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Development of a BALB/c mouse model for food allergy: comparison of allergy-related responses to peanut agglutinin, β-lactoglobulin and potato acid phosphatase

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The purpose of this study was to develop a BALB/c mouse model for comprehensively assessing food allergies. Serum specific IgE and IgG1 antibodies against protein (PNA, β-LG, and PAP) were induced in intraperitoneally sensitized BALB/c mice. On day 28, blood was collected to obtain the serum, and the splenocytes were cultured. On day 30, mice were challenged with antigen by intraperitoneal injection or intragastric administration, and the physiological and immunological responses to the antigen were studied. A general finding was that allergenicity-related parameters in the mice treated with PNA were statistically higher than those in the mice treated with PAP ($P < 0.05$ for IL-4; $P < 0.05$ for specific IgE; $P < 0.001$ for specific IgG1), whereas parameters in those treated with β-LG were between the other two. Statistically higher histamine release was observed in PNA and β-LG-sensitized mice than in control mice challenged with the same protein by i.p. injection. Intraperitoneal challenge with PNA and β-LG in sensitized mice induced edema in the ear and inflammatory cell infiltration in the lung, which were not observed with the control mice. The results show a new model that covers many features of clinical food allergies that are not seen in other models. The order of potential allergenicity might be PNA > β-LG > PAP, and the intraperitoneal challenge could be more sensitive to induced systemic food allergy.

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1 Introduction

Food allergy, an immediate hypersensitivity response to an otherwise harmless food or food component, affects approximately 2–6% of infants and 1–2% of adults.[1] The number of food allergy occurrences is increasing and as such, increasing attention is being focused on allergies. With the development of novel foods, including foods and food products from genetically modified crops, one of the major issues is whether the novel foods have the potential to induce allergic diseases.[2,3]

The IFBC/ILSI decision tree, jointly developed by the International Food Biotechnology Council (IFBC) and the International Life Sciences Institute (ILSI), was the best known systematic approach for assessing the allergenic properties of novel proteins.[4] However, a universal, reliable and relevant *in vitro* or *in vivo* test to study the potential allergenicity of novel proteins was not available. Therefore, it was suggested that the most direct way to determine the potential allergenicity of novel proteins should be the development and validation of a widely accepted animal model. In 2001, FAO/WHO revised the previous decision tree, and the FAO/WHO 2001 decision tree was developed, in which animal models were included.[5] This decision tree was rapidly revised two years later[6] and further revised in 2009,[7] as no single test could be predictive of allergenicity. This new approach is called the weight of evidence approach. The use of the animal model is not mandatory but it is recognized that a robust model may help in identifying any potential *de novo* sensitizers.[8]

An ideal animal model for the food allergy study should meet the following criteria. First, it should include the induction of allergic parameters clinically relevant to humans, and antibody responses that are directed to similar proteins/epitopes as found in patient sera.[9] Second, a model without adjuvants seems preferable, since adjuvants may reduce a false-positive IgE response to a non-allergenic food/protein[10] and a model with adjuvants is unable to determine the inherent

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potential of a given protein inducing allergic sensitization. Third, an animal model should be easy to operate, and reproducible across laboratories over time.[8]

Mice have been used for the development of food allergy models without adjuvants. One of the advantages of using mouse models instead of rat models is that significant allergic symptoms can be easily induced in mice.[11] In particular, the BALB/c mouse, a so-called high IgE responder strain, has been studied. Many researchers have used BALB/c mice in a model where the production of specific IgE was studied after intraperitoneal injection with allergens such as rice seeds, milk whey protein, tree nuts and cashew nuts.[11–14]

Theoretically, any food containing a protein could elicit an allergic reaction; however, eight common foods are responsible for >90% of food allergies.[15] It is reasonable to suppose that not all proteins are equally allergenic. Only a small part of food proteins consumed regularly is related to allergic diseases. Indeed, most cases of food allergy in the USA and Western Europe are caused by a relatively limited range of food. These suggest that there is a spectrum of allergenicity among food proteins.[16] FAO/WHO suggested that the potential allergenicity of the expressed protein be ranked against well-known strong and weak food allergens in the animal models.[17]

In our previous study, the BALB/c mouse model was initially established for assessing the potential allergenicity of proteins.[8] In the present study, to further validate our model, we studied proteins with different allergenicities to determine whether a comparable spectrum of proteins is recognized by the mouse immune systems. Peanut agglutinin (PNA), β-lactoglobulin (β-LG) and potato acid phosphatase (PAP) were used to develop the BALB/c model, and ovalbumin (OVA) was regarded as a positive control protein. Mice were sensitized by intraperitoneal injection, and two routes of challenge were compared: intraperitoneal (i.p.) injection and intragastric (i.g.) administration. IL-4, IFN-γ, histamine, serum protein-specific IgE and IgG1 were measured and histopathology (ear, lung and jejunum) was analyzed.

2 Materials and methods

2.1 Animals

Young adult (6–8 weeks old) female BALB/c strain mice (Vital River, Beijing, China) were used throughout these studies. The mice were housed in an animal room maintained at 23 ± 3 °C and 50 ± 10% relative humidity with alternating 12:12 h light–dark cycle. The mice were housed in stainless-steel wire cages (32 cm × 20 cm × 12 cm) in groups of 10, and had free access to diet (without PNA, β-LG, PAP and OVA) and water. The diet did not contain any allergens that could influence the outputs of the experiments. The study design was approved by the Institutional Animal Care and Use Committee (IACUC) of the Chinese Center for Disease Control and Prevention. All of the animals received humane care according to the criteria outlined in the Guide for the Care and Use of the Animal Management Rules of the Health Ministry of the People's Republic of China.

