

Allergic and Anaphylactic Response to Sesame Seeds in Mice: Identification of Ses i 3 and Basic Subunit of 11s Globulins as Allergens

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Key Words

Sesame · Food allergy · Cytokines, type 2 · IgE

Abstract

Background: Allergy to sesame seeds is an emerging food allergy of a serious nature due to a high risk of systemic anaphylaxis. Although a mouse model to study sesame anaphylaxis is desirable, currently it is not available. Here, using a transdermal exposure model system, we tested the hypothesis that sesame seed elicits IL-4-associated IgE antibody response with consequent clinical sensitization in mice. **Methods:** Groups of BALB/c mice were exposed to sesame seed extract or saline or a control food (vanilla bean extract) by transdermal applications. Systemic IgE, IgG1 and IgG2a antibody responses were examined using preoptimized ELISA. Type 2 and type 1 cytokine responses were evaluated by ex vivo antigen-mediated activation of spleen cells. Clinical response to oral sesame challenge was studied. Western blot and N-terminal amino acid sequence analyses were performed to identify the sesame allergens. **Results:** Transdermal exposure to sesame elicited robust IgE and IgG1 but very little IgG2a antibody responses. IgE re-

sponse to transdermal exposure in two high-IgE responder mice strains with disparate MHC confirmed the intrinsic allergenicity of sesame seed. Transdermal sensitization was associated with activation of IL-4 but not IFN- γ . Furthermore, oral exposure to sesame resulted in clinical signs of systemic anaphylaxis. Western blot and sequence analysis identified four allergens including Ses i 3 and the basic subunit of 11s globulins. **Conclusion:** These data argue that transdermal exposure to sesame seed can result in IL-4 activation, IgE response and clinical sensitization for systemic anaphylaxis.

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Introduction

Food allergy afflicts 6–8% children and ~2–4% adults in many westernized countries [1]. Prevalence of this disorder appears to be increasing consistent with other atopic disorders for reasons that are not completely clear at present [1, 2].

Whereas global prevalence of sesame allergy in particular is unknown, recent evidence suggests that it may be on the rise [3]. Thus, there are a growing number of reports of sesame allergy from many countries including the USA, the UK, Finland, France, Germany, Italy, the Netherlands, Spain, Switzerland, Israel and Japan as re-

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viewed recently [3]. Furthermore, sesame allergy is often associated with systemic anaphylaxis similar to peanut and tree nut allergies [1, 3]. Accordingly, sesame is considered as a major allergenic food for labeling purpose by the European Union as well as the Canadian Food Safety Inspection Agency [4, 5].

In the recent past, enormous efforts have been undertaken to develop animal models of food allergy. Consequently, at present, there are animal models for some food allergies including peanut, hazelnut, milk, egg and fish [6–9]. In contrast, currently we are not aware of an animal model reported to study sesame seed allergy.

Although sensitization to sesame is thought to occur via the oral route in humans, there are reports suggesting that occupational exposure to sesame in the baking industry may also result in sensitization [10, 11]. Furthermore, a recent study suggested that sensitization to peanut via skin may occur in infants and children due to the use of peanut-containing diaper rash creams or baby lotions [12]. Thus, it remains possible that transdermal exposure may be relevant in sensitization to food proteins. However, there is a lack of direct demonstration whether sesame can cause transdermal sensitization in animal models. Here we tested this possibility using a mouse model.

Although a mouse model to study sesame allergy is desirable, currently it is unclear whether sesame can elicit IgE antibody response in mice as it does in humans. Here, using a transdermal exposure model system, we tested the hypothesis that sesame seed elicits classical IL-4-associated allergic (IgE antibody) response with consequent clinical sensitization in mice.

Materials and Methods

The following materials were purchased from sources as indicated in parentheses: sesame seed and vanilla bean protein extract (Greer Labs, Lenoir, N.C., USA); food extracts were tested for protein content by Lowry's method; biotin-conjugated rat anti-mouse IgG1, IgE and IgG2a antibody; paired antibodies and recombinant standards for mouse IL-4, IL-5, and IFN- γ (BD PharMingen, San Diego, Calif., USA); *p*-nitro-phenyl phosphate (Sigma, St. Louis, Mo., USA); streptavidin alkaline phosphatase (Jackson ImmunoResearch, West Grove, Pa., USA).

