari kelompok kedua. Metode sensitisasi alergi kelompok perlakuan kedua lebih baik dari sensitisasi alergi kelompok perlakuan pertama

Kata kunci: alergi; mencit; ovalbumin; total IgE.

Introduction

Allergy is a hypersensitivity reaction caused by a certain immune mechanism. In various regions in Indonesia, the incidence of allergies varies from 3% to 60%. Although influenced by subjects morbidity characteristics and design of the study, the figures above clearly indicate that more and more allergic cases are reported.¹ Allergic manifestation are asthma, rhinitis, conjunctivitis, anaphylaxis, drug-, food, insects' allergy etc. According to World Health Organization data, hundreds millions of people have rhinitis all over the world and it is estimated that 235 million people have asthma.² Pharmacotherapy by using antihistamine, corticosteroids and several symptomatic drugs have been shown to be effective in reducing symptoms however do not treat underlying allergy and disease progression.^{2,3}

Immunotherapy was reported as a highly potential treatment for allergy. Sub cutaneous and sublingual immunotherapy were approved by World Allergy Organization and still under development studies. Many researches have been conducted to establish new approach of allergy therapy. These studies explored about Toll like receptor agonists, cytokine blocker and transcription factor inhibitor.³ Allergy mouse model became important for in vivo study model. Therefore, this study focuses to establish allergy mouse model by using ovalbumin sensitization.

Methods

This study was an in vivo experimental study on Balb/c mice, and approved by *Animal Care and Use Committee* (ACUC) no. R.04-16-IR. Balb/c mice was obtained from PT. Indo Ani Lab. Animal was adapted and given food ad libitum for 2 weeks in room temperature. Animal experiment was conducted in PT. Bimana Indomedical animal study facility Bogor on June until September 2016. Hematology measurement was conducted in pathology laboratory, Primate Research Center-Bogor Agriculture University, Indonesia. While total IgE concentration was measured by sandwich ELISA method in Biochemistry laboratory, Faculty of Medicine Universitas Indonesia (FMUI).

Six Balb/c mice were divided into 2 groups, induced by ovalbumin to prepare allergic mice. First group was injected by subcutaneous 100ug ovalbumin in 2% alhydrogel on day-0 followed by intraperitoneal injection of 50 ug ovalbumin in 2% alhydrogel on day-14 (as shown in figure 1A). One hundred micrograms ovalbumin was given to Balb/c mice by drops intranasal on day-21.⁴ Second group of mice was injected by intraperitoneal 10 ug ovalbumin in 2% alhydrogel on day-0 and day 7 respectively. Ten micrograms ovalbumin was given to Balb/c mice by drops intranasal on day-21, 22 and 23 respectively (as shown in Figure 1B).⁵

