Allergic rhinitis behavioral changes after Indonesian house dust mites allergenic extract administration as immunotherapy

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Abstract

Background: Allergy is a hypersensitivity reaction that is generally mediated by Immunoglobulin E (IgE). More than 25% of the world's population is suspected of having these various diseases, and the prevalence and progression of these diseases have continued to increase significantly in recent years. Among these allergy-related diseases, allergic rhinitis and food allergy are the types of allergies with the highest prevalence. Clinical manifestations of allergic rhinitis include sneezing, rhinorrhea, nasal itching, and nasal congestion.

Objective: This study aimed to determine the behavioral changes of allergic rhinitis after Indonesian House Dust Mites (IHDM) allergenic extract administration as an immunotherapy

Methods*:* Eight male BALB/c mice aged 6-8 weeks in each group were treated for seven groups. The sensitization phase is given intraperitoneal, the desensitization phase is given by subcutaneous, and the challenge phase is given intranasal. The allergic parameters were observed, such as nose rubbing and sneezing. The parameters were observed for 15 minutes after the challenge administration.

Results: The results showed that the administration of Indonesian House Dust Mites as immunotherapy decreased the frequency of nose rubbing and sneezing after the administration of immunotherapy compared to the allergic rhinitis model.

Conclusions: The administration of the Indonesian House Dust Mites as immunotherapy decreased the allergic rhinitis immune response by altering the behavioral parameter.

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Introduction

Allergy is a hypersensitivity reaction commonly mediated by Immunoglobulin E (IgE).[1] Hypersensitivity reactions triggered by these immunological mechanisms conduct to the progression of various diseases such as allergic rhinitis, allergic asthma, food allergy, urticaria, and angiodermatitis. More than 25% of the world's population is suspected of having these various diseases, and the prevalence and progression of these diseases have continued to increase significantly in recent years.[2,3] In addition, these various allergic diseases significantly impact the quality of life of affected people, especially in interaction with their work and social life, leading to various comorbidities and an increased need for medical services.[4,5] Clinical manifestations of allergic rhinitis include sneezing, rhinorrhea, nasal itching, and nasal congestion.[6,7] The term allergy refers to a specific immune response that occurs dynamically after exposure to certain allergens. Allergens that cause allergic rhinitis generally include proteins and glycoproteins.[8] One of the sources of indoor allergens that are the leading cause of allergic rhinitis cases is the House Dust Mite (HDM). This happens because they are easily found worldwide and have a high allergenic potential for these allergens.[9,10]

Rapid exposure will stimulate specific immune responses

mediated by allergen-specific IgE and IgE antibodies. Allergens will simultaneously activate receptors by stimulating Protease-Activated Receptors (PARs) and Toll-Like Receptors (TLRs). This enhances T Helper Cells Type 2 (Th2)-mediated allergic reactions. On the other hand, the concentrations of allergen-specific IgE bound to the surface of mast cells and serum IgE increase with continued allergen exposure.[3] In addition, there is cross-linking between allergens, IgE, and FcεRI receptors that cause degranulation of mast cell vesicles. Mast cell degranulation is followed by the release of proinflammatory mediators such as histamines, leukotrienes, and prostaglandins,[11] leading to early-stage allergic reactions. In the early stages of allergic rhinitis, the allergic reaction manifests as sneezing, excessive and watery nasal discharge, nasal itching, and local inflammation due to sensory nerve irritation, leading to interstitial edema and acute airway obstruction due to the release of proinflammatory substances.[9,12-14]

Many components of HDM can act as Pathogen-Associated Molecular Patterns (PAMPs) that can bind to Pattern-Recognition Receptors (PRRs) on epithelial and antigen-presenting cells, including dendritic cells. On first exposure, PRRs recognize PAMPs as foreign bodies that will continue the signal to induce Th2 and IgE cell production. PAMPs known from HDM are feces and their bodies contain chitin, DNA, and endotoxins.[15] Chitin contained in the HDM exocytoskeleton can induce the innate immune system through PRRs, including TLR-2 and C-type lectin, which will induce the production of Th2 cells.[16] When chitin enters mammals, it stimulates the production of Acidic Mammalian Chitinase (AMC), which breaks down chitin and chitinase-related protein YKL-40, which binds to chitin. In asthmatic patients, the concentration of AMC and YKL-40 will be high and correlate with asthma severity.[17] Chitin induces an increase in pulmonary eosinophils and fibrosis, whereas YKL-40 induces bronchial smooth muscle proliferation and is involved in airway remodeling.[18,19]