Mice Immunization and Bleeding

All mice were purchased from the Jackson Lab (Bar Harbor, Me., USA). Only adult animals (6–7 weeks age) were used in the study. All animal procedures used were in accordance with the Michigan State University policies. Groups of mice ($n = 3$ –7) were exposed to saline or food extracts via transdermal exposure as described earlier [6, 13]. In each mouse the reagent was applied to the back skin where hair had been clipped off and was covered with

nonlatex bandage for 3 days. Following 4 days of rest, the cycle of exposure to saline or control food or sesame seed extract was continued for 6–13 weeks. Weekly blood samples were collected and plasma was used in the antibody analysis.

Measurement of Sesame-Specific IgE, IgG1 and IgG2a Antibodies in the Plasma

We have previously described optimization of enzyme-linked immunosorbent assay (ELISA) for food specific IgG1, IgE and IgG2a antibody analyses [14]. The ELISA procedure used in this study was essentially as described by us before using pooled plasma samples [14].

Spleen Cell Culture and Cytokine Analyses

Spleen cells were harvested and standard cell cultures were set up essentially as described [6]. Briefly, spleen cells were cultured (7.5 million/ml) in the absence and presence of sesame protein extract (100 and 500 μ g/ml). Cell culture in the presence of medium alone served as a negative control. Cell culture supernatants were harvested at indicated time points for use in cytokine analyses using preoptimized ultrasensitive assays (assay sensitivity: IL-4: 0.1 pg/ml; IL-5: 2.8 pg/ml; IFN- γ : 0.72 pg/ml).

Induction of Systemic Anaphylaxis, Clinical Scoring, Measurement of Rectal Temperature

Mice were gavaged with either sesame (13 μ g/mouse; 0.5 ml volume) or saline (0.5 ml/mouse) and observed for clinical signs of systemic anaphylaxis during the next 30 min by 2 individuals in a blinded manner according to the method described previously [8]. The scores were: 0 = no symptoms; 1 = scratching and rubbing around the nose and head; 2 = puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3 = wheezing, labored respiration, cyanosis around the mouth and the tail; 4 = no activity after prodding, or tremor and convulsion, and 5 = death. Rectal temperature was measured using a temperature probe (Yellow Springs Instrument, Yellow Springs, Ohio, USA) before and after oral challenge.

Western Blot and N-Terminal Amino Acid Sequence Analysis

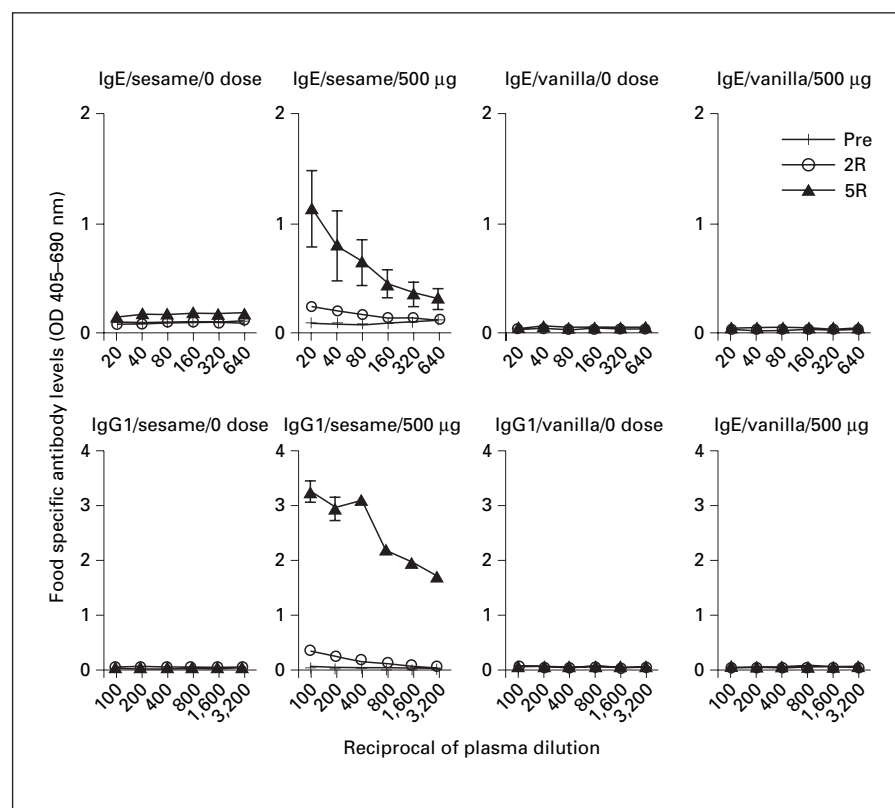
Sesame protein extract was subjected to 12% SDS-PAGE and then electrophoretically transferred onto PVDF membrane (Bio-Rad) for 12 h at 4°C. The blot was blocked (4% nonfat dry milk) and then incubated with Protein G (Pharmacia)-treated plasma samples. Following washing (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20), protein-bound IgE was detected using biotin-labeled rat anti-mouse IgE followed by streptavidin-HRP conjugate (Jackson Immuno-Research, Pa., USA). The blot was developed with a chemiluminescence detection kit (Super Signal West Femto Maximum Sensitivity Substrate, Pierce Biotech., USA).