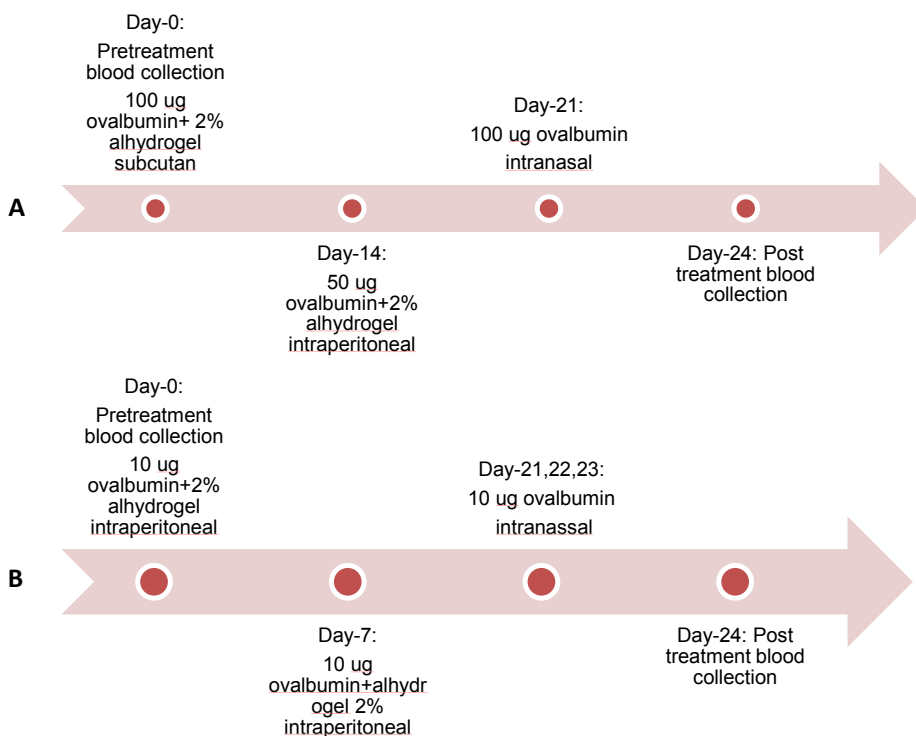


Figure 1. Sensitization Procedure of Allergy Mice.
A. First Group Treatment;
B. Second Group Treatment

On day-0 before treatment, mice body weight was measured by using digital scale (ACIS). Mice were anaesthetized by using 0.03 ml ketamine (120 mg/kg) and 0.01 ml xylazine (5 mg/kg) intraperitoneally. After anesthesia procedure, $\pm 100 \mu\text{L}$ blood was collected from orbital vein for hematology and total IgE measurements.

On day-24 allergy induction procedure according to the protocol, mice body weight was measured by using digital scale (ACIS). Mice were anaesthetized by using intraperitoneal 0.03 ml ketamine (120 mg/kg) and 0.01 ml xylazine (5 mg/kg) then intracardial blood samples were obtained by syringe injection directly to mice cardiac under anesthesia.

After blood collection, cervical dislocation was done for euthanasia of mice. Blood was divided into

2 parts, $\pm 100 \mu\text{L}$ for hematology examination and the rest was processed by 1000x g centrifugation for 10 minutes, then blood plasma was stored at -20°C . Hematology examination was performed in pathology laboratory of Primate Research Center-Bogor Agriculture University. While total IgE level measurement was performed in Department of Biochemistry FMUI laboratory by sandwich ELISA kit (eBioscience) following manual instruction.

Allergy sensitization results are as follows, from 6 mice sensitized with ovalbumin following procedure, there were no pain symptoms that required particular treatment or death of all treatment group until day-24. Procedure for allergic induction is presented in the Figure 2.