2.2 Protein

Ovalbumin (OVA, catalogue number: A5503), peanut agglutinin (PNA, catalogue number: L0881), β-lactoglobulin (β-LG, catalogue number: L3908) and potato acid phosphatase (PAP, catalogue number: P3752) were obtained from Sigma-Aldrich Co. LLC. (St Louis, USA), and reconstituted in phosphate buffered saline (PBS) at 0.2 mg mL^{-1} (10 mg protein was solubilized in 50 mL PBS), 4 mg mL^{-1} (40 mg protein was solubilized in 10 mL PBS) and 200 mg mL^{-1} (1000 mg protein was solubilized in 5 mL PBS), respectively. Solutions were prepared before use and mixed thoroughly to ensure they were homogenous and stable.

2.3 Study design

Schematic representation of the sensitization and challenge in this study is provided in Fig. 1.

2.3.1 Sensitization. The mice (n = 30, each group) received intraperitoneal injections of 0.05 mg protein (OVA, PNA, β-LG and PAP, respectively) in 0.25 mL PBS (that is, 0.25 mL of 0.2 mg mL^{-1} protein solution) on days 0, 3, 6, 9, 12. Control animals (n = 90) were treated intraperitoneally five times (days 0, 3, 6, 9, 12) with 0.25 mL PBS.

2.3.2 Sampling of serum. Blood samples from 10 mice in each group were taken from the orbital plexus on day 28. Blood samples were centrifuged at 3000 rpm for 15 min at 4 °C to obtain serum. Individual serum samples were stored at −80 °C until analysis.

2.3.3 Spleen cell culture and cytokine analyses. On day 28, after blood was drawn, the spleen cells of the mice (n = 10 for each group) were harvested and standard cell cultures were set up as described.[12,14,18] Briefly, spleen cells were cultured (4 × 10^6 cells per ml) in the presence of protein (0.5 mg ml^{-1}, OVA, PNA, β-LG and PAP, respectively) for 48 hours.[19] Cell culture supernatants were harvested for use in cytokine analyses, including IL-4 with the mouse IL-4 high sensitivity ELISA kit (eBioscience, Vienna, Austria) and IFN-γ with the mouse IFN-gamma quantikine ELISA kit (R&D Systems Inc., Minneapolis, USA).

2.3.4 Elicitation of the allergic reaction, clinical observation and measurement of plasma histamine level. On day 30, two methods of elicitation of allergic reaction were compared: intraperitoneal injection and intragastric administration.

The mice were challenged by intraperitoneal injection (10 mice in the protein group and 10 in the control) with 1 mg protein (OVA, PNA, β-LG and PAP, respectively) in 0.25 mL PBS (that is, 0.25 mL of 4 mg mL^{-1} protein solution), and by intragastric administration (10 mice in the protein group and 10 control mice) with 50 mg protein (OVA, PNA, β-LG and PAP, respectively) in 0.25 mL PBS (that is, 0.25 mL of 200 mg mL^{-1} protein solution).

Mice were observed for signs of systemic anaphylaxis over the next 40 minutes. The symptoms of hypersensitivity were evaluated by a scoring system described previously, and were scored as follows: 0 indicates no symptoms; 1 indicates scratching and rubbing around the nose and head; 2 indicates puffiness around the eyes and mouth, diarrhea, pilar erecti,

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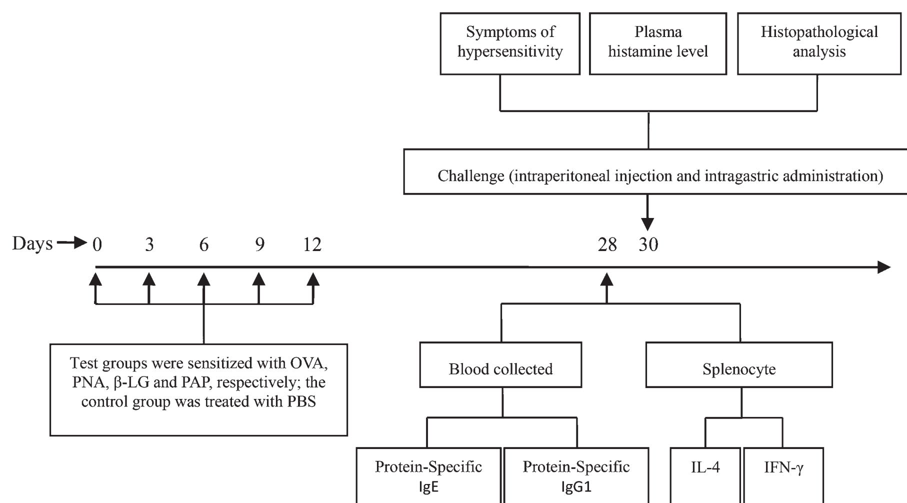

Fig. 1 Schematic of the sensitization and challenge in this study. Groups of mice (n = 30) were intraperitoneally sensitized with 0.25 mL of 0.2 mg mL^{-1} of protein (OVA, PNA, β-LG and PAP, respectively) solutions on days 0, 3, 6, 9, 12. On day 28, ten mice were randomly chosen from each group, then their blood samples were collected, spleen cells were cultured and cytokines were analyzed. On day 30, the challenge was performed and two methods of elicitation of allergic reaction (10 mice for intraperitoneal injection and 10 mice for intragastric administration in each protein group) were compared through the following parameters: symptoms of hypersensitivity, plasma histamine level and histopathologic analysis. The mice in the control group were intraperitoneally sensitized with PBS and challenged with the corresponding proteins in two methods of elicitation.

reduced activity, and/or decreased activity with increased respiratory rates; 3 indicates wheezing, labored respiration, cyanosis around the mouth and the tail; 4 indicates no activity after prodding or tremor and convulsion; and 5 indicates death.[13]

In order to evaluate the plasma histamine level, mice blood samples were collected in centrifuge tubes containing 10 μL of 7.5% potassium ethylenediamine tetraacetic acid (EDTA-K_2) at 50 min after challenge. After centrifugation, plasma was collected and frozen at −80 °C until use. Plasma histamine levels were determined using an immunoassay kit (IBL-America Inc., Minneapolis, USA).

