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Food Proteins from Different Allergen Families Sensitize Balb/C Mice Induced Family-specific Immunological Response

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Abstract

Background: The classification of food allergens based on the structure and function of proteins contributes to the study of the relationship between bioinformatics and potential allergenicity of allergens. Food allergens always share sequence similarity with the allergens in the same allergen families. For that reason, food proteins from different allergen families may induce different patterns of immune responses in animal models.

Methods: 3-4-wk-old female Balb/c mice were sensitized with food proteins once per week for 4 wk, and then challenged 2 wk later (on Day 42 of study). Serum levels of histamine and protein-specific IgG₁ and IgE antibodies, and measures of vascular permeability were performed 20 min thereafter. Five food proteins (11S globulin, OVA, HSA, LRP and Cry 1Ab/Ac) of different allergen families were used to assess patterns of immune responses for each allergen family and then bioinformatics and digestive stability in simulated gastric fluid were employed to assess the overall utility of the Balb/c.

Result: The assay results indicated that in this model, histamine and protein-specific IgE antibodies levels, and vascular permeability, could be used to identify allergenicity of 11S globulin, OVA and PAP only. However, the result of protein-specific IgG₁ measures could only distinguish allergic food protein with negative control.

Conclusion: Based on bioinformatics, the five different food proteins clearly distinct patterns of immune responses in the Balb/c model.

Keywords: Mice model; Allergen family; Food protein; Allergenicity

Introduction

Food allergy is a serious and apparently growing problem worldwide. The results of epidemiology surveys indicate that up to 6% of young children and 3-4% of adults suffer from food allergy [1]. Food-induced allergic reactions may be responsible for different clinical symptoms including mild urticaria, diarrhea or anaphylactic shock.

Food allergy is an important manifestation of type I allergic reactions and mainly mediated by Immunoglobulin E (IgE). Protein specific IgE can be induced by ingesting food allergens and binding of these allergens by specific IgE on effector cells, such as basophils and mast cells, leads to mediator release (i.e. histamine) and a subsequent cascade of pathologic event. Currently, food allergens are classified into plant and animal types, and >500 have been identified to date.

Bioinformatics analysis is widely used to evaluate the potential allergenicity of protein. And it is recommended by FAO/WHO for assessing the allergenicity of exogenous proteins [2]. The principle of bioinformatic analysis is the contrast of amino acid sequence of testing proteins with known allergens in the database. Simulated Gastric Digestion (SGF) is also recommended by FAO/WHO in the guideline of the assessment of potential allergenicity of exogenous proteins [2]. Previous studies suggested that those proteins that can induce anaphylaxis possess the characteristic of digestion-stability [3].

Animal model is a research focus of the assessment of allergenicity of food protein in the last decade. The studies of the classifications of food allergens show that food allergens from the same family always have similarity in structures or functions [4,5]. The differences of structures or functions may lead to different patterns of immune response. In this study, we attempted to establish the utility of a Balb/c mouse model for evaluating the allergenicity of food proteins. In addition, this model

was used to investigate if there were also different patterns of immune responses induced by food proteins from diverse allergen families.

Materials and Methods

Preparation of food proteins

OVA (Ovalbumin, Grade VI) and PAP (Potato acid phosphatase) were purchased from Sigma-Aldrich (St. Louis, MO). 11S globulin (Gly m 6) was extracted from de-fatted soybean meal that was extracted in 0.02 M phosphate-buffered saline (PBS [pH 8.5]; soybean meal:PBS=1:16 [w/v]) for 45 min at 40°C. After centrifugation 2000×g (10 min), the supernatant was collected, adjusted to pH 6.2, and placed in an ice bath for 12 hr. The extract was then centrifuged at 2000×g (10 min) and precipitates bearing 11S globulin collected [6]. The recombinant HSA (human serum albumin), LRP and Cry 1Ab/Ac were produced by our laboratory (as outlined in Cao et al.) [7] had been expressed in BL21 E. Coli (TianGen, Beijing, China) and purified from His Trap™ FF gel column (Amersham Biosciences, Uppsala, Sweden).

The classification of food allergens

mllFam (<http://www.meduniwien.ac.at/allergens/allfam/>) is based on Allergome database which is the currently most comprehensive

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collection of allergen data. The list of 528 food allergens (including the isoallergens) is gained from SDAP (<http://fermi.utmb.edu/SDAP/index.html>). Pfam ID of the allergen is also obtained from the database in SDAP. With the shared Pfam ID as a standard, allergens in the same family could be identified and retrieved from AllFam. The method of classification described above was applied to obtain the allergen family to which OVA, 11S glycinin, HSA, LRP and Cry 1Ab/Ac belong.