The IgE binding proteins were isolated and used in sequence analysis. N-Terminal amino acid sequencing was performed at the macromolecular structure facility (Michigan State University) on a Perkin Elmer/Applied Biosystems 494cLC Procise Protein Sequencer which utilizes Edman Degradation Chemistry.

Statistical Analysis

Student's *t* test was used to evaluate significance using Analyse-It™ software program (Analyse-It software, Leeds, UK). The statistical significance level was set at 0.05.

Fig. 1. Immune response to sesame seed following transdermal exposure in BALB/c mice. Groups of mice (n = 4 per group) were exposed to saline or sesame seed extract or a control food (vanilla) extract by transdermal applications. Food-specific IgE (**a**) and IgG1 (**b**) antibodies were measured using preimmune plasma and following 5 weeks of exposure. Data is shown from preimmune plasma, secondary response (2R) and fifth response (5R).



Results

Characterization of Sesame-Specific Antibody Responses in BALB/c Mice

Preimmune plasma had neither detectable sesame- nor vanilla-specific IgE antibodies (mean titer <20) or significant amounts of IgG1 antibodies (mean titer <100). Following several transdermal exposures to food extracts, elevated levels of both IgE and IgG1 antibodies appeared only to sesame protein but not in saline control or vanilla-exposed mice (fig. 1). The dose-response and time-course of antibody responses to sesame is shown in figure 2. As evident, the dose of 5 µg/mouse failed to elicit detectable IgE or IgG1 antibodies. The dose of 50 µg/mouse elicited only IgG1. The dose of 500 µg/mouse elicited robust IgE as well as IgG1 responses. Repeated measurements confirmed the memory immune response to sesame seed. Measurement of sesame-specific IgG2a antibody response revealed the following levels (titers): pre-immune serum: <100 titer; 4th and 5th responses: ≤200 to 400 at 500 µg/mouse dose in sesame-exposed mice; <100 titer at all time points in saline-exposed mice.

IgE Antibody Response to Sesame Seeds Is Independent of Host Strain Genetics

Immune response to sesame was examined in two inbred strains of mice with different MHC haplotypes (BALB/c H-2^d and ASW/SnJ H-2^s). As evident, sesame elicited a robust IgE antibody response in both strains (fig. 3).

Characterization of Sesame-Driven Type 2 Cytokine Responses in Mice

We examined sesame-specific type 2 cytokine responses in saline versus sesame-sensitized mouse spleen cells following ex vivo activation. Sesame significantly activated the prototypic type 2 cytokine (IL-4 and IL-5) responses in spleen cells from sesame-sensitized mice but not from the control mice (fig. 4). In contrast, IFN-γ levels in cultures activated with sesame proteins were less than the background IFN-γ level observed in cultures with culture medium alone (culture medium alone: 11.452 ± 0.862 ng/ml; sesame 100-µg dose: 9.936 ± 3.12 ng/ml, sesame 500-µg dose: 6.0411 ± 0.3258 ng/ml; day 3).

Fig. 2. Dose-response and time-course analyses of type 2-dependent antibody responses following transdermal exposure to sesame seeds in BALB/c mice. Groups of mice (n = 3–4 per group) were exposed to sesame protein extracts via transdermal application at indicated dose. Sesame binding IgE (**a**) and IgG1 (**b**) responses were measured over a 5-week period. Data shown is geometric mean $\pm$ SE specific antibody titers. Where titers were identical error bars are not shown. Pre = Preimmune plasma; 1R, 2R, 3R, 4R, 5R = primary, secondary, tertiary, fourth and fifth responses.

