Preventive effect of kefir in BALB-c (Mus musculus) induced by ovalbumin towards relative amount of B cell-Ig-E

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Abstract. Food allergy or hypersensitivity is defined as "all reactions that are triggered by immune cells after food intake, including hypersensitivity type 1 reactions, mediated by IgE produced by Plasma B cells. IgE will bind to mast cells causing degranulation and release of inflammatory products resulting in tissue damage. This study aims to determine the preventive effects of Kefir on Balb-C Mice (Mus Musculus) induced by ovalbumin on the relative number of B-Ig-E cells. The research method is a true experimental laboratory control post design only consisting of 5 treatments and 4 replications, consisting of negative control (healthy): placebo NaCl Physiological, positive control (ovalbumin at a dose of 20 μg/mice and adjuvant Al (OH)₃ 1000 μg on days 8 and 15 and re-induced orally with a dose of 60 mg /mice and 10 mg /mice Al (OH)₃ on the 29th day), T1, T2, T3 (kefir for 14 days with doses of 300, 600 and 900 mg /kg body weight and ovalbumin). Data on the relative amount of B+ cell-Ig-E are quantitative then analyzed using the One-Way ANOVA test with a 95% confidence level to find out the differences between treatments significantly. The results showed that administration of kefir and ovalbumin to the relative number of B+ cells were not significantly different between the treatment groups while the decrease in the relative amount of IgE+ occurred in the T1 group. The conclusion shows that administration of kefir can decrease B+ cell-Ig E+ in ovalbumin-induced mice.

1. Introduction

Food allergies or hypersensitivity are defined as "all reactions that are chained down by immune cells after food intake [1], including type 1 hypersensitivity reactions, mediated by IgE [2]. Allergies due to food consumption take place very quickly, some minutes (3-5 minutes) and the process occurs in the intestine which will stimulate the activity of Th2 cells so that the activity of Ig E. occurs. Initially, the capture of antigens in the form of food is mediated by dendritic cells (CD13) which are receptors of human intestinal epithelial cells. to B cells, then B cells will differentiate into plasma cells and produce
antibodies M (Ig M) IgM will switch to form Ig E. Ig E will bind to mast cells causing degranulation and release of inflammatory products CD23 (Ig E receptors) triggers pro-inflammatory epithelial signals such as IFN-γ and release of chemokines (interleukin (IL-8 and CCL20) [3]. These chemokines can then be used and recruitment of inflammatory cells such as basophil cells, and eosinophil cells as part of the final phase reaction, and recruitment of dendritic cells to respond to immune and antigen responses.

Food allergic reactions are estimated to be around 1% - 6% of all dog and cat diseases in general practice and 10% - 20% of all cases seen in dermatology practice. Research shows that 33% of dogs studied were less than 1 year old when clinical signs of food allergies were obtained. Certain dog breeds are more susceptible to food allergies such as soft-coated terrier wheaton, Dalmatians, White West Highland terriers, collies, shar-peis, Lhasa apsos, cocker spaniels, spring spaniels, miniature schnauzers, Labrador feeders, dachshunds, puddles, German shepherds, golden retriever. The average age for clinical signs in cats is 4 to 5 years [2].

Confirmation of the diagnosis of food allergies through a thorough patient's medical history including dietary history and complete physical examination. Tentative diagnoses of food allergies are other pruritic disorders, such as atopy and allergic dermatitis. So it is needed supporting diagnoses such as skin scrapings, cytological samples, and fungal culture, depending on the physical examination findings as well as the clinical signs and history of the patient [2]. Handling food allergies can by avoiding these allergens and taking anti-histamine drugs.

Allergic disease is characterized by Th2 cell immune responses in response to excessive influx of antigens or proteins in the body such as food and ectoparasites. The Th2 response will suppress Th1 activity to help maintain an allergic phenotype [4]. Eosinophils function to maintain intestinal homeostasis, which can have a significant impact on the nature of endogenous intestinal microflora and response to intestinal pathogenic microbes [5].

Microbial exposure functions to repair intestinal microbes through signals to develop the mucosal immune system [4]. Intestinal dysbiosis (intestinal microbial imbalance) precedes the onset of clinical allergies, through changes in immune regulation. Probiotics contain beneficial bacteria and yeast in the intestine which play a role in stimulating Th1 cells and inhibiting Th2 through IFN-stimulus stimuli produced by Th cells so that they can reduce inflammation as a result of allergic responses through bioactive peptide content [6] besides kefir also contains vitamins and bacteriocin [7] Goat milk contains higher fat which is 4.2% compared to cow's milk, while the percentage of carbohydrate and water is lower [8]. According to research conducted by Ruei-Liu et al [9] that kefir consumption from cow's milk and soy milk can reduce the response of Ig E and IgG1 and alter the normal flora of the intestine in preventing food allergies and increasing resistance to pathogenic microorganisms in the gastrointestinal.