Digestive stability in simulated gastric fluid

Simulated gastric fluid (SGF) was prepared. In brief, 0.2 g NaCl and 62.4 mg pepsin (4220 U/mg; Sigma) in 70 ml distilled water had its pH adjusted to 1.2 with HCl, add then the mixture volume was brought 100 ml with distilled water. For the digestive analyses, the mixing ratio of test protein and SGF was 1:19 (v/v). Upon introduction of the protein, the reaction was allowed to proceed for 15 sec, 2 min, 30 min and 60 min at 37°C, at which time reaction was inactivated by addition of 70 μ l 0.2 M NaHCO₃. The resulting products were in the samples were then underwent SDS-PAGE analyses.

Mice

The alb/c (female, 3-4-wk-old) mice were purchased from Vital River (Beijing, China) and then allowed to acclimate to their new housing for 1 wk before beginning experimental protocols. Mice were housed under specific pathogen-free conditions (23-26°C, 40-70% relative humidity, and 12-hr light/dark cycle) with *ad libitum* access to standard rodent diet (free of any of test proteins) and filtered water during the course of the study. The Beijing Municipal Science and Technology Commission of China (NO SYXK 2010-0036) approved all of the animal studies described herein.

Sensitization and challenge protocols

Mice of each group (n=8) received a dose by gavage of 1 mg test protein (11S globulin, OVA, PAP) in 100 μ l saline once per week on 4 consecutive weeks. All the diets for the mice are free of test protein. Retro-orbital plexus samples were obtained at Day 0 and at the end of each week, and processed for sera/plasma (see below). After this induction period, the mice were not exposed to protein for 2 wk. On Day 42 of the study, all sensitized animals were orally challenged with 10 mg protein. The negative control group was treated with the same volume of saline. Mice were exsanguinated 20 min after challenge. The tubes containing EDTA-K₂ were used to collect plasma for histamine analysis. Blood samples were obtained from the orbital plexus at day 0 and each week. Common tubes were used for sera collection; each sample was allowed to clot for 1 hr at room temperature. In all cases, the blood samples were centrifuged at 3000×g (10 min, 4°C) to obtain plasma for histamine measures or sera for use in antibody analyses. Individual serum and plasma samples were prepared and stored at -20°C until analysis.

40 min after the challenge, mice was injected with 3 ml of PBS, 10 mM EDTA. After a 1 min abdominal massage, a small incision was made on the peritoneal membrane and the peritoneal lavage (PL) fluid was gently aspirated. The aspirate was centrifuged at 600×g for 6 min at 4°C and the supernatant was collected and frozen at -70°C until analyzed [8].

Determination of protein-specific IgG₁ and IgE levels

Protein-specific IgG₁ and IgE antibodies were detected using indirect enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well microtitre plates (Costar, Corning, NY) were coated with 10 mg given test protein/L in 0.05 M sodium carbonate buffer (pH 9.6) and

incubated at 4°C. After blocking the wells with PBS/0.3% BSA, serum samples were applied to the wells, and IgE binding was detected by biotin-conjugated rat anti-mouse IgE antibodies (Abcam, Cambridge; diluted 1:2000) and then a detector, Horseradish Peroxidase (HRP)-conjugated streptavidin (Thermo Scientific, Waltham, MA; diluted 1:4000). For detection of IgG₁, HRP-conjugated rat anti-mouse IgG₁ (eBioscience, San Diego, CA; diluted 1:2000) was used. For detection in both scenarios, after washing away unbound detector or primary, 100 μ l TMB (tetramethylbenzidine; KPL, Gaithersburg, MD) was added for 10 min and the reaction was stopped by 50 μ l of 2 M H₂SO₄. The titres of protein-specific antibodies were then estimated as described previously in Sun et al. [9].

Determination of histamine levels

Plasma histamine levels were determined using a mouse Histamine (HIS) ELISA Kit (Cusabio Biotech, Wuhan, China), according to the manufacturer's instructions. The sensitivity of the kit was 7.8 pg/ml.

Vascular leakage

Vascular leakage was determined by measuring albumin levels in the PL fluid 40 min after challenge as described previously [8]. The content of albumin was quantified in the supernatants using a Bicinchoninic Acid kit (Biomiga, San Diego, USA). Albumin concentration was calculated according to a BSA standard curve.

Results

The classification of food allergens

456 of the 528 food allergens listed in SDAP belong to corresponding allergen families in AllFam. Further 60% of these 456 belonged food allergens belong to five allergen families: Prolamin superfamily, EF hand domain, Cupin superfamily, Profilin and Bet v 1-related protein. Twenty-eight Allergen families contain fewer than three food allergens, and make up 60% of all the food allergen families. That suggests that most of the food allergens could be categorized into the main food allergen families, an analysis consistent with the results of an analysis of Mills et al. [10].