Regarding allergic reaction control, the bulk of healing control nonetheless makes a specialty of the management of medication primarily based totally on symptom control (inclusive of antihistamines, leukotriene receptor antagonists, and corticosteroids), in which those capsules do now no longer deal with the motive of the disorder itself.[20,21] Furthermore, the pharmacological results of those symptomatic healing methods will cease quickly after the treatment is discontinued. Allergen-Specific Immunotherapy (AIT) is the most straightforward treatment that could deal with the underlying motive of allergic reactions. AIT acts immediately on suitable objectives via immunological mechanisms concerning the induction of allergen-particular immune tolerance.[22,23] Therefore, AIT is a capable healing alternative for treating allergic reaction-associated diseases. Thus, in this study, we observed the effect of IHDM allergen extract administration as a desensitization agent towards allergic rhinitis behavior.

Materials and Methods

Materials

The materials needed were IHDM allergenic extract 5 mg/mL from the previous research (Indonesia), NaCl 0.9%, Water For Injection (PT Widatra Bhakti, Indonesia), Aluminum Hydroxide,

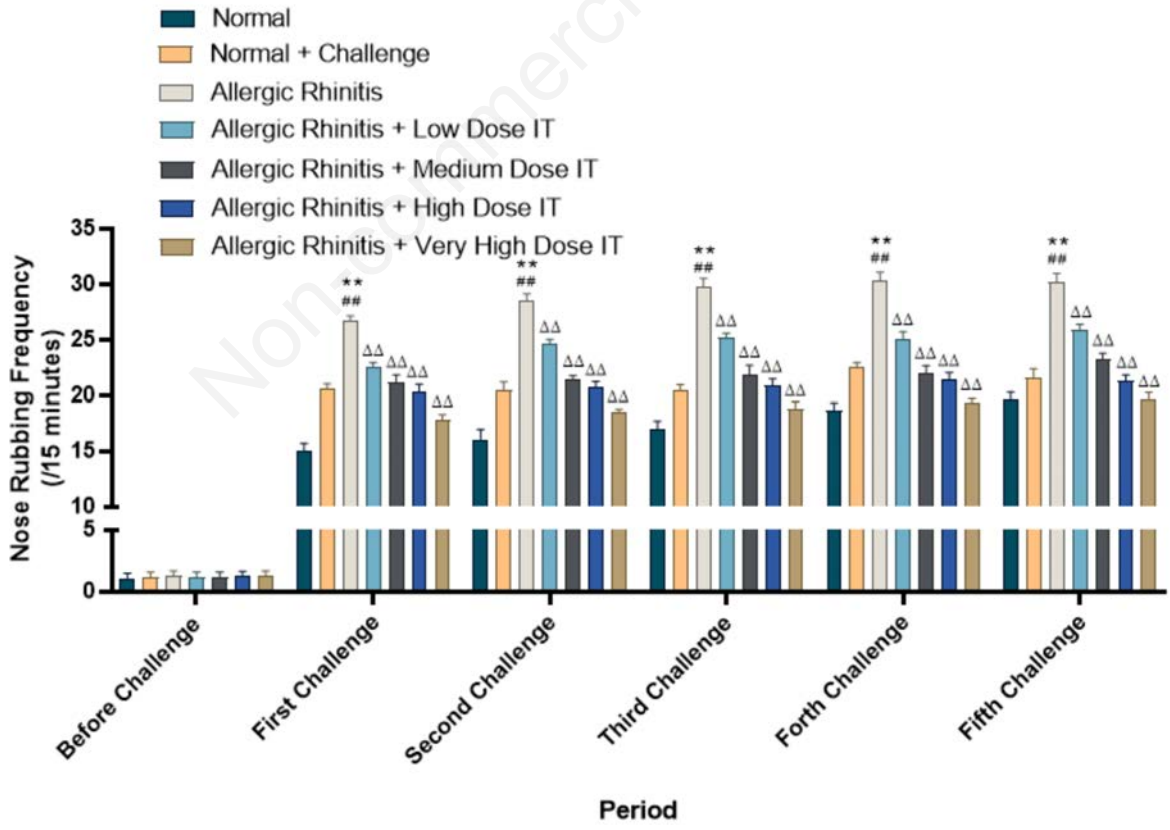

Figure 1. Frequency of nose rubbing in the treatment group. **p<0.01 against the normal group; ##p<0.01 against normal + challenge group; p<0.01 against allergic rhinitis group. The data is the mean ± SEM of 8 mice/group. Statistical tests were performed using One-way ANOVA. IHDM, Indonesian House Dust Mites allergenic extract; IT, Immunotherapy with IHDM.

Sodium Phosphate Dibasic and Monobasic (Merck, USA).

Animals

Experimental animals were adapted by keeping mice for a week before being carried out in the study. Mice were placed in groups in cages measuring 30 cm x 30 cm x 20 cm and were covered with 6 mm wire gauze. All test animals were carried out the same way, received the same food, and were kept in a well-ventilated room. The room temperature was around 22.5±2.5ºC, and the cage was cleaned daily and replaced with new husks every three days. The lighting was regulated with a 12 hours cycle of light and 12 hours of dark.

