Development of gastro-food allergy model in shrimp allergen extract-induced sensitized mice promotes mast cell degranulation

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Abstract

Background: Food allergies have become more common in the last decade. Shrimp is one of the most dominant food allergy triggers in Asian countries, including Indonesia. After ingesting allergens, B cells will produce allergen-specific Immunoglobin E (IgE). In the sensitization period, repeated allergen exposure promotes Mast Cell (MC) degranulation in intestinal tissue and releases several inflammatory mediators, thereby causing hypersensitivity reactions. Shrimp Allergen Extract (SAE) is an immunotherapy and diagnostic agent currently being developed in Indonesia. In this study, we investigated the effect of SAE administration on eliciting an MC immunological response.

Methods: Mice were divided into a non-sensitized and sensitized group. The non-sensitized group only received 1 mg of alum (i.p), whereas the sensitized group received 1 mg of alum and 100 µg of SAE on days 0, 7, and 14. Then, both groups were challenged with 400 µg SAE (p.o) on days 21, 22, and 23 following systemic allergic symptom observation.

Results: We showed that SAE was able to increase systemic allergic symptoms significantly in the sensitized mice through repeated challenge (1.33±0.21; 1.83±0.17; and 2.00±0.00), compared to non-sensitized mice (0.17±0.17). Moreover, histopathological analysis showed that the SAE administration causes an increase of MC degranulation in the ileum tissue of the sensitized mice (44.43%±0.01), compared to non-sensitized mice (35.45%±0.01)

Conclusions: This study found that SAE could induce allergic reactions in mice by influencing critical effector cells, MCs.

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Introduction

Food allergy is an immune-mediated allergic reaction to specific dietary proteins.[1] In the last decade, food allergies have become more frequent. Seafood allergies are one of the most common food allergies. The opportunities vary according to consumption level, age, and geographic location. Seafood consumption, particularly crustaceans, contributes to two-thirds of global food production in Asia, including Indonesia. This condition makes seafood a cause of food allergies in Asia, with a prevalence reaching 7.7%. Meanwhile, seafood allergies affect approximately 2.3% of the population in the United States. Current meta-analysis studies also show that crustacean allergies, especially shrimp, are more common globally than mollusk allergies.[2-4] According to reports, shrimp allergies are the primary factor in the severity of allergic reactions that can potentially be life-threatening. Nearly 50% of patients have at least once been rushed to an emergency room for intensive care because of allergies.[5]

The gastrointestinal tract is the primary route of allergen exposure; it involves a complex immunoregulation mechanism involving the role of Gut-Associated Lymphoid Tissue (GALT).[6] Allergens can induce GALT to cause local and systemic allergic reactions such as gastrointestinal, dermatological, and ocular symptoms, as well as respiratory and anaphylactic reactions.[5-8] In the case of food allergies, ingesting allergens can cause B cells to produce allergen-specific Immunoglobin E (IgE). Additionally, the allergen-specific IgE produced will bind directly to high-affinity

IgE receptors (FcεR1) on Mast Cells (MCs) surface. This stage is also known as sensitization because the body reacts to the allergen. Repeated allergen exposure promotes cross-linking between allergens and specific IgE bound to FcεR1. Cross-linking causes MC degranulation in intestinal connective and mucosa tissue and the release of allergen-specific biologically active mediators. These events, in turn, cause hypersensitivity reactions.[9-12] Systemic symptoms and anaphylactic shock are linked to MC degranulation in connective tissue. Experimental studies in rodent models of anaphylaxis have shown that FcεR1/MCs play a significant role in IgE-mediated anaphylaxis. The expansion of MCs in the intestinal tract triggers increased intestinal permeability and absorption of systemic antigens, activating systemic MCs and stimulating respiratory and cardiovascular collapse.[13,14] Meanwhile, mast cell degranulation in the mucosa is associated with gastrointestinal symptoms that are functional, acute, and rapidly reversible.[15,16]