The bioinformatics analysis and classification of tested food proteins

As a result of the search in AllFam database, it was determined that OVA and 11S globulin belonged to Serpin serine protease inhibitor, and Cupin superfamily respectively. The sequences of LRP, HSA and Cry 1Ab/Ac were searched in Allergen Online database. The results of those analyses indicated that HSA was homologous with Bos d 6 (Figure 1A), which belongs to serum albumin, and that LRP shared a homology with Gly m 4 (Figure 1B), which belongs to Bet v 1-related protein. The result of Cry 1 Ab/Ac suggested it was not a potential food allergen (Figure 1C).

Kinetics of protein digestion by pepsin

The results showed that with 11S globulin (composed of two subunits, A [acid subunit] and B [basic subunit]), the A subunit was digested between 2 min and 30 min, whereas B subunit could resist digestion until 60 min (Figure 2A). In contrast, OVA (Figure 2B) and Cry 1Ab/Ac (Figure 2C) were completely digested between 2 min and 30 min, suggesting that each has weak digestion stability. The results of HSA and LRP digestion assays indicated that each could be pepsinized rapidly (Figures 2D and 2E) and thus each is thus not digestion-stable.

A

80mer Sliding Window Search Results

Hit #	Defline	Species	Best %ID	# Hits > 35%	Full Alignment E-val	Length	Links NCBI Details
1	gi 1543794 sp P35747.1 ALBU_HORSE RecName: Full=Ser	Equus caballus	92.50%	506of506	2.4e-190	76.20%	gi 1543794 GO
2	gi 1351908 sp P49064.1 ALBU_FELCA RecName: Full=Se	Felis catus	91.20%	506of506	1.6e-201	81.80%	gi 1351908 GO
3	gi 6687188 emb CAB64867.1 albumin [Canis lupus fa	Canis familiaris	90.00%	506of506	1e-197	79.90%	gi 6687188 GO
4	gi 1351907 sp P02769.4 ALBU_BOVIN RecName: Full=Se	Bos taurus	90.00%	506of506	2.3e-189	75.60%	gi 1351907 GO
5	gi 633938 gb AAB30434.1 albumin [Canis lupus fami	Canis familiaris	90.00%	276of506	1.1e-088	82.20%	gi 633938 GO
6	gi 3319897 emb CAA76841.1 albumin [Canis lupus fa	Canis familiaris	90.00%	506of506	1.3e-196	79.40%	gi 3319897 GO
7	gi 3336842 emb CAA76847.1 bovine serum albumin [B	Bos taurus	90.00%	506of506	4.6e-189	75.50%	gi 3336842 GO

Number of sequences with at least one 8mer match = 8

gi|3336842 Hits:88 -- Def: bovine serum albumin [Bos taurus]
 gi|1351907 Hits:88 -- Def: RecName: Full=Serum albumin; AltName: Full=BSA; AltName: Allergen-Bos d 6; Flags: Precursor
 gi|3319897 Hits:95 -- Def: albumin [Canis familiaris]
 gi|6687188 Hits:100 -- Def: albumin [Canis familiaris]
 gi|543794 Hits:83 -- Def: Serum albumin precursor (Allergen Equ c 3)
 gi|1351908 Hits:127 -- Def: Serum albumin precursor (Allergen Fel d 2)
 gi|113575 Hits:1 -- Def: Serum albumin precursor (Alpha-livetin) (Allergen Gal d 5)
 gi|633938 Hits:33 -- Def: albumin [Canis familiaris]