2.3.5 Histopathological analysis. After blood samples were collected at 50 min after challenge, the mice were sacrificed. The left ear, lung and jejunum were embedded in paraffin after fixation in 10% formaldehyde. Five-micrometer sections were stained with hematoxylin and eosin (H&E). The morphologies of the left ear, lung and jejunum were observed using a microscope (Olympus BX 41, Tokyo, Japan).

2.4 Measurement of serum protein-specific IgE

Food protein-specific IgE was assayed by enzyme-linked immunosorbent assay (ELISA). Plates (Corning Inc., New York, USA) were coated overnight at 4 °C with rat anti-mouse IgE (0.05 mg mL^{-1}; Chondrex Inc., Washington, USA). After washing, wells were blocked with phosphate buffered saline (PBS)-30% goat serum for 1 h at room temperature (RT). After washing, mouse sera (optimal sample dilutions avoiding assay saturation were determined as 1 : 1 for IgE) were incubated overnight at 4 °C. Biotinylated-protein was obtained according to the instruction of biotinylation reagents and kits (ThermoFisher Scientific Inc., Massachusetts, USA). The biotinylated-protein (1 μg mL^{-1}) was added to the plate for 1.5 h at RT, followed by incubation with streptavidin peroxidase (1 : 200; Chondrex Inc., Washington, USA) for 1 h at RT. Plates were developed with a tetramethylbenzidine substrate. The reaction was stopped with 2 N H_2SO_4. Optical densities were measured at 450 nm.

2.5 Measurement of serum protein-specific IgG1

Protein-specific IgG1 was determined by ELISA. Briefly, plates (Corning Inc., NewYork, USA) were coated with protein (0.01 mg mL^{-1}) overnight at 4 °C. Wells were washed with PBS containing 0.05% Tween and blocked with PBS containing 30% goat serum overnight at 4 °C. Diluted serum samples (optimal sample dilutions avoiding assay saturation were determined as 1 : 1000 for IgG1) were incubated for 1 h at 37 °C, followed by incubation for 1 h with an HRP-labelled goat anti-mouse IgG1 antibody (1 : 20, Cayman Chemical, Michigan, USA). The reaction was stopped with 2 N H_2SO_4. Optical densities were measured at 450 nm.

2.6 Statistics

The SPSS Statistical System (SPSS for Windows 18.0, Chicago, USA) was used to analyze the data. If two groups had homogeneous variances, the t-test was used to compare the difference between them. If variances were not equal, median levels were compared between two groups through the Mann–Whitney test. For comparison of more than two groups with equal variances, one-way analysis of variance was used, followed by the Bonferroni t-test if statistically significant. For the comparison of more than two groups with unequal variances, the Kruskall–Wallis test was used followed by Dunn's test if statistically significant. All statistical tests were performed at the $P < 0.05$ level of significance.

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3 Results

3.1 Cytokine analysis

3.1.1 IL-4. Higher, statistically significant IL-4 responses were observed in protein-treated (OVA, PNA, β-LG and PAP) mice than in healthy control mice ($P < 0.01$).

The ratio of IL-4 in the protein group and control group was used to better compare IL-4 levels among different protein groups, and was obtained as follows: IL-4 levels in protein groups were divided by the averages of the corresponding control groups (the average of IL-4 in each control group was used as the reference value of IL-4 in the corresponding protein group). As was shown in Fig. 2, mice in the OVA group and PNA group had statistically higher IL-4 levels than mice in the PAP group ($P < 0.05$). The order of protein groups from high to low IL-4 levels was PNA > β-LG > PAP.

3.1.2 IFN-γ. There were no significant differences in IFN-γ between protein groups and control groups.

The ratio of IFN-γ in the test group and control group was used to better compare IFN-γ levels among different protein groups. The ratio of IFN-γ in the protein group and control group was obtained as follows: IFN-γ levels in protein groups were divided by the averages of those in the corresponding control groups (the average of IFN-γ in each control group was used as the reference value). There was no significant difference in IFN-γ levels between protein groups. The results are shown in Fig. 3.

3.2 Measurement of serum protein-specific IgE

Higher, statistically significant protein-specific IgE levels were observed in protein-treated mice than in healthy control mice ($P < 0.001$, OVA; $P < 0.01$, PNA; $P < 0.01$, β-LG; $P < 0.05$, PAP).

The ratio of protein-sIgE in the protein group and control group was used to better compare protein-sIgE levels among different protein groups, and was obtained as follows: protein-sIgE levels in protein groups were divided by the averages of those in the corresponding control groups (the average of protein-sIgE in each control group was used as the reference value). Mice in the OVA group ($P < 0.01$) and PNA group ($P < 0.05$) had statistically higher protein-sIgE levels than mice in the PAP group. The order of protein groups from high to low protein-sIgE levels is PNA > β-LG > PAP. The results are shown in Fig. 4.