2. Material and methods

2.1. Animals

Male mice strain of Bulb C aged 8-12 weeks with body weight of mice between 20-25 grams from the Biosciences Laboratory of Brawijaya University Malang, kefir after obtaining from the Faculty of Animal Husbandry, Brawijaya University, Ovalbumin vial preparations (Sigma Company), B cell antibodies and Ig E anti-mice and pellet feed.

2.2. Ovalbumin induction

BALB/C mice were injected with 10 μg OV /head IP (Ova A5503; Sigma-Aldrich) mixed with 500 μg aluminum hydroxide (Alum) gel (AlH₃O₃; Sigma). Ovalbumin dose of 20 μg/head and adjuvant Al (OH)₃ 1000 μg in 0.5 ml of sterile distilled water IP on the 8th and 15th day and then returned to OVA on the 29th day through PO (dose of 60 mg / head and 10 mg / head of Al (OH)₃ in 1 ml of sterile aquades. The severity of the allergic response is assessed 30-45 minutes after the last induction by measuring changes in body temperature and evaluating clinical scores (component of the severity of diarrhea) 10]. The study group consisted of 5 treatments: Positive control (ovalbumin), Negative control...
Healthy mice were given a placebo NaCl Physiologically orally (0.5 ml / head) on days 1-14), T1 (kefir dose 300 mg/kg bw for 14 days+ ovalbumin), T2 (kefir dose 600 mg /kg bw for 14 days+ovalbumin), and T3 (kefir dose 900 mg /kg bw for 14 day + ovalbumin). Research using a treatment of experimental animals has obtained ethical approval from the Research Ethics Commission No 959 KEP Brawijaya University.

2.3. Preparation of Kefir
1 Liter of skim milk mixed with 100 gram (10%) kefir grain fermented at 40-50ºC for 18-24 hours. Furthermore, the finished kefir is mixed with 5% pasteurized sheep's milk so that the concentration of lactic acid bacteria is 107 cfu / g and fermented again at 18-25ºC for 12-15 hours and stored at 1-3ºC until used [11].

2.4. Flow cytometry analysis
The mice were then extracted and the spleen followed by flow cytometer test to calculate the relative number of B cells and Ig E. Intracellular cells for fixation and increased membrane permeability using CytoFix / CytoPerm kit buffer (eBioscience) [10].

3. Results and discussion
3.1. Relative amount of B cell
The results showed that positive control had a relatively lower number of B cells compared to negative control (figure 1). This shows that in healthy conditions, mice cells undergo activation and proliferation and differentiate into plasma B cells and produce antibodies. The antibodies formed in the mucosa of the digestive tract are Ig A antibody secretory. B cells will regenerate and mature from naïve B cells from bone marrow, and will carry out an apoptosis program to kill damaged cells [12]. In conditions exposed to ovalbumin, plasma B cell proliferation occurs to produce Ig A antibodies.

![Figure 1. Relative amount of B cells after Kefir treatment. Different notation (a, b, c) shows that Relative number of B cells is significantly different between treatment groups (P <0.05)](image-url)

In the treatment group that was given kefir as preventive, after mice induced with ovalbumin showed increased activation and proliferation of B cells compared to the positive control group that was only given ovalbumin. T3 group showed the most optimal for B cell activation compared to the other treatment groups. Kefir functions to accelerate the activation of plasma B cells through Th cells. This is in accordance with research conducted by Wold [13], that Lactobacillus probiotics can accelerate the
humoral immune response through the activation of antibodies in the mucosa to produce Ig A as a protection against allergens attached to the mucosa.

3.2. Relative Amount of Ig E antibody
The results showed that the relative amount of Ig E antibody in positive control was higher than negative control (figure 2). IgE antibodies play a role in 'immediate hypersensitivity' of mucosal tissue. Ig E antibodies will bind to IgE receptors (FcεRI) in mast cells and basophils in the blood circulation. Sensitization will then occur to cause anaphylactic degranulation, which is characterized by the release of inflammatory mediators from arachidonic acid metabolism. Effects of inflammatory metabolism include vasodilation and increased muscle permeability which will appear clinical symptoms such as rhinitis, asthma, atopic dermatitis, and food allergy [12]. In the preventive group by giving kefir showed a decrease in all treatment groups, but the one that was not significantly different from the negative control (healthy mice) was the T1 group.