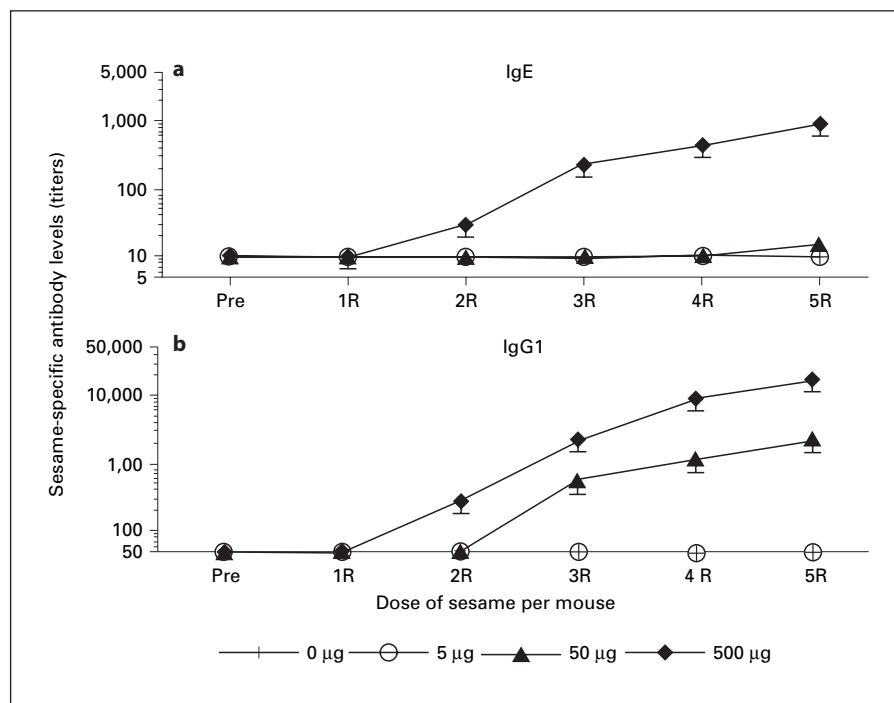
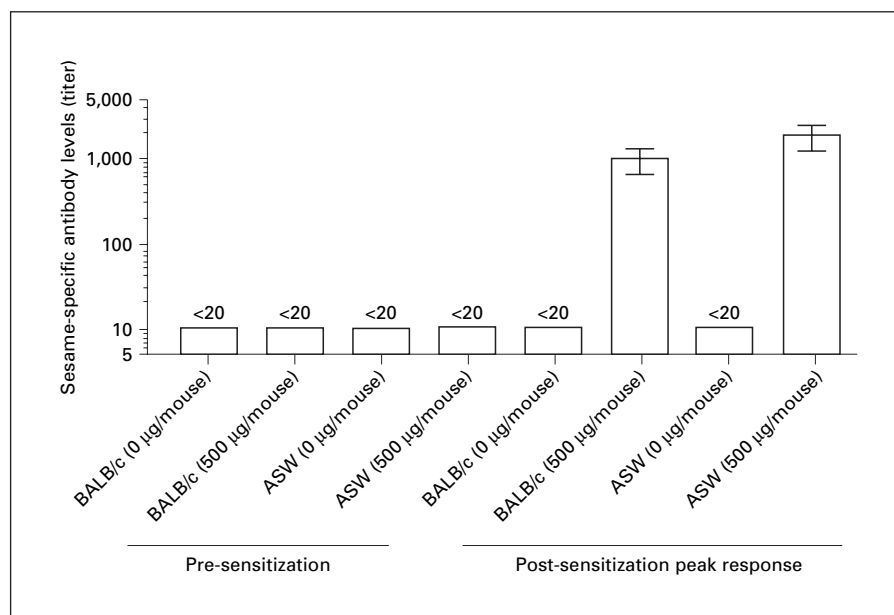


Fig. 3. Sesame-specific IgE antibody responses in mice strains with differing MHC haplotype. Groups of BALB/c (H-2^d) and ASW (H-2^s) mice (n = 3–7 per group) were exposed to saline or sesame seed protein extract via transdermal application. Sesame-specific IgE responses were measured before and after exposure to sesame seed extracts over a 13-week period. Peak antibody responses are shown as geometric mean $\pm$ SE of specific antibody titers. Where titers were identical error bars are not shown.