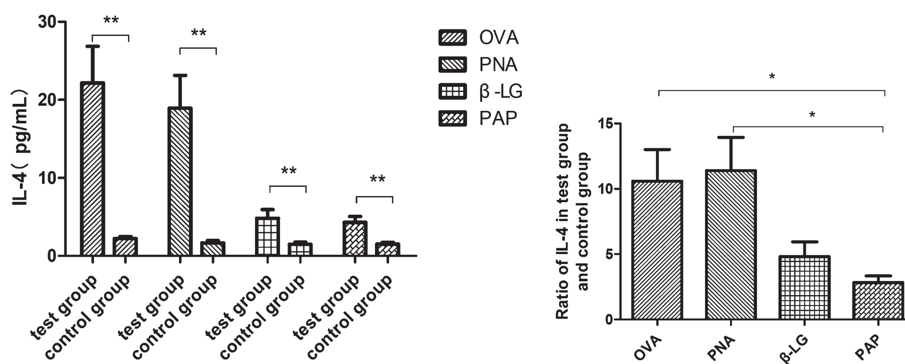

Fig. 2 IL-4 (type-2 cytokine, left), and the ratio of IL-4 in the protein group and control group (right). Spleen cells were isolated from female BALB/c mice treated intraperitoneally five times with 0.05 mg and 0 mg protein in 0.25 mL PBS on day 28 ($n = 10$). Spleen cells were cultured with protein (0.5 mg ml^{-1}) for 48 hours. After cultivation, the culture supernatants were collected for use in IL-4 analyses. The level of IL-4 was measured by ELISA. ** represents $P < 0.01$, * represents $P < 0.05$.

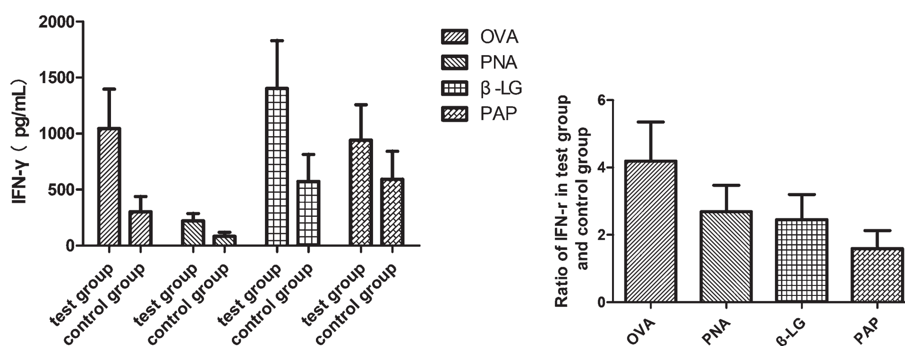

Fig. 3 IFN-γ (type-1 cytokine, left), and the ratio of IFN-γ in the protein group and control group (right). Spleen cells were isolated from female BALB/c mice treated intraperitoneally five times with 0.05 mg and 0 mg protein in 0.25 mL PBS on day 28 ($n = 10$). Spleen cells were cultured with the corresponding protein (0.5 mg ml^{-1}) for 48 hours. After cultivation, the culture supernatants were collected for use in IFN-γ analyses. The level of IFN-γ was measured by ELISA.

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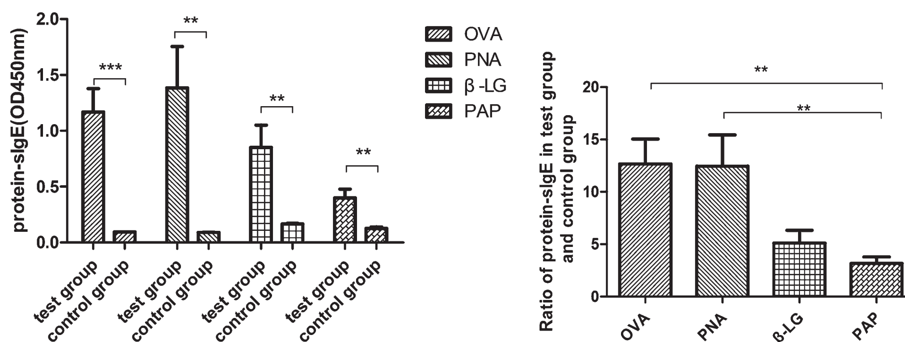

Fig. 4 Protein-specific IgE (left) and the ratio of protein-sIgE in the protein group and control group (right). Female BALB/c mice were treated five times intraperitoneally with 0.05 mg and 0 mg of protein in 0.25 mL of PBS, and blood was drawn on day 28 (n = 10). Protein-sIgE in serum was measured by ELISA. ** represents P < 0.01; *** represents P < 0.001.

3.3 Measurement of serum protein-specific IgG1

Higher, statistically significant protein-specific IgG1 levels were observed in protein-treated (OVA, PNA, β-LG and PAP) mice than in healthy control mice (P < 0.001).

The ratio of protein-sIgG1 in the protein group and control group was used to better compare protein-sIgG1 levels among different protein groups. The ratio of protein-sIgG1 in the protein group and control group was obtained as follows: protein-sIgG1 levels in protein groups were divided by the averages of those in the corresponding control groups (the average of protein-sIgG1 in each control group was used as the reference value). Mice in the OVA group and PNA group had statistically higher protein-sIgE levels than mice in the PAP group (P < 0.001). The order of protein groups from high to low protein-sIgG1 levels is PNA > β-LG > PAP. The results are shown in Fig. 5.

3.4 Hypersensitivity symptoms induced by the i.p. and i.g. challenge

At 40 min after the i.p. challenge, the OVA, PNA and β-LG treated mice exhibited significant signs of systemic anaphylaxis that were scored as described in the Study design section; three PAP-treated mice showed scratching and rubbing around the nose and head, but no obvious symptoms were observed in the control mice.

At 40 min after the i.g. challenge, the mice (2 for OVA, 2 for PNA, 1 for β-LG, and 1 for PAP) showed scratching and rubbing around the nose and head; no obvious symptoms were observed in control mice. The results are shown in Fig. 6.

3.5 Plasma histamine level

At 50 min after the i.p. challenge, higher, statistically significant histamine levels were observed in the protein-treated mice compared to the control mice (P < 0.01, OVA; P < 0.001, PNA; P < 0.001, β-LG; P < 0.01, PAP). At 50 min after the i.g. challenge, there was no significant difference in the histamine levels of protein-treated mice compared to the control mice. The results are shown in Fig. 7.