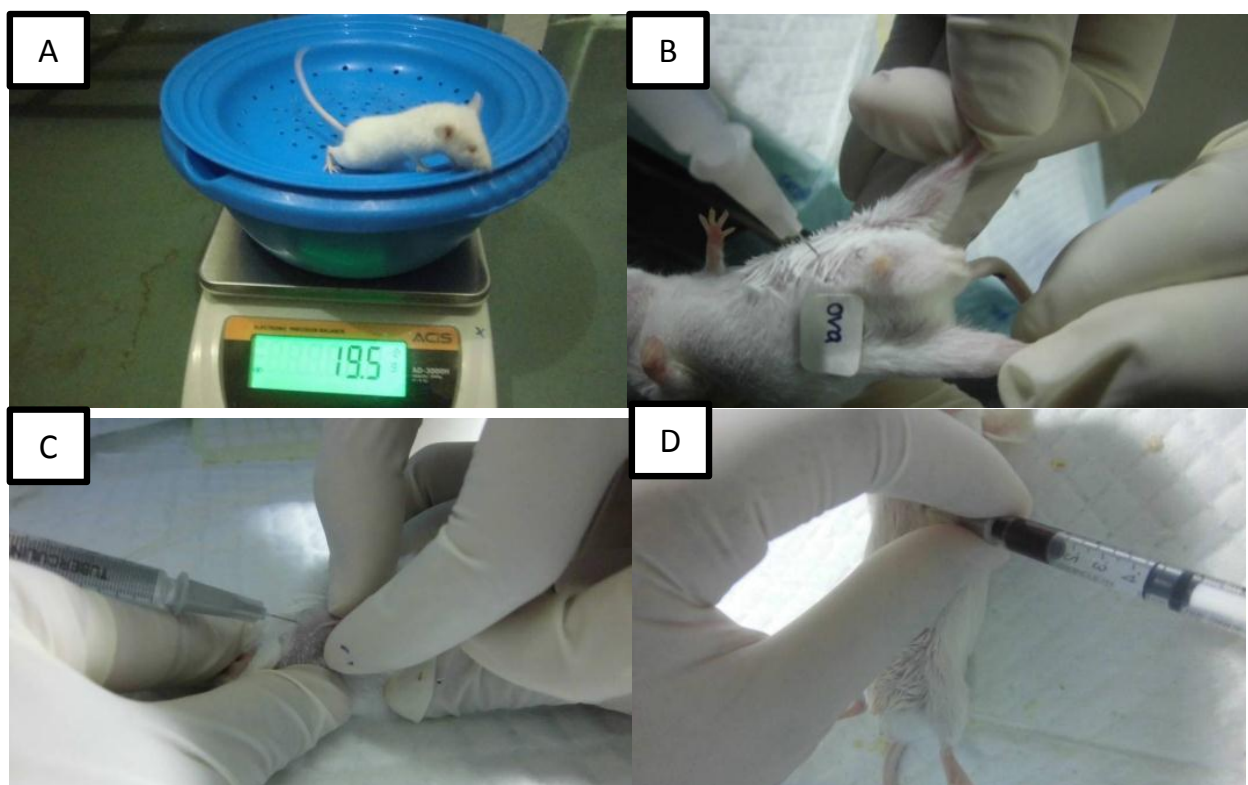


Figure 2. Allergy Sensitization Procedure of Mice, A. Weighing of Balb / C mice; B. Intraperitoneal Injection of Ovalbumin; C. Subcutaneous Ovalbumin Injection; D. Intracardial End Point Blood Collection

Results

Comparison of mice body weight in the first treatment group were significantly different between pre and post treatment ($p < 0.05$) by using paired t

test. Whereas in the second treatment group there were no differences in body weight before and after treatment ($p < 0.05$, paired t test). Comparison of body weight between the first and the second treatment groups is presented in Figure 3.

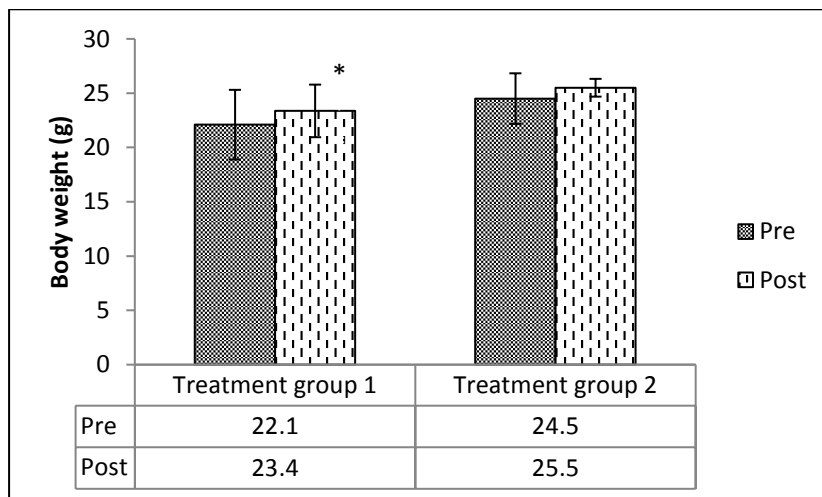


Figure 3. Comparison of Ovalbumin Induced Allergy Mice Body Weight

Figure 4 shows the comparison of hematology in ovalbumin induced allergy mice. Both of treatment group did not show statistical difference

of white blood cells, red blood cells, hemoglobin and hematocrit between pre and post treatment ($p > 0.05$, paired t test).