Allergen

The allergenic extract from previous research was standardized with the concentration of Der p1 is 6.13±0.78 ng/mL.

Methods

HDM-induced allergic rhinitis model and treatment protocol

Mice were given three kinds of treatment, namely: sensitization, immunotherapy, and challenge. In sensitization, mice were injected with 200 µL PBS pH 7.4 or IHDM allergenic extract (1250 µg/mL) + aluminum hydroxide (10 mg/mL) intraperitoneally on days 1, 8, and 15. As for the immunotherapy stage, the mice were injected with 200 µL PBS pH 7.4 or IHDM allergenic extract (625, 1250, 2500, 5000 µg/mL) subcutaneously on days 21, 23, and 25. Furthermore, in the challenge, mice were given 25 µL PBS pH 7.4 or IHDM allergenic extract (1250 µg/mL) intranasally on days 36, 37, 38, 39, 40, and 41. The grouping of mice was based on the simple random allocation method, in which 56 were divided into seven groups, each consisting of eight mice.

Allergic rhinitis behavioral observation

The nose-rubbing and sneezing behavior of mice were recorded for 15 minutes on days 20, 35, 36, 38, 40, 42, and 44. Furthermore, the frequency of nose-rubbing and sneezing of mice were recorded in the recordings and was calculated by two observers who were blinded by the grouping of experimental animals in this study.[24]

Statistical analysis

Data analysis using GraphPad Prism Vers. 9.0.2 to analyze the variable relationship between nose rubbing and sneezing responses after each challenge were analyzed using One-way ANOVA with a 95% confidence level. The values for all measurements were expressed as mean ± standard error of the mean.

Results

Nose rubbing behavior

The findings obtained in this study are divided into two main parts. The first part is to see the trend of nose-rubbing behavior in