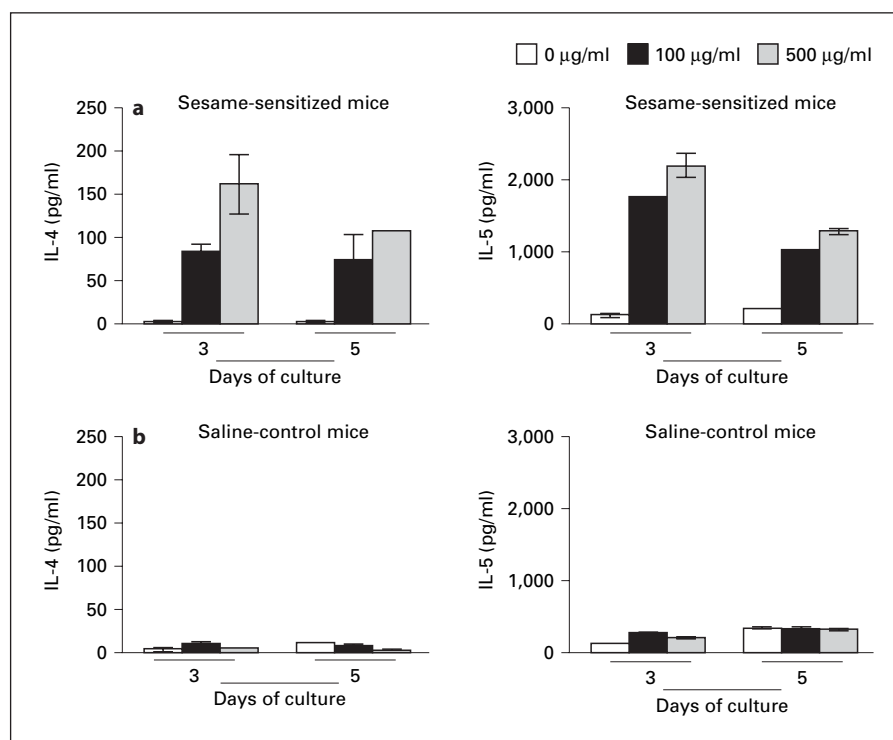
Sesame-Sensitized Mice Exhibit Systemic Anaphylaxis following Oral Challenge

Oral challenge of sesame-sensitized mice resulted in significant clinical scores and a drop in rectal temperature suggesting systemic anaphylaxis (fig. 5).

Identification of Ses i 3 and Basic Subunit of 11s Globulins as Allergens

We performed Western blot analysis using control plasma versus sesame-sensitized plasma and identified four allergens: 49, 35, 24 and 21 kDa (fig. 6). Subsequently, N-terminal amino acid sequence analysis revealed identity with Ses i 3, a 7S vicilin-type globulin and the

Fig. 4. Sesame-driven type 2 cytokine (IL-4 and IL-5) responses in BALB/c mice sensitized by transdermal application with sesame seed extract. Groups of BALB/c mice ($n = 3-4$ per group) were sensitized with sesame seed (500 μg per mouse) or saline via transdermal exposure over a 13-week period. One week following the last exposure, spleen cells were harvested and cultured with indicated dose of sesame seed protein extract. Cell culture supernatants were harvested at indicated time points and analyzed for cytokines using ELISA. Data shown is average $\pm$ SE of duplicate analyses from sesame-sensitized mice (**a**) and from saline control mice (**b**). Student's t test: for IL-4: sesame 100 and 500 $\mu\text{g}/\text{ml}$ vs. culture medium alone, and sesame-sensitized vs. control mice, day 5 at 500 $\mu\text{g}/\text{ml}$ dose, $p < 0.05$; for IL-5: sesame 100 and 500 $\mu\text{g}/\text{ml}$ vs. culture medium, and sesame-sensitized vs. control mice, days 3 and 5 at both doses, $p < 0.01$.



two isoforms of the basic subunit of 11s globulin. The 35-kDa protein could not be sequenced (likely due to blockage of N-terminus).