3.6 Histopathology

3.6.1 Ear. The histopathology results of studies on the mice ear segments (sensitized with 0.05 mg and 0 mg of protein) i.p. and i.g. challenged with OVA, PNA, β-LG and PAP, respectively, are shown in Fig. 8.

Histopathological examination of the ear segments revealed venous distention (shown in mice sensitized and i.p. challenged with OVA), edema (present in sensitized mice i.p. challenged with OVA, PNA and β-LG, as well as i.g. challenged with β-LG), mild edema (shown in sensitized mice i.p. challenged

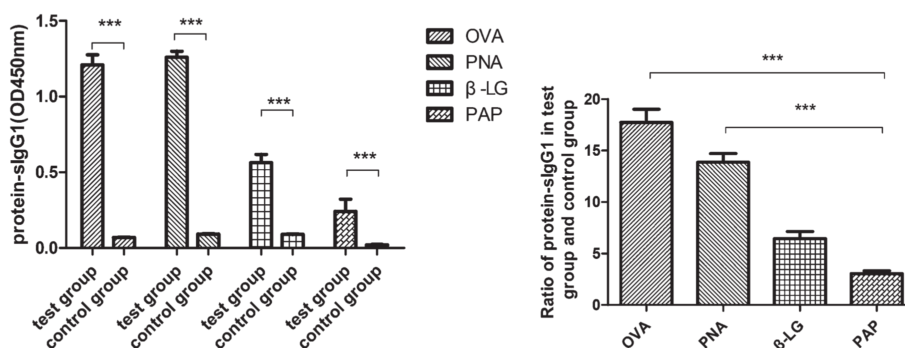

Fig. 5 Protein-specific IgG1 (left) and the ratio of protein-sIgG1 in the protein group and control group (right). Female BALB/c mice were treated five times intraperitoneally with 0.05 mg and 0 mg protein in 0.25 mL PBS, and blood was drawn on day 28 (n = 10). Protein-sIgG1 in serum was measured by ELISA. *** represents P < 0.001.

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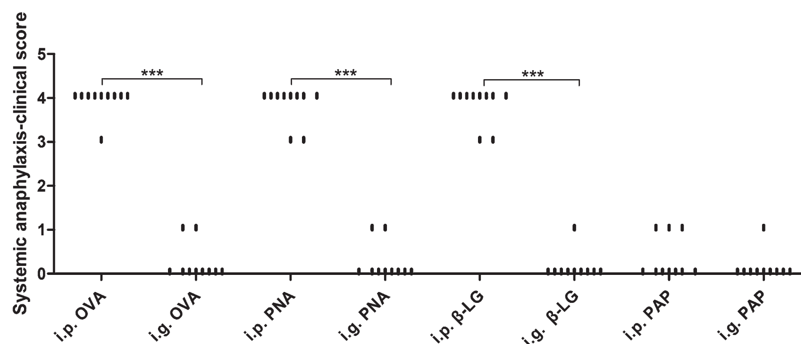

Fig. 6 Clinical scores present in a scatter plot, with each symbol representing one mouse. Female BALB/c mice were treated five times intraperitoneally with 0.05 mg protein in 0.25 mL PBS. On day 30, the challenge was conducted and two methods of elicitation of allergic reaction (intraperitoneal injection and intragastric administration) were compared through the hypersensitivity symptom score. *** represents $P < 0.001$.

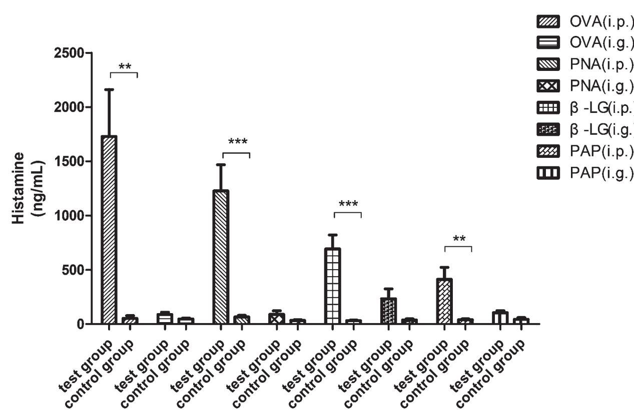

Fig. 7 Plasma histamine levels in the protein and control groups. Female BALB/c mice were treated five times intraperitoneally with 0.05 mg and 0 mg protein in 0.25 mL PBS. On day 30, two methods of elicitation of allergic reaction were compared: intraperitoneal injection and intragastric administration. The mice were challenged by intraperitoneal injection (10 mice in the protein group and 10 control mice) with 1 mg protein (OVA, PNA, β-LG and PAP, respectively) in 0.25 mL PBS (that is, 4 mg mL^{-1} of protein solution), and were challenged by intragastric administration (10 mice in the protein group and 10 control mice) of 50 mg of protein (OVA, PNA, β-LG and PAP, respectively) in 0.25 mL PBS (that is, 0.25 mL of 200 mg mL^{-1} protein solution). ** represents $P < 0.01$; *** represents $P < 0.001$.

with PAP, as well as i.g. challenged with OVA and PNA), inflammatory cell infiltration (shown in sensitized mice i.p. challenged with OVA, PNA and β-LG, as well as i.g. challenged with PNA, β-LG), occasional inflammatory cell infiltration (shown in sensitized mice i.g. challenged with OVA). In mice sensitized and i.g. challenged with PAP, as well as in control mice, no significant lesions were showed in the histopathologic examination of ear segments.

3.6.2 Lung. The histopathological results of mice lung segments (sensitized with 0.05 mg and 0 mg protein) i.p. and i.g. challenged with OVA, PNA, β-LG and PAP, respectively, are shown in Fig. 9.