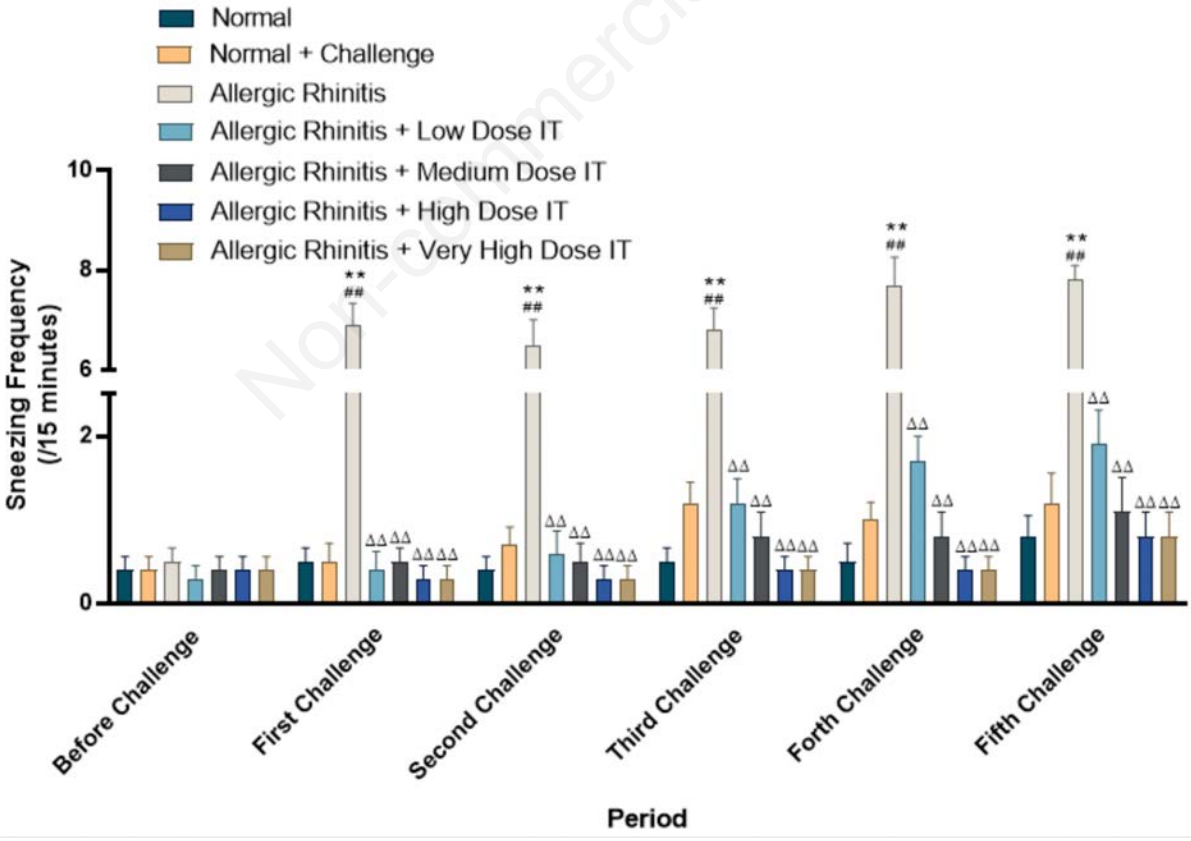

Figure 2. Frequency of sneezing in the treatment group. **p<0.01 against the normal group; ##p<0.01 against normal + challenge group; p<0.01 against allergic rhinitis group. The data is the mean ± SEM of 8 mice/group. Statistical tests were performed using One-way ANOVA. IHDM, Indonesian House Dust Mites allergenic extract; IT, immunotherapy with IHDM.

the treatment group. This can indicate that the number of challenges given is sufficient to induce an allergic response in experimental animals. The second part shows the difference in the frequency of nose-rubbing behavior between treatment groups. These results indicate no significant difference in all experimental animal groups regarding nose-rubbing responses on day 35 (without challenge). Then, there was also no significant difference in the group of normal mice compared to the group of normal mice that were given the challenge. Furthermore, there was an increase in the nose-rubbing behavior response of the allergic mice group compared to the normal group of mice and the normal group of mice that were given the IHDM challenge in normal mice. This also applies to the 2nd, 3rd, 4th, and 5th challenges.

Another result obtained was that the group of allergic mice that were given low and moderate doses of IHDM immunotherapy showed a decrease in the nose rubbing behavior response in this group after the 2nd, 3rd, 4th, and 5th challenges (treatment days 38, 40, 42, and 44) compared with a group of allergic mice that did not receive IHDM immunotherapy. In line with this, the group of allergic mice that were given high and very high doses of IHDM immunotherapy showed a decrease in the response of nose rubbing behavior compared to the group of allergic mice that did not receive immunotherapy, namely after the 1st, 2nd, 3rd, 4th, and 5th challenges (treatment days 36, 38, 40, 42, and 44). Overall, the data related to the effect of giving IHDM immunotherapy on the nose-rubbing behavior of experimental animals can be seen in Figure 1.