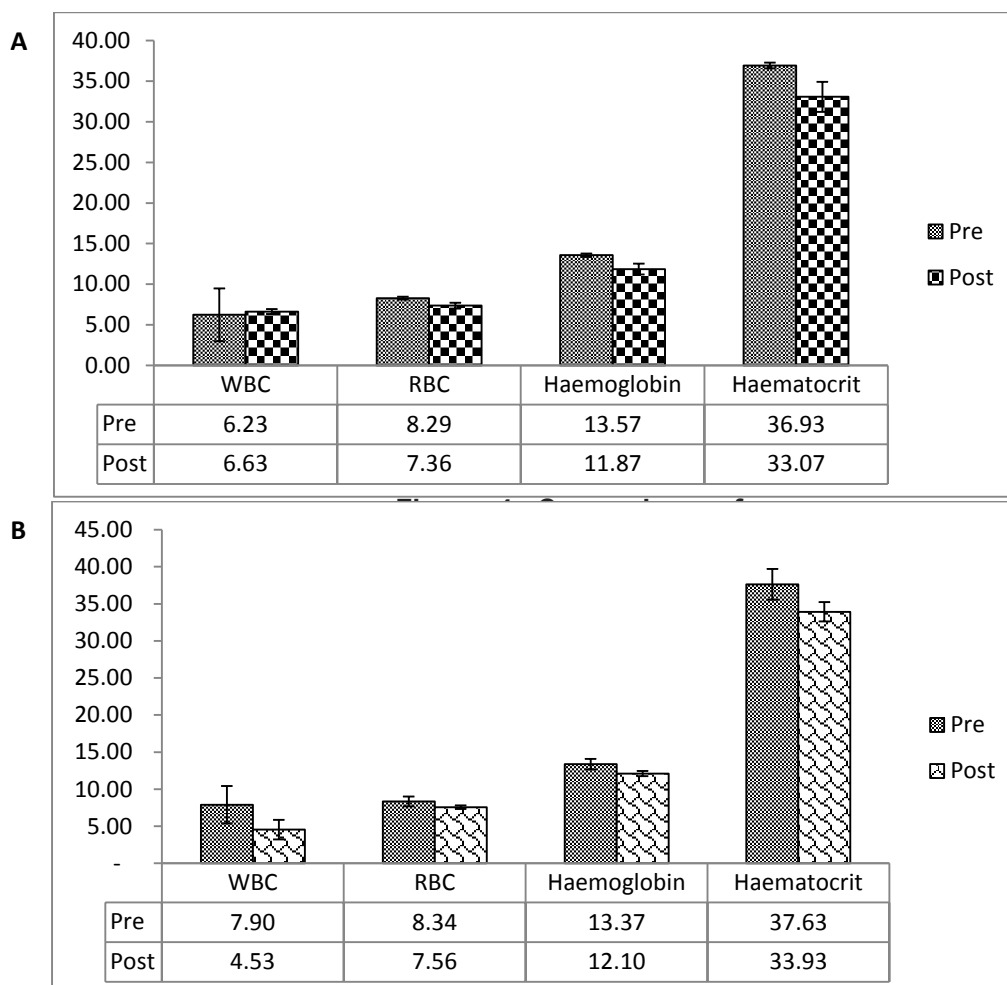


Figure 4. Comparison of Ovalbumin Induced Allergy Mice Hematology. A. Treatment Group 1; B. Treatment Group 2. WBC: White Blood Cells ($\times 10^3$ cells/uL). RBC: Red Blood Cells ($\times 10^6$ cells/uL)

Comparison of total IgE concentration is presented in Figure 5. Post treatment total IgE concentration (2495.69±2761.38 ng/mL) of treatment group 1 was higher than the pretreatment total IgE concentration (847.70±515.52 ng/

mL) although it was not statistically significant. Meanwhile, post treatment total IgE concentration of treatment group 2 (10606.32±4343.65 ng/ mL) was significantly higher than the pretreatment total IgE concentration (5676.72.70±4804.57 ng/mL) by using paired t-test (p<0.05).