Histopathological examination of the lung segments revealed dilated space between veins and around tissues (shown in sensitized mice i.p. challenged with OVA, PNA and β-LG, as well as i.g. challenged with PNA, β-LG), widened alveolar septa (shown in sensitized mice i.p. challenged with OVA), inflammatory cell infiltration (shown in sensitized mice i.p. challenged with OVA, i.g. challenged with PNA, β-LG), occasional inflammatory cell infiltration and some dilated space between veins and around tissues (shown in sensitized mice i.g. challenged with OVA). In sensitized mice i.p. and i.g. challenged with PAP, as well as in control mice, no significant lesions were showed in the histopathological examination of the lung segments.

3.6.3 Jejunum. The histopathological results of jejunum segments in mice (sensitized with 0.05 mg and 0 mg protein) i.p. and i.g. challenged with OVA, PNA, β-LG and PAP, respectively, are shown in Fig. 10.

Histopathological examination of the jejunum segments revealed structure looseness, edema (shown in sensitized mice i.p. challenged with OVA, PNA, PAP, as well as i.g. challenged with PNA, β-LG, PAP), and inflammatory cell infiltration in submucosa (shown in sensitized mice i.p. challenged with OVA), villi swelling, separation between the epithelial layer and lamina propria (shown in sensitized mice i.g. challenged with OVA), inflammatory cell infiltration (shown in sensitized mice i.p. challenged with β-LG, PAP, as well as i.g. challenged with β-LG, PAP), mild inflammatory cell infiltration (shown in sensitized mice i.p. and i.g. challenged with PNA). No significant lesions were observed in control mice.

4 Discussion

Food allergies have become an important health issue of growing interest. IgE-mediated food allergy is the most common type of food allergy and consists of two separate phases: first sensitization and later elicitation.[20] In the sensitization phase, allergy proteins result in Th2-prone immunological reactivity, which induces an isotype switch, making B cells become IgE producing plasma cells after antigen stimulation in persons that are susceptible to allergens. Allergen-specific IgE binds to IgE receptors (Fc receptors) on the cell surface of the mast cells. Mast cells play an important role in

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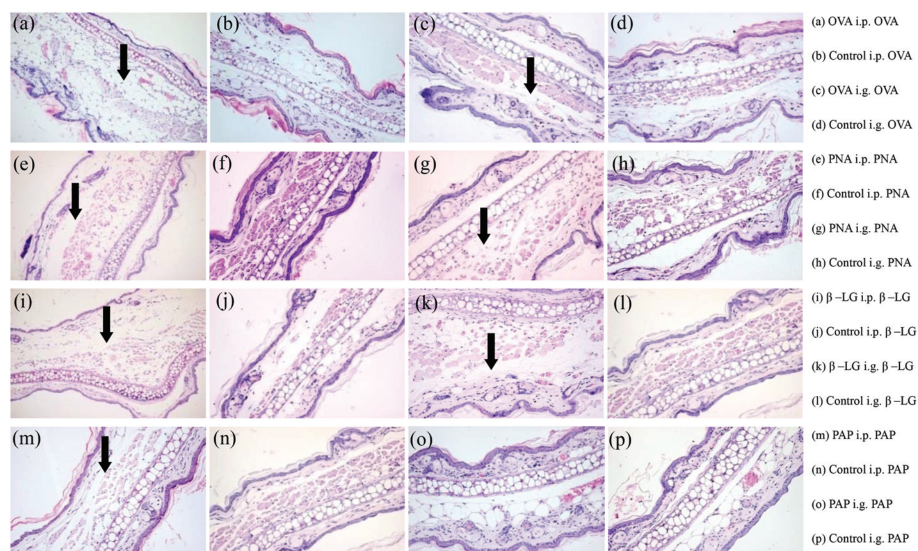

Fig. 8 The histopathology results of mice ear segments (sensitized with 0.05 mg and 0 mg protein) i.p. and i.g. challenged with OVA, PNA, β-LG and PAP, respectively. Severe ear damage is linked to clinical signs of systemic anaphylaxis in this model. Solid arrows indicate edema. (a), (e), (i), (m) Ear segments from mice i.p. sensitized and i.p. challenged with OVA, PNA, β-LG and PAP, respectively. (b), (f), (j), (n) Ear segments from control mice challenged with OVA, PNA, β-LG and PAP, respectively. (c), (g), (k), (o) Ear segments from i.p. sensitized and i.g. challenged with OVA, PNA, β-LG and PAP, respectively. (d), (h), (l), (p) Ear segments from control mice i.g. challenged with OVA, PNA, β-LG and PAP, respectively.