![Figure 2](image)

**Figure 2.** Different notations (a, c, d) indicate the Relative number of Ig E antibodies significantly different between treatment group

Exposure to oral antigens will stimulate an increase in Th2 cells. Th2 cells will initiate an allergic response quickly through the release of interleukins, especially IL-4 and IL-5, which will induce IgE production, regulate eosinophil production and function, and stimulate the growth of mucosal-type mast cells. Instead Th1 cells are involved in classic delayed hypersensitivity which inhibits Th2 cells [14]. Ig E will bind with mast cells to remove inflammatory products that can cause diarrhea [3]. Allergens are captured by presenting cell antigens then presented to B cells, and switching antibodies occur to produce Ig E antibodies.

The incidence of allergies is influenced by several factors including: type of allergen, animal strain, route of administration, dosage of administration, and environment. Types of allergens such as including lipid transfer protein (LTP) and albumin 2S or superfamily cupin, including 11S and 7S globulin. Animal food allergies are dominated by parvalbumin, tropomycin and casein proteins. Protein structure and IgE binding contribute to allergens [15].

Animal mice are important for measuring sensitization to new foods in the form of protein in vivo. Higher peanut specific IgE concentrations were found in BALB/c mice compared to other strains. Otherwise, there was no difference between BALB/c and C3H/HeOuJ mice on the IgE response to β-lactoglobulin (BLG) but C3H / HeOuJ mice specific IgE levels of protein are higher. As an alternative to mice, Brown Norway (BN) which has a strong IgE antibody response can be used in addition to their large size making it possible to monitor serum kinetics of specific antibody responses in individual
animals. On the negative side, oral doses of mice require more protein compared to mice which affects the cost of the experiment besides that less immunological reagents are available for mice [15].

There are several routes used to induce allergic sensitization food allergens including intraperitoneal (i.p.), oral, intranasal (i.n.) and skin. The delivery route can change the response that is generated. For example, i.p. sensitization with wheat protein will induce a specific IgE response similar to Epitope that binds to IgE in humans, sensitization with OVA by I.P causes binding of IgE to be more specific than oral sensitization. Sensitization to food allergens such as beans or cow’s milk can occur in the intestine with oral sensitization. Oral exposure of peanut extract in C3H / HeJ mice (PE) combined with cholera toxin toxin is able to induce specific IgE in serum and show systemic symptoms of anaphylaxis. subcutaneous sensitization with OVA, without aluminum hydroxide, results in higher levels of antibodies compared to via i.p. administration of OVA with aluminum hydroxide [15].

Kefir is fermented milk produced from kefir grains consisting of a specific and complex mixture of bacteria and yeast that live in a symbiotic relationship. The nutritional composition of kefir varies according to the composition of milk, the microbiological composition of the kefir grains used, the time and temperature of fermentation and storage conditions. Kefir originates from the Caucasus and Tibet [16]. Kefir seeds or kefir grains are gelatinous protein masses and polysaccharide structures that contain various species of lactic acid bacteria, acetic acid bacteria, and yeast, which form a natural microbial ecosystem. Kefir has a different taste because of the presence of various compounds produced during the fermentation process. Lactic acid is the main metabolite produced; Other important metabolites produced are carbon dioxide and ethanol at low concentrations and flavor components such as acetaldehyde and acetoin, bioactive peptides, vitamins, exopolysaccharides, and bacteriocin. This compound can act directly or in combination to provide many beneficial health effects associated with kefir consumption [7].

The benefits of kefir as health include increasing digestive metabolism and tolerance to lactose, antibacterial, hypo cholesterol, plasma glucose control, anti-hypertension, anti-inflammation, antioxidant activity, anti-carcinogenic activity, anti-allergic activity and healing [16]. Probiotics bind to toll-like receptors causing macrophage activation. Macrophages will produce cytokines IL-12 to induce Th1 cells and NK cells to produce IFN-γ. Cytokines will inhibit Th2 to prevent the activation of B cells to produce IgE which plays a role in the allergic process [17].

4. Conclusion
Giving kefir can increase the activation of the relative number of B cells and decrease IgE in mice induced by ovalbumin.

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