SAE is an allergen extract currently being developed as an immunotherapy agent and diagnostic kit in Indonesia. This SAE product has been used in clinical practice and administered to patients subcutaneously. However, considering long-term immunotherapy, non-invasive SAE product designs are required to ensure patient comfort and ease of use. Tropomyosin (TPM), the primary allergen found in shrimp, is present in SAE.[17,18] The developed SAE product contains a TPM level of 0.16-0.27 ng/mL in the crude extract. Previous studies have utilized this SAE product and shown its efficiency as an immunotherapy agent and diagnostic tool in a gastro-food allergy model in mice.[19] The parameters observed included the levels of IgE and IgG2a and the relative expression of IL-5 and IL-10 mRNA. Considering that different allergen extract manufacturers can impact allergen and product potential, standardizing SAE products in development was performed to ensure safety and effective therapy.

In this study, we used standardized SAE to develop a gastro-food allergy model in mice. The current study is the initial step in investigating the effect of SAE administration in triggering the critical effector cells in immune response, specifically MCs, in allergic mice.

Materials and Methods

Mice

Female BALB/c (*Mus musculus*) mice aged 6–8 weeks were used in this study. Mice were obtained from the Animal Laboratory, Faculty of Pharmacy, Airlangga University, Surabaya. All mice were treated under controlled conditions, fed a shrimp-free diet, and kept in a pathogen-free environment.

Preparation of SAE

A 500 g of shrimp muscles are cut into pieces and then ground. Then, 500 mL of acetone (1:1 w/v) was added to the sample to remove lipids and pigments. The process is repeated until no color is visible and filtered. The obtained residue was dried in a fume hood (25°C) until the acetone evaporated completely. The defatted samples were extracted using the maceration method in PBS pH 7.4 (1:10 w/v, 25°C). Then, centrifugation was carried out to take the supernatant. The resulting extract is collected in a sample bottle, labeled, and stored at -20°C until the following procedure. Furthermore, the extract will be prepared in 200 µL PBS with 100 µg and 400 µg SAE concentrations.

Standardization of SAE

Litopenaeus vannamei, ages 90-20 days, was used as the raw material in this study. SAE standardization is accomplished by measuring TPM levels in extracts using the Human Tropomyosin alpha-1 chain ELISA kit (Bioassay Technology Laboratory) following the manufacturer's protocol. The concentration of TPM in the crude extract ranged from 0.219 to 0.425 ng/mL.

Treatment

Previous research was used to develop a gastro-food allergy model.[19-22] In the sensitization phase, non-sensitized mice only received 1 mg of aluminum hydroxide (alum) in PBS intraperitoneally (i.p.). Meanwhile, sensitized mice received 100 µg SAE in PBS and 1 mg alum (i.p.). The treatments were administered on days 0, 7, and 14. Then, a repeated challenge using 400 µg SAE (p.o.) was administered to both groups on days 21, 23, and 25, followed by 30 minutes of systemic allergy symptom observation, to assess the success of developing a gastro-food allergy model in mice (Figure 1A). The administration of the first challenge stage (day 21) is designed to induce allergic sensitization in sensitized mice. The second stage of the challenge (day 23) aims to elicit an allergic response in sensitive mice. Furthermore, the third challenge stage (day 25) is intended to ensure that sensitized mice experience sustained effects of allergic reactions. Finally, termination was carried out on day 59.

Histopathology of Degranulated MCs

Ileum tissue samples were fixed using Carnoy's solution. The tissue was then submerged in paraffin after being washed twice for three minutes with xylene. Next, paraffin blocks were sliced to a thickness of 5 µm. The MCs were stained with 0.5% toluidine blue to identify degranulation, and the number of MCs in the ileum was measured in 10 microscopic fields randomly selected from six mice per group. Toluidine-positive cells with five or more stained granules entirely distinct from other cells are called degranulated MCs. The percentage of degranulated MCs was calculated as follows:

$$\frac{\text{Number of degranulated MCs}}{\text{Total number of MCs}} \times 100\%$$