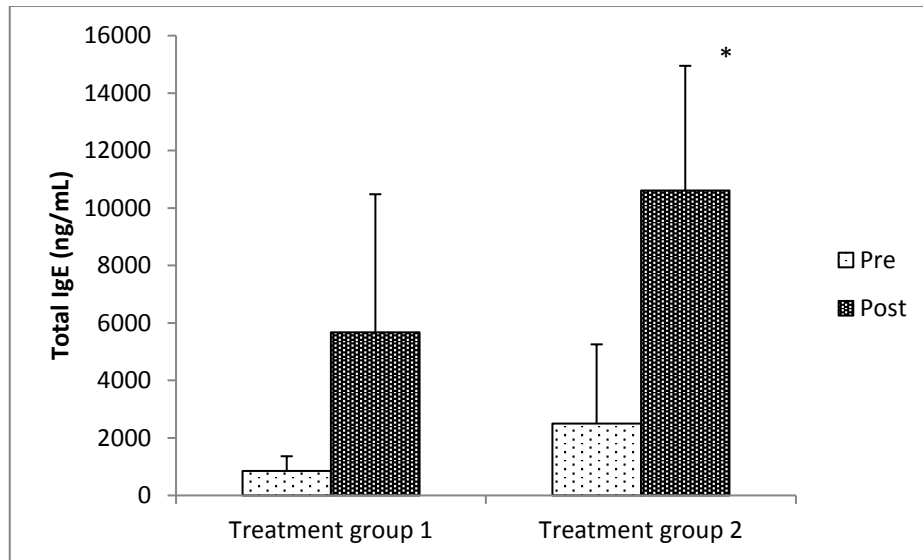


Figure 5. Comparison of Ovalbumin Induced Allergy Mice Total IgE Concentration

Discussions

Animal model of acute allergic response has been widely studied.⁶ Extended studies of allergy including pathogenesis, diagnosis, and drug development have been conducted. Drug development study needs animal model to investigate the effects of drug candidate. It is important to establish allergy mouse model which could mimic allergy response in human before drug application in clinical trial. In vivo animal models are the most appropriate tool for studying drug due to intact immune system and respiratory system.⁷

Mice is a popular animal model of the most common clinical manifestation of allergy namely asthma. Mice have several advantages compared with other species including better understanding on genetic, easy to manipulate outcomes by using transgenic technology, relatively cheap and easily sensitized by using a number of antigens.⁷

In this study, we used Balb/c mice as allergy animal model. The most commonly used strain of mouse for antigen challenge is Balb/c due to ability to develop Th2 biased immune response.⁷ However, the other strain (C57BL/6 and A/J) reported to be alternative strain for allergy as well. Balb/c mice

produce higher level of specific antibody anti-ovalbumin than C57BL/6 and A/J. Furthermore, Th2 cytokines production including IL-4 and IL-5 concentration higher than these strain.⁸

Ovalbumin was used in this study as allergen for mice sensitization. Ovalbumin is derived from chicken egg, frequently used for allergic pulmonary inflammation in laboratory rodent. However, ovalbumin is not a common allergen of human asthma. The other alternative allergen which have clinical relation in human is house dust mite and coackroch.⁹ Use of ovalbumin free adjuvant intra peritoneally was reported to induce allergy immune response on Balb/c mice.¹⁰

It is well known that allergy is a chronic disease. To mimic allergy pathogenesis, mouse model should be treated with sensitization phase which induce acute immune response and followed by challenge to induce late phase immune response mediated by cytokines and chemokines.¹¹

In this study we compared 2 methods which both use ovalbumin as allergen for short term ovalbumin challenge. Differences of these methods are route of administration, dosage of ovalbumin and method of challenge. In the first treatment group, ovalbumin

was injected subcutaneous on day-0 followed by intraperitoneal injection on day-14 and challenge by single dose of intranasal ovalbumin. While in the second treatment group, ovalbumin was injected intraperitoneally on day-0 and day-7. This treatment followed by 3 dosage intranasal ovalbumin challenge on day-21, 22 and 23.