Sneezing behavior

In this study, it was found that there was no significant difference in response to the mice's sneezing behavior between all groups on day 35 (without challenge). On the other hand, there was no significant difference in response to the sneezing behavior of mice from the normal group compared to the normal group of mice that were given the IHDM challenge either without the challenge or after being given the challenge. Furthermore, it was found that there was an increase in the response of sneezing behavior in the group of allergic rhinitis mice compared to the group of normal mice and the group of normal mice that were given the IHDM challenge either after the 1st, 2nd, 3rd, 4th, or 5th challenge. In the group of allergic rhinitis mice that were given IHDM immunotherapy at low, medium, high, or very high doses, the mice showed a decrease in the sneezing behavior response of mice compared to allergic rhinitis mice, namely on days 36, 38, 40, 42, and 44 (consecutively the 1st, 2nd, 3rd, 4th, and 5th challenges). Based on the data obtained, the group of allergic rhinitis mice given IHDM immunotherapy tended to approach the sneezing behavior response produced by normal mice. Overall data related to the effect of giving IHDM immunotherapy on sneezing. Overall, the data related to the effect of giving IHDM immunotherapy on the sneezing behavior of experimental animals can be seen in Figure 2.

Discussion

Research on strategies to find new therapies in the management of allergies is increasing. Most current management of allergic rhinitis still focuses on the administration of drugs based on symptom control, such as leukotriene receptor antagonists, oral antihistamines, intranasal antihistamines, intranasal corticosteroids, nasal decongestants.[25,26] Allergy mechanisms, both cellular and molecular, have been described in many studies. Allergies occur due to exposure to allergens which the body responds to by producing IgE. IgE produced by the body will bind to mast cells and the incoming antigen to form the IgE-FAB (Facilitated Antigen Binding) complex. The incoming antigens will bind to IgE through the FcεRI receptor. After the complex is formed, the mast cells will degranulate and release proinflammatory mediators, such as histamine, prostaglandins, and leukotrienes. The released proinflammatory mediators will then induce an allergic response, such as vasodilation of blood vessels that cause the body part to look swollen and red.[3,26-28]

Allergic conditions are characterized by sneezing behavior, nose-rubbing, runny nose, and red and watery eyes. In this study, which observed the frequency of sneezing and rubbing the nose for 15 minutes, it was found that the normal and normal-challenge groups significantly increased in the allergy group. It also happened in the allergy and immunotherapy groups, where there was a significant decrease in the frequency of these behaviors. These results are similar to several existing studies,[29-34] due to allergic conditions releasing histamine, prostaglandins, and leukotrienes, which result in an acute allergic response.

Generally, the decrease in the frequency of nose rubbing and sneezing behavior can be suspected as a result of a decrease in the late immune response process during repeated exposure which causes complex binding between mast cells or basophil-IgE-allergens.[3,35,36] As a result, mast cells and basophils do not degranulate or release their proinflammatory mediators. The decreasing parameters can also be due to a shift ratio of Th1 and Th2. Initially, the allergy process is due to the induction of Th2 cells after the release of alarmin due to allergens. By giving the same allergen at a specific dose and time, it can shift the ratio of Th2 to Th1 so that the stimulation of B cells becomes stronger. The result is the induction of IgA and IgG, which compete with IgE for binding to mast cells or basophils. As a result, mast cells and basophils that bind to IgA or IgG will not degranulate.[3,25,27]

Until now, most of the treatment used to treat cases of allergies is still limited to treatment based on symptom reduction. Allergy treatment aimed at the immune response has not been widely developed, namely immunotherapy.[25,26] Immunotherapy has now been developed to treat allergies by shifting the immune response mechanism. Research conducted by Joudi *et al*.[37] stated that the administration of subcutaneous immunotherapy in adults with persistent allergic rhinitis could provide good clinical outcomes with improved patient quality. This is also in line with research conducted by Lundberg *et al*.,[38] which states that the use of specific allergen immunotherapy can improve clinical outcomes in allergic rhinitis patients. Therefore, AIT is a potential therapeutic option for treating allergy-related diseases.[8,9] In some guidelines, immunotherapy has been included as a line of treatment for allergic diseases. However, assistance to allergic patients to determine the right concentration and time of use is still a note in these guidelines.[20,39,40]

Conclusions

The administration of the Indonesian House Dust Mites as an immunotherapy can decrease the allergic rhinitis immune response by altering the behavioral parameter. The nose rubbing and sneezing behavior are some parameters that can reflect the physiological changes of allergy. The decrease of the parameters in this study is because of the shift ratio of Th2 to Th1 so that it could induce the IgA and IgE against the competitive binding with IgG to mast cells or basophils.

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