All observations were made with a 400x magnification light microscope, and images were captured with a digital microscope camera at 1000x magnification (OPTIKA, B-190TBPL Digital Binocular Microscope, Italy).[23,24]

Assessment of systemic allergy symptoms

Systemic allergy symptoms were measured for 30 minutes following the challenge using the scoring system described below: no symptoms (0); scratching and rubbing around nose and head (1); puffiness around the eyes, reduced activity with or without increased respiratory rate (2); wheezing, labored respiration, cyanosis around the mouth and tail (3); no activity after producing or tremor and convulsion (4); and death (5).[20-25]

Statistics

The data is presented as the mean ± SEM and analyzed using the Unpaired T-test for % MC degranulation and the Mann-Whitney U test for systemic allergy symptoms scores, followed by a post hoc test using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA). A p-value <0.05 was considered statistically significant.

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Results

SAE sensitization increases systemic allergy symptoms in mice

Figure 1B represents systemic allergy symptoms following sensitization. Repeated challenge (400 µg, p.o) after sensitization with SAE (100 µg, i.p.) increased systemic allergy symptom scores significantly in the sensitized mice (1.33±0.21; 1.83±0.17; and 2.00±0.00), compared to non-sensitized mice (0.17±0.17). These results show that gastrointestinal allergy modeling in mice was successful after the sensitization phase.

SAE sensitization increases the number of degranulated MCs in the ileum tissue

The number of degranulated MCs in the ileum tissue correlates with the severity of food allergy symptoms.[26-27] In addition, as previously mentioned, the degranulation of MCs triggers the release of various inflammatory mediators. Therefore, this study also evaluated the number and morphology of MCs in the ileum tissue of mice using toluidine blue staining. Histological analysis showed that a significant increase in degranulated MCs occurred in the sensitized mice (44.43%±0.01), compared to non-sensitized mice (35.45%±0.01) (Figure 2A).

Figure 2B shows the morphology of MCs in ileum tissue. Intact MCs have many viscous intracellular granules that stain intensely with toluidine blue in the cytoplasm and appear violet in color. On the other hand, degranulated MCs have blurred cell membrane boundaries, increased cell membrane shrinkage, and scattered granules throughout the cells.[28,29]

Discussion

In this study, SAE was used as an agent to induce a gastro food allergy model in experimental animals. During the three challenge periods, the SAE-sensitized group experienced an increase in systemic allergic symptoms. The success of this *in vivo* gastrointestinal allergy modeling demonstrates that SAE has a high potential for development as a diagnostic agent. This result was consistent with previous studies that developing a food allergy model using female BALB/c mice induced with TPM (Pen a 1) or its recombinant (rMet e 1) and cholera toxin increased systemic allergic symptoms such as scratching or scratching the head, swelling in the eye area, shortness of breath, and seizures.[5,20,21,30,31]

It has been reported that MCs can play a critical role in developing the acute manifestations of allergic disorders. MCs are long-lived cells whose number, distribution, phenotype, and function are all influenced by innate and adaptive immune response factors. The most critical mechanism of MC activation in allergic disease