Discussion

There are four important findings from this study: (1) transdermal exposure to sesame seed can elicit IgE response associated with IL-4, but not IFN- γ response; (2) the ability of sesame to elicit IgE response is an intrinsic property because IgE response was demonstrable in two mice strains with different MHC haplotype without the use of an adjuvant; (3) transdermal exposure to sesame also resulted in clinical sensitization as evidenced by signs of systemic anaphylaxis following oral challenge with sesame, and (4) four sesame allergens were identified including Ses i 3 and the basic subunit of 11s globulins.

In this study we chose BALB/c as the primary mouse strain to examine allergic response to sesame seeds. This is because BALB/c mice have been widely used in the allergy studies including food-allergic sensitization model development for other foods although it was not clear whether they would respond to sesame seed with specific IgE antibodies and type 2 cytokine responses [6, 15, 16].

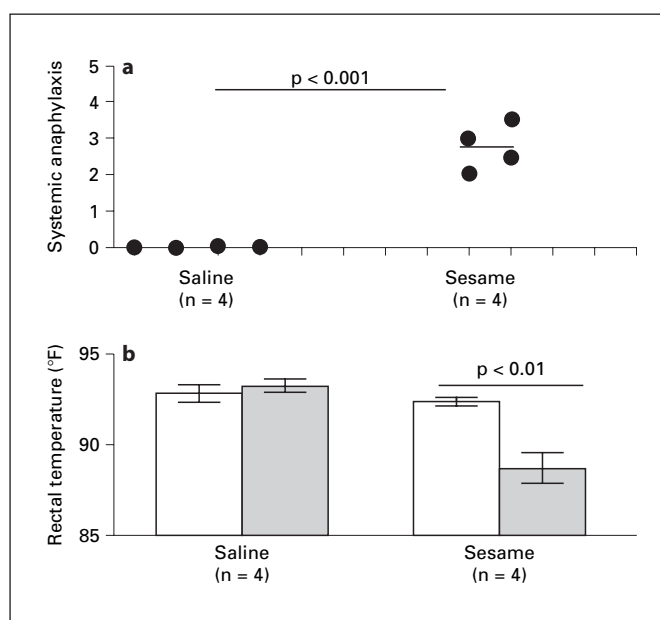


Fig. 5. Systemic anaphylaxis to sesame in transdermally sensitized mice. Groups of BALB/c mice ($n = 4$ per group) were sensitized with sesame seed (500 μg per mouse) or saline via transdermal exposure for 6 weeks and confirmed for IgE response. **a** Clinical scores of systemic anaphylaxis. **b** The profile of rectal temperature before and at 30 min following oral challenge with saline or sesame (13 mg per mouse). Differences were compared using Student's t test.