Subcutaneous, intraperitoneal and intravenous administrations are the most common route of substance injection in mice. The rate of absorption depends on route of administration. The most rapid absorption of substance will be achieved by intravenous injection. While intraperitoneal injection absorption rate is one-quarter to one-half of intravenous route due to the large surface area of abdominal cavity. Subcutaneous injection absorption rate is lower than intravenous and intraperitoneal administration.¹² In contrary, recent study reported that subcutaneous injection was superior than the intraperitoneal route. Both methods increased house dust mite (Der p) specific IgE however only subcutaneous route shows significant airway remodelling.¹³

Alhydrogel was used as adjuvant in this study due to availability and ease in preparation. Adjuvant is a substance that enhances T and B cell activation of antigen-presenting cells. Alhydrogel contain alum which is regularly used as adjuvant in mouse model. However, a study reported that subcutaneous OVA adjuvant free protocol generates a phenotype similar to OVA intraperitoneal protocol as well.¹⁴

Dosage of ovalbumin which was used in the first treatment group was higher than the second treatment group. From both methods, there were no incidence of death. Post treatment body weight of the first group was significantly higher than the pretreatment. The same result was seen on second group as well although not statistically significant. These results showed that ovalbumin sensitization did not cause severe systemic response on mice. This is characterized by no decrease of mice appetite and body weight.

Hematology examination results of both methods including red blood cells count, white blood cells count, hemoglobin count and hematocrit percentage show no difference between pretreatment and post treatment. Reference value for mice white blood cells count is 6000-15000 cells/uL, hemoglobin 10.2-16.6 g/dL and hematocrit 39-49%.¹⁵ This results show no important effect of ovalbumin on hematology parameters. Although decrease of white blood cells and hematocrit value

on second treatment group, it was not statistically different between pre and post treatment.

Post treatment total IgE of both methods showed higher concentration than pretreatment. Meanwhile, second treatment group showed significantly statistical difference by pair t-test. Total IgE increase show effective induction of allergy. In allergy individual, IgE is highly produced as response against allergen. IgE synthesis regulation depend on individual tendency to increase Th2 response against allergen since Th2 type cytokines induce isotype switching of heavy chain to IgE class on B cells.¹⁶ Total IgE level usually used for atopy marker in human although approximately half of allergic patients show normal range IgE.³ Therefore, increase of total IgE could be an important parameter of allergy. Ideally, this result should be followed up by specific IgE measurement. In this research, considering the limitation of diagnostic kit for this purpose, total IgE level was used as allergy parameter in mice.

IgE antibody production is induced by Th2 and Tfh after allergen stimulation. Two of cytokines secreted by Th2 and Tfh are IL-4 and IL-13. IgE bind FcεRI on mast cells and basophil.^{16,17} Subsequent allergen stimulation caused mast cells and basophil activation. Activated mast cells and basophil induce three biological responses namely granule release, synthesis and secretion of lipid mediators and synthesis and secretion of cytokines. This causes various clinical symptoms of allergies.¹⁷ IgE antibodies are responsible for mast cell sensitization and antigen recognition for immediate type hypersensitivity reactions. IgE is an antibody isotype contain ε heavy chain. IgE is the most efficient in Fc receptors binding on mast cells and activating these cells compared to other isotypes.^{16,17}

In this study, allergy sensitization of both group successfully increased total IgE concentration. Allergy stimulation of second group seems superior than first group as shown by enhancement of total IgE production. The other parameters showed no significant difference between post treatment compared to pretreatment. Whereas total ovalbumin concentration volume needed for the allergy sensitization of second treatment group (50 ug/mouse) was lower than first treatment group (250 ug/mouse).

Conclusion

In this preliminary study, allergy sensitization of both treatment groups successfully increase total IgE concentration which is one of the important parameters of allergy condition. Considering rise of total IgE concentration and total albumin volume, second treatment method by using intraperitoneal sensitization and intranasal challenge is superior than first treatment method which uses combination of subcutaneous and intraperitoneal sensitization followed by intranasal challenge.

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