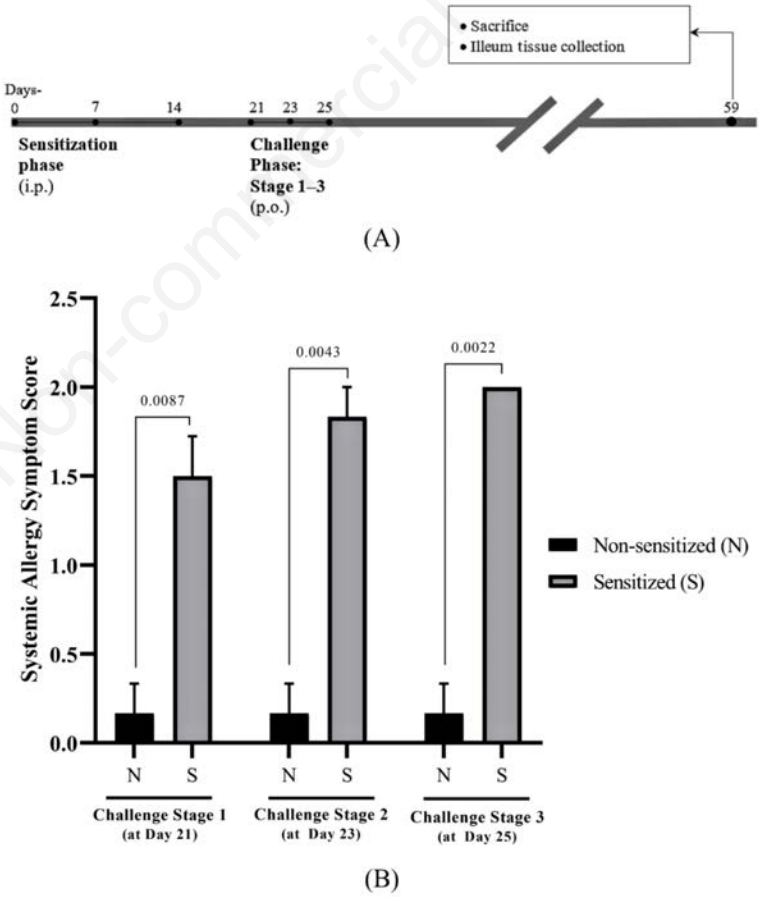

Figure 1. SAE causes systemic allergic reactions in sensitized mice. (A) Experimental protocol. The gastro-food allergy model is induced during the sensitization phase and assessed by challenges administration. (B) Effect of SAE on systemic allergy symptoms after the sensitization phase. Each bar represents the mean ± SEM value (n=6).

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is through FcεR1. Cross-linking of FcεR1 can cause MCs to degranulate, which leads to the release of various inflammatory mediators (cytokines, histamine, platelet-activating factor, and prostaglandins). The release of these mediators can influence tissue remodeling and function and regulate inflammation. Thus, MCs play an essential role as effector cells in IgE-mediated allergic reactions. In allergic conditions, degranulation can occur within minutes.[32]

Meanwhile, cytokines can be derived from granule storage or, more commonly, transcription and produced within 2-6 hours. FcεR1-mediated reactions were increased by the cytokines IL-3, IL-4, and IL-5. Furthermore, degranulation of MCs induced by IgE and allergens results in a temporary loss of Treg function, resulting in histamine-mediated impaired peripheral tolerance. In animal studies, MCs in the intestinal mucosa have previously been shown to be important effector cells in IgE-mediated food allergies.[26,33-36] In line with previous research, our study found an increased percentage (%) of degranulated MCs in the ileum mucosa of allergic mice. Thus, the present study confirmed that SAE administration can induce allergic reaction.

Since there is no active allergy medication, the prospect of development therapy is still under investigation. Nevertheless, the present study can serve as a starting point for developing safe and effective food allergy treatment strategies. AIT has recently emerged as a promising treatment option for IgE-mediated food allergy. This strategy involves administering specific food allergens, such as TPM, and gradually increasing doses until a maintenance dose is reached. Allergen accumulation can alter how the immune system responds to allergens by turning allergies off, reducing allergic symptoms, and inducing allergic tolerance. The event was caused by a shift in the Th2/Th1 ratio. It occurred when the number of Th2 cells decreased due to apoptosis and anergy, followed by a decrease in inflammatory cytokines. Meanwhile, the number of Th1 cells has increased. Finally, the body develops an immunity to allergens.[37-40] According to the AIT paradigm, thus we contend that SAE also can be developed as an immunotherapy agent. Therefore, further research into the role of SAE in allergic mechanisms is required.

Conclusions

In conclusion, this study found that SAE can be used to induce allergic reactions in mice by influencing critical effector cells, which are MCs. However, more studies on the role of SAE in allergic mechanisms are required to identify and develop attractive therapeutic targets or strategies for food allergies in the future.