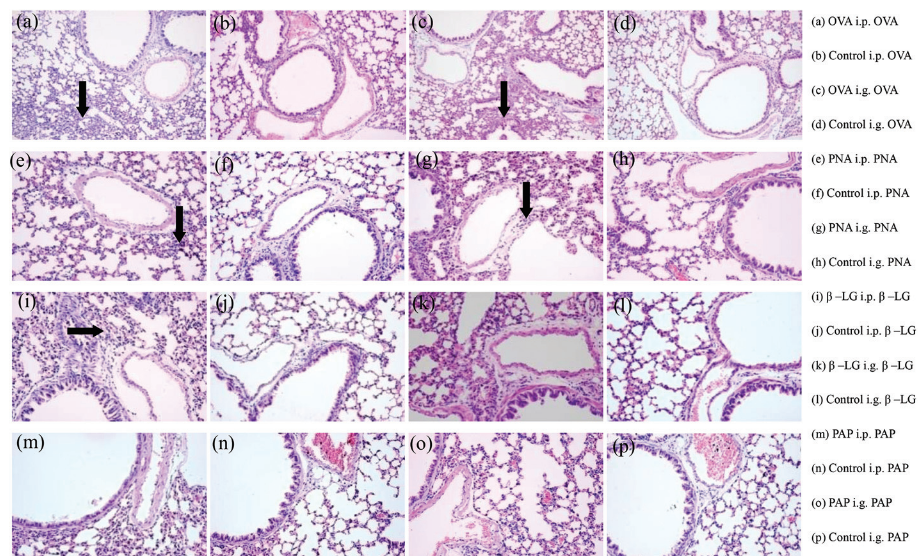

Fig. 9 The histopathology results of lung segments in mice (sensitized with 0.05 mg and 0 protein) i.p. and i.g. challenged with OVA, PNA, β-LG and PAP, respectively. Severe lung lesions are bound up with clinical signs of systemic anaphylaxis in this model. Solid arrows indicate inflammatory cell infiltration. (a), (e), (i), (m) Ear segments from mice i.p. sensitized and i.p. challenged with OVA, PNA, β-LG and PAP, respectively. (b), (f), (j), (n) Ear segments from control mice challenged with OVA, PNA, β-LG and PAP, respectively. (c), (g), (k), (o) Ear from i.p. sensitized and i.g. challenged with OVA, PNA, β-LG and PAP, respectively. (d), (h), (l), (p) Ear segments from control mice i.g. challenged with OVA, PNA, β-LG and PAP, respectively.

the development of clinical symptoms that are caused during renewed exposure to the food allergen. In this elicitation phase, allergen-specific IgE becomes cross-linked on the surface of mast cells after the allergen binds to allergen-specific IgE, and intracellular signaling events are initiated, activating the mast cells. The granules of the activated mast cells are rapidly released, with preformed mediators such as histamine. Mast cells synthesize and secrete various other mediators (such as cytokines, chemokines and leukotrienes) into the immediate extracellular environment. Circulating basophils, eosinophils, neutrophils and Th2 lymphocytes are attracted to the site of mast cell activation by these mediators, where the immune reaction is magnified and the symptoms of food allergy occur.[8]

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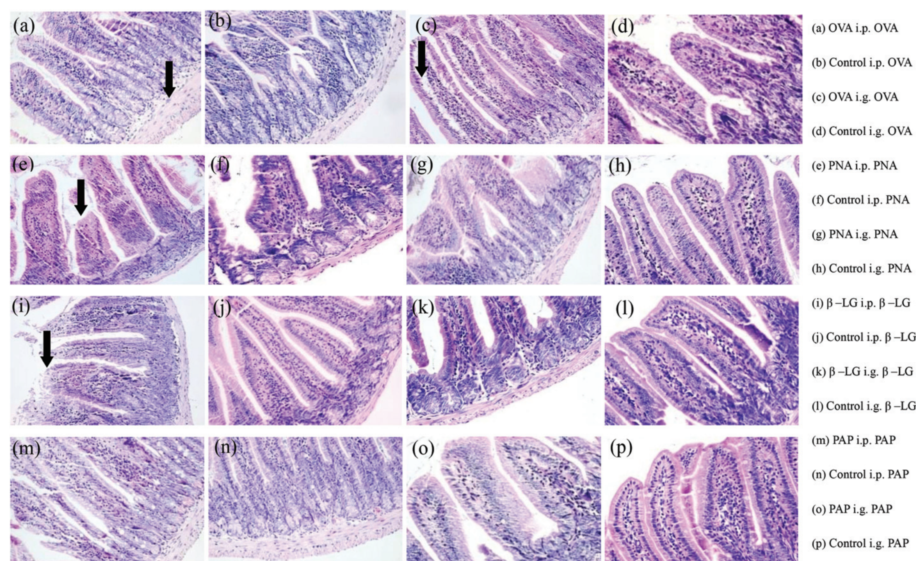

Fig. 10 The histopathology results of jejunum segments in mice (sensitized with 0.05 mg and 0 mg protein) i.p. and i.g. challenged with OVA, PNA, β-LG and PAP, respectively. Severe jejunum lesions are linked to clinical signs of systemic anaphylaxis in this model. The solid arrow in (a) indicates inflammatory cell infiltration in submucosa; the solid arrow in (c) indicates villi swelling; the solid arrows in (e) and (i) indicate structure looseness. (a), (e), (i), (m) Ear segments from mice i.p. sensitized and i.p. challenged with OVA, PNA, β-LG and PAP, respectively. (b), (f), (j), (n) Ear segments from control mice challenged with OVA, PNA, β-LG and PAP, respectively. (c), (g), (k), (o) Ear segments from mice i.p. sensitized and i.g. challenged with OVA, PNA, β-LG and PAP, respectively. (d), (h), (l), (p) Ear segments from control mice i.g. challenged with OVA, PNA, β-LG and PAP, respectively.

Naive CD4+ T cells can differentiate into T helper 1 (Th1) cells or Th2 cells, dependent on the cytokine profile that is induced: IFN-γ drives the maturation in the direction of Th1, while IL-4 drives the maturation in the direction of Th2. Typically, Th1 cells are related to cell-mediated immunity for eliminating cancer cells and fighting against viruses and other intracellular pathogens, while Th2 cells are predominantly involved in humoral immunity and up-regulate antibody production to direct against extracellular organisms.[21] Allergy proteins result in Th2-prone immunological reactivity to release cytokines including IL-4, IL-5, IL-10 and IL-13, which induce an isotype switch making B cells become IgE producing plasma cells.[12,14] In line with the above, our study showed that significantly higher IL-4 responses were observed in protein-allergic mice than in healthy control mice, while there was no significant difference in IFN-γ between the protein group and control group. The order of protein groups from high to low IL-4 levels is PNA > β-LG > PAP. The results of cytokine analysis indicated that proteins might induce Th2-prone immunological reactivity instead of Th1-prone immunological reactivity, and the order of protein inducing Th2-prone immunological reactivity might be PNA > β-LG > PAP. To definitely draw the above conclusion on the Th1/Th2 shift, more cytokine measures could be included, like IL-5, IL-10 and IL-13, in further experiments.