B

80mer Sliding Window Search Results

Hit #	Defline	Species	Best %ID	# Hits > 35%	Full Alignment E-val	Length	Links NCBI Details
1	gi 1134194 sp P26987.1 SAM22_SOYBN RecName: Full=St	Glycine max	87.50%	79of79	4.7e-044	83.50%	gi 1134194 GO
2	gi 60418924 gb AAK19889.1 pathogenesis-related pr	Vigna radiata	87.50%	79of79	4.8e-039	78.10%	gi 60418924 GO
3	gi 37499626 gb AAQ91847.1 Ara h 8 allergen [Arach	Arachis hypogaea	76.23%	79of79	1.7e-036	71.60%	gi 37499626 GO
4	gi 169786740 gb ACA79908.1 Ara h 8 allergen isofo	Arachis hypogaea	75.00%	79of79	2.1e-035	68.40%	gi 169786740 GO
5	gi 110676574 gb ABG85185.1 pathogenesis-related p	Arachis hypogaea	71.30%	79of79	3.4e-034	66.50%	gi 110676574 GO
6	gi 534898 emb CAA54696.1 1 Sc-3 [Betula pendula]	Betula pendula	60.04%	79of79	4.8e-028	58.10%	gi 534898 GO
7	gi 5726304 gb AAD48405.1 AF136945.1 major allergen	Corylus avellana	60.00%	79of79	9e-028	59.50%	gi 5726304 GO
8	gi 11762106 gb AAG40331.1 AF323975.1 major allergen	Corylus avellana	60.00%	79of79	9e-028	59.50%	gi 11762106 GO
9	gi 11762102 gb AAG40329.1 AF323973.1 major allergen	Corylus avellana	58.80%	79of79	2.5e-027	58.20%	gi 11762102 GO
10	gi 1813216 gb AAC02632.1 cherry-allergen PRIMA1 [P	Prunus avium	58.80%	79of79	5.6e-026	55.60%	gi 1813216 GO
11	gi 145904610 gb ABP97433.1 Ara h 8 allergen isofo	Arachis hypogaea	58.80%	79of79	6.4e-024	51.00%	gi 145904610 GO
12	gi 212291470 gb ACJ23864.1 Fag s i pollen allergen	Fagus sylvatica	58.80%	79of79	1.2e-025	55.10%	gi 212291470 GO

Number of sequences with at least one 8mer match = 140

gi|37499626 Hits:10 -- Def: Ara h 8 allergen [Arachis hypogaea]
 gi|169786740 Hits:10 -- Def: Ara h 8 allergen isoform 3 [Arachis hypogaea]
 gi|110676574 Hits:2 -- Def: pathogenesis-related protein 10 [Arachis hypogaea]
 gi|60418924 Hits:32 -- Def: pathogenesis-related protein 10 [Vigna radiata]
 gi|145904610 Hits:4 -- Def: Ara h 8 allergen isoform [Arachis hypogaea]
 gi|730049 Hits:6 -- Def: Major pollen allergen Car b 1 isoform 2 (Car b 1)
 gi|1545895 Hits:7 -- Def: pollen allergen Car b 1 [Carpinus betulus]
 gi|1545897 Hits:7 -- Def: pollen allergen Car b 1 [Carpinus betulus]

C

80mer Sliding Window Search Results

No Matches of Greater than 35% Identity Found

No sequences found with an exact 8mer match

Figure 1: The results of FASTA of HSA, LRP, Cry 1Ab/Ac on Allergen Online. A represents the result of HSA, which is homologous with Bos d 6. B represents the result of LRP, which shares a homology with Gly m 4. C represents the result of Cry 1Ab/Ac, which is not a potential food allergen.

Balb/c mouse model can distinguish different allergenicity of food proteins

Balb/c mice were sensitized over 4 wk by oral gavage. OVA are a model food allergen in rodent model. The results of OVA group and negative group were compared to assess the Balb/c model in our study. The results of protein-specific IgG₁ and IgE antibodies, level of histamine and vascular permeability are shown in figure 3. All the indexes of OVA group were significant higher than negative group. That

suggested that a T-helper (T_H)-2 cellular response was induced in OVA-sensitized mice.

The mice here were sensitized with one of three proteins (PAP, OVA and 11S globulin) of different allergenicity in order to certain if this model could distinguish differing allergenicities of food proteins. PAP exist little evidence for allergenicity, while OVA and 11S globulin are well-known as food allergens. OVA were apparently easy to develop oral tolerance against in mice model, and 11S globulin has