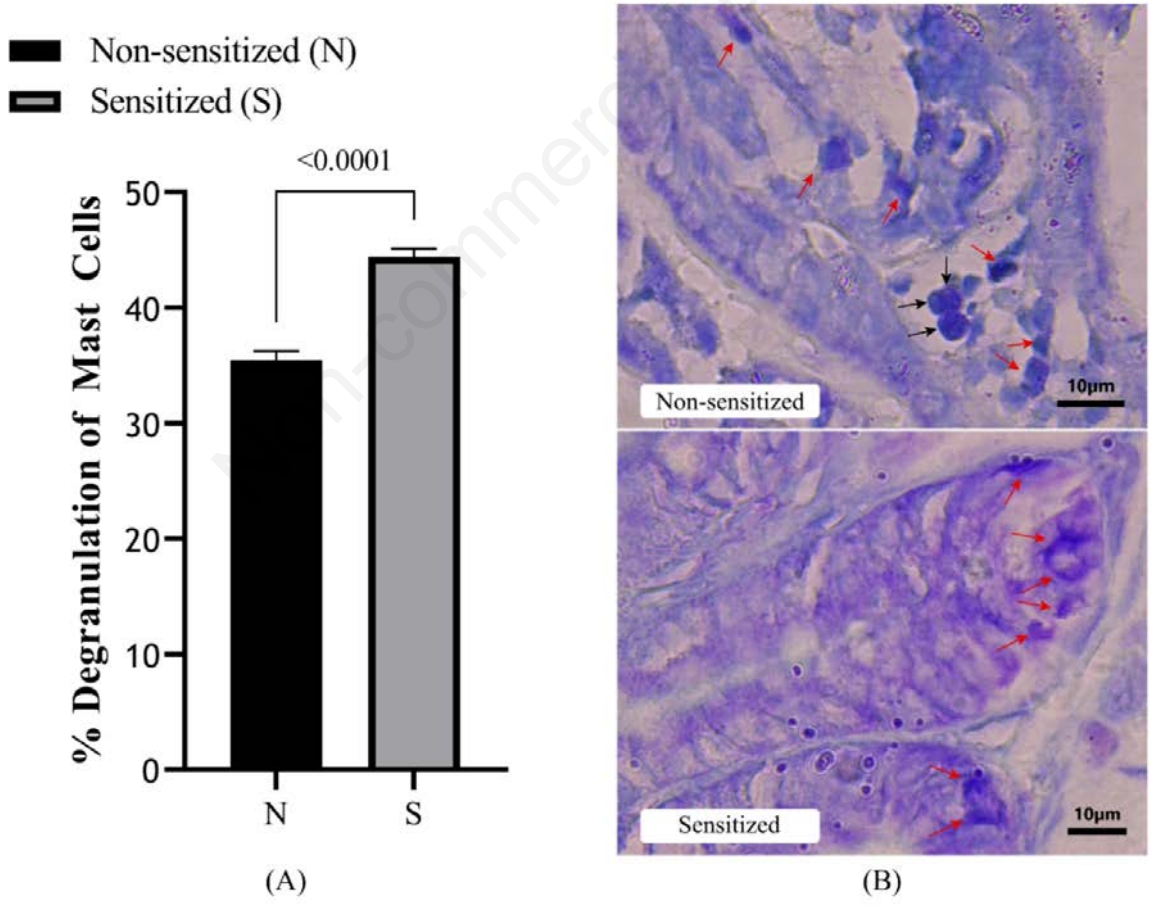

Figure 2. SAE stimulates MCs in gastro-food allergy mice. (A) The percentage of degranulated MCs in the intestinal tissue of the experimental animal. Each bar represents the mean ± SEM value (n=6). (B) Representative ileum specimens stained with toluidine blue. Sections were photographed at 1000x magnifications using a light microscope. Intact mast cells showed as black arrow, and degranulated MCs showed as red arrow.

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