Based on the mechanism of food allergy, producing a high content of allergen-specific IgE when mice are dosed with a strong allergen is of utmost importance in order to develop a BALB/c mouse model for food allergy. The order of protein groups from high to low specific IgE levels is PNA > β-LG > PAP, which is consistent with the result of IL-4.

Immunoglobulin E (IgE), mast cells, and histamine are most likely responsible for most human allergies; an alternative pathway mediated by IgG might mediate anaphylaxis in persons repeatedly exposed to large quantities of allergen and might be more important in the elicitation of the allergy.[22–24] Mice, like human beings, could suffer from IgE/FcεRI/mast cell-mediated gastrointestinal and systemic anaphylaxis. With the exception of the classic pathway, mice also have allergies through another pathway requiring IgG antibodies, macrophages or basophils, FcγRIII and platelet-activating factor (PAF).[24,25] Systemic anaphylaxis in mice can be mediated largely through IgG1 and FcγRIII.[26] One type of IgG1 has allergic activity, and its synthesis is IL-4 dependent (IL-4 was produced by Th2-like cells), which is similar to IgE.[27] In IgG1-mediated systemic anaphylaxis, antigens can form complexes with specific IgG1, macrophages or basophils can efficiently capture the IgG1-allergen complexes *via* FcγRIII expressed on their surface and then macrophages or basophils release PAF upon stimulation with IgG1-allergen complexes that cross-link cell FcγRIII. PAF increases vascular permeability with 1000- to 10 000-fold more potency than histamine.[28] The order of protein groups from high to low specific IgG1 levels is PNA > β-LG > PAP, which is consistent with the results of IL-4 and specific IgE.

The results of the above parameters (IL-4, IFN-γ, specific IgE and IgG1) showed that the order of the potential allergenicity of proteins (from high to low) might be PNA > β-LG > PAP. In line with the results, many studies[16,29–34] showed that PNA was regarded as a reference allergen like OVA (potent allergen), β-LG was considered as a moderate allergen and PAP was used as a non-allergen. It was indicated that the BALB/c mouse

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model might be a useful model to rank proteins with inherent potential allergenicity.

Severe hypersensitivity symptoms were observed in mice of the protein groups OVA, PNA and β-LG at 40 min after the i.p. challenge. Symptoms of hypersensitivity included reduced activity, bristled fur, tremor and convulsion, while no obvious symptoms were observed in PAP-treated mice and control mice. At 40 min after i.g. challenge, no obvious symptoms were observed in protein-treated mice and control mice. It is obvious that the i.p. challenge was more sensitive than the i.g. challenge.

Histamine is a potent mediator of numerous biological reactions. In the human organism, it is virtually ubiquitous in tissues and body fluids, being mainly stored in its inactive form in the metachromatic granula of mast cells and basophilic leucocytes. On release, histamine functions as a potent mediator of numerous physiological and pathophysiological processes in nearly all organs and tissues.[17,35,36] Histamine has been clearly implicated as a primary mediator of "immediate type" allergic reactions (IgE-mediated allergic sensitization). At 50 min after the i.p. challenge, higher, statistically significant histamine levels were observed in protein-treated mice, compared to control mice, while at 50 min after i.g. challenge, there was no significant difference in histamine levels in the protein-treated mice compared to the control mice. It is obvious that the i.p. challenge was more sensitive than the i.g. challenge, which is consistent with the results of the observation of clinical symptoms and histopathology analysis.

Food allergies affect multiple organs (*e.g.* lungs, intestines, ears and skin) and induce many clinical symptoms. At 50 min post i.p. challenge, inflammation occurred in the ear, lung and jejunum of mice sensitized with PNA, β-LG and OVA; there were more severe histopathological lesions after the i.p. challenge than the i.g. challenge with PNA, β-LG and OVA. In line with the results, Husain's study[33] showed that massive ear swelling was observed as early as 30 min post i.p. challenge and peaked 1 h post i.p. challenge. For further study, immunohistochemistry, *e.g.* using Ly6G-antibody for neutrophils, might be a good marker to verify the cell infiltration in the ear, lung and jejunum.

Herein, we presented a murine model of food allergy in BALB/c mice through intraperitoneal sensitization, i.p. and i.g. challenges. Our model demonstrated an IgE mediated hypersensitivity reaction in mice. This study showed that the BALB/c mouse model might be a valuable model to identify the allergenicity of different proteins and research clinical symptoms of food allergy. However, it is necessary to further assess the model. In particular, allergenic whole foods or their protein extracts are needed to further validate the BALB/c mouse model, before the model is used for the evaluation of food allergy.

5 Conclusion

In this study, the BALB/c mouse model was used to assess the potential allergenicity of three proteins (PNA, β-LG and PAP; OVA was used as a positive control protein) and two ways of elicitation of allergic reaction (intraperitoneal injection and intragastric administration) were compared. A general finding was that the order of potential allergenicity might be PNA > β-LG > PAP; the i.p. challenge could be more sensitive to induced food allergy than the i.g. challenge and the parameters (IL-4, specific IgE, histamine, histopathology) should be chosen. Data in this study show that it is possible to develop a mouse model that will be valuable in identifying the inherent potential allergenicity of proteins; however, further studies, including reproducibility and other conditions, are required before using the BALB/c mouse model for food allergies.

Abbreviations

OVA	Ovalbumin
PNA	Peanut agglutinin
β-LG	β-Lactoglobulin
PAP	Potato acid phosphatase
ELISA	Enzyme-linked immunosorbent assay
i.p.	Intraperitoneal injection
i.g.	Intragastric administration

Conflicts of interest

There are no conflicts of interest to declare.

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