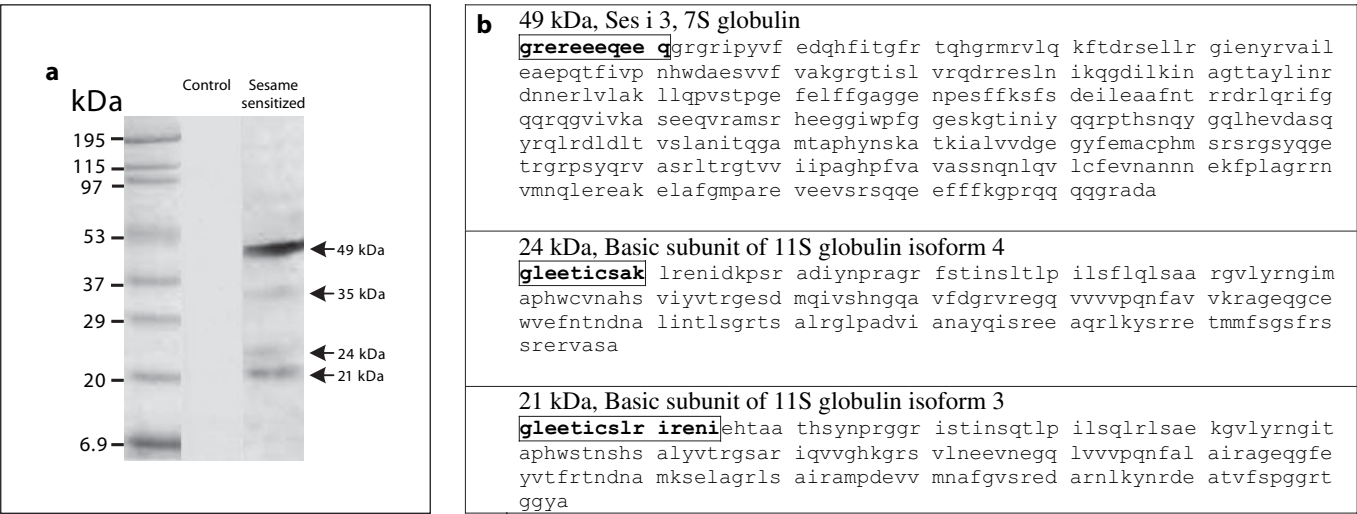


Fig. 6. Western blot analysis and N-terminal amino acid sequence analysis of IgE-binding sesame seed protein. **a** Sesame seed protein extract was subjected to SDS-PAGE followed by Western blot analysis using protein G-treated plasma from sesame-sensitized vs. control mice. Bound IgE was detected using anti-IgE antibody. First lane: Molecular weight markers; second lane: control mouse serum; third lane: serum from sesame-sensitized mice. **b** GenBank sequence of allergens with boxed area corresponding to the N-terminal sequence that we obtained in our analysis.

Furthermore, BALB/c strain may represent a host with genetic predisposition to develop high IgE response – a feature similar to atopic humans. Although we have used the BALB/c strain of mice in this study, others have used other strains such as C3H/HeJ for peanut and cows milk food allergy model development [7, 8, 17]. Therefore, it is difficult to conclude that BALB/c is the best strain for all types of food allergy model development.

In this study, we have characterized IgE and IgG1 antibody responses as readouts of type 2 immune responses. This is because both the IgG1 and IgE isotype of antibody production is dependent on the prototypic type 2 cytokine IL-4, although dependence of IgG1 is not complete [18, 19]. Interestingly, sesame elicited both IgG1 and IgE antibody responses. In mice, although both IgE and IgG1 antibodies can participate in systemic anaphylaxis, it remains unclear whether sesame-specific IgG1 antibodies have any disease relevance [20]. There was very little or no evidence for the activation of IgG2a or IFN-γ response, respectively.

Previous studies on food allergy mouse model development have used sensitization methods such as intraperitoneal injection with alum adjuvant or oral sensitization with cholera toxin as an adjuvant [7, 8, 15, 21]. In this study we used the transdermal exposure method to examine allergic sensitization because previously studies

have found that exposure via this route in BALB/c mice elicits a robust IgE antibody response to ovalbumin and hazelnut [6, 13, 16].

Other studies have shown that oral exposure to antigens in mice leads to oral tolerance rather than allergic sensitization unless powerful mucosal adjuvant (such as cholera toxin) or antiulcer drugs were administered [7–9, 15, 22]. In this study using the transdermal exposure method where neither adjuvant nor drugs are necessary for inducing allergic sensitization, we found a robust allergic response to sesame seeds.

It is noteworthy that sesame seed is an allergenic food (not an allergen per se) as it represents a source of many allergens ranging in molecular weight from 7 to 67 kDa including the following as reviewed recently: Ses i 1 (9 kDa, 2S albumin), Ses i 2 (7 kDa, 2S albumin), and Ses i 3 (45 kDa, 7S vicilin-type globulin) [3]. We found evidence for four sesame allergens in our model that included Ses i 3 and two isoforms of the basic subunit of 11s globulin. Notably Ses i 3 has been reported to be a human sesame allergen recognized by 75% of patients [23]. Although 20- to 25-kDa proteins were reported to be a sesame allergen in humans, whether those proteins are indeed the basic subunit of the 11s globulins remains unclear [23–27].

Our data argue that transdermal exposure to sesame proteins can result in activation of IL-4 leading to allergic sensitization due to production of sesame-specific IgE antibodies. It is not completely clear at present whether food proteins can cause allergic sensitization in humans via transdermal route of exposure. Previous studies reported allergic sensitization to sesame in people working in the baking industry presumably via skin contact [10, 11] and sensitization to peanut among infants and children due to the use of peanut-containing diaper creams or skin lotions [12]. Thus our data using sesame proteins and a mouse model along with previous mouse model reports of transdermal sensitization to ovalbumin and hazelnut

raises the possibility of sensitization to food proteins via skin contact occurring in humans [6, 16].

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