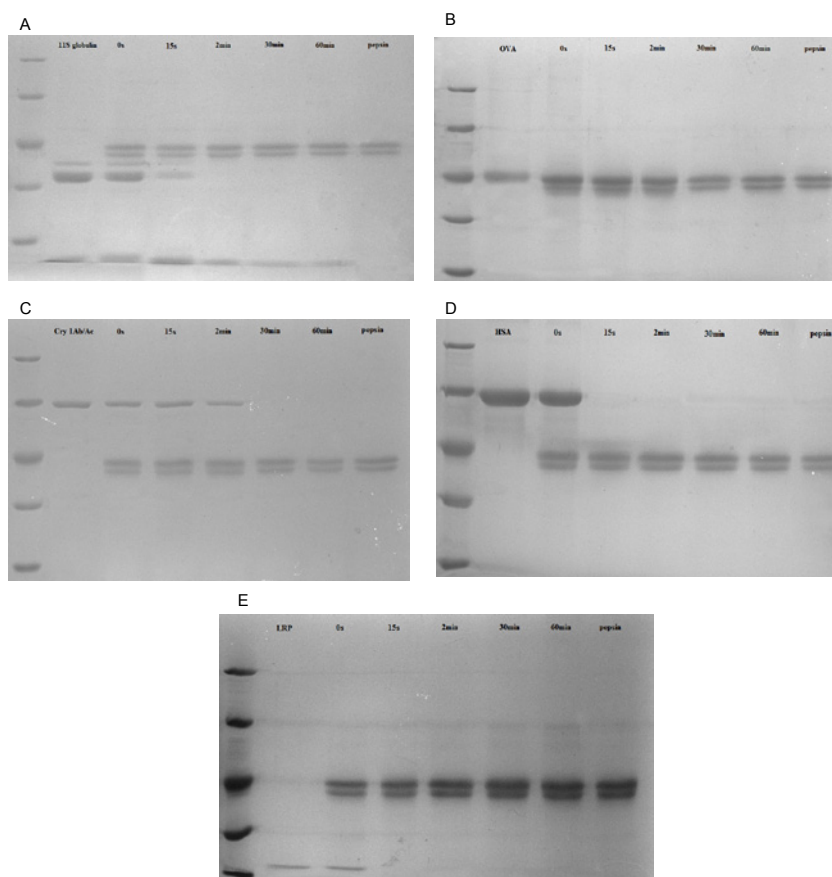


Figure 2: The results of SGF of 11S globulin, OVA, Cry 1Ab/Ac, HSA and LRP. The marker represents 116 kD, 66.2 kD, 45 kD, 35 kD and 25 kD respectively. **A** represents 11S globulin, whose B subunit can resist digestion until 60 min. **B** represents OVA and **C** represents Cry 1Ab/Ac, which were completely digested after 2 min. **D** represents HSA and **E** represents LRP, which were pepsinized rapidly.

strong allergenicity. The results of protein-specific IgE antibodies (Figure 4A), histamine (Figure 4B) and vascular leakage (Figure 4C) were in accordance with the allergenicity of these three proteins (11S globulin>OVA>PAP). However, histamine levels of OVA and 11S globulin group have no significant difference. In contrast, a strong response of PAP-specific IgG₁ antibodies was induced in our experimental condition (Figure 4D).

The data summarized above reveal that the Balb/c model in this study has the capacity not only to distinguish allergenic food proteins from non-allergenic food proteins via oral exposure but also to identify the allergenicity of food allergens. Nevertheless, protein-specific IgG₁ is not appropriate for this model.

Food proteins from different allergen families induce different patterns of immunological response

On the basis of the results of bioinformatic analysis, 11S globulin, OVA, HSA and LRP belong to, respectively, the cupin superfamily, serpin serine protease inhibitor, and serum albumin and Bet v 1-related protein. Cry 1Ab/Ac was not a potential food allergen according to the analysis. Different patterns of immune response were induced with the five protein. The 11S globulin has strong allergenicity. High levels of 11S globulin-specific IgE and histamine could be observed in mice, and vascular leakage was also significant increase up to Day 35 (Figures 5A and 5B), and decreased thereafter (Figure 5C). In contrast, the level

of 11S globulin-specific IgG₁ antibodies was relatively low, and no significant weekly differences were noted (Figure 5D).

As an allergen that can induce oral tolerance easily, while OVA induced a strong specific IgG₁ response, specific IgG₁ levels were decreased at Day 21 and 28 (Figure 5D). The level of specific IgE was relatively lower than 11S globulin group, and it declined from day 28 (Figure 5C). Moreover, the level of histamine and vascular leakage in the OVA-sensitized mice increased significantly in this period (Figures 5A and 5B). Compared with OVA and 11S globulin, relatively weak responses in histamine and vascular leakage were induced by three exogenous proteins (HSA, LRP, Cry 1Ab/Ac) (Figures 5A and 5B). The level of LRP-specific IgG₁ was higher than before at Day 42 (Figure 5D). The result of LRP-specific IgE was quite low, and the level was had no significant difference weekly (Figure 5C). A relatively weak response of specific IgG₁ and IgE are induced by HSA (Figures 5C and 5D). The level of HSA-specific IgG₁ reached the maximum value at day 21 (Figure 5D), whereas the level of specific IgE increased from day 28 (Figure 5C). The result of specific IgG₁ and IgE antibodies of Cry 1Ab/Ac, as a non-allergenic exogenous protein, had no significant difference weekly (Figures 5C and 5D).

Discussion

In this study, we used five food proteins from different allergen families to study family-specific pattern of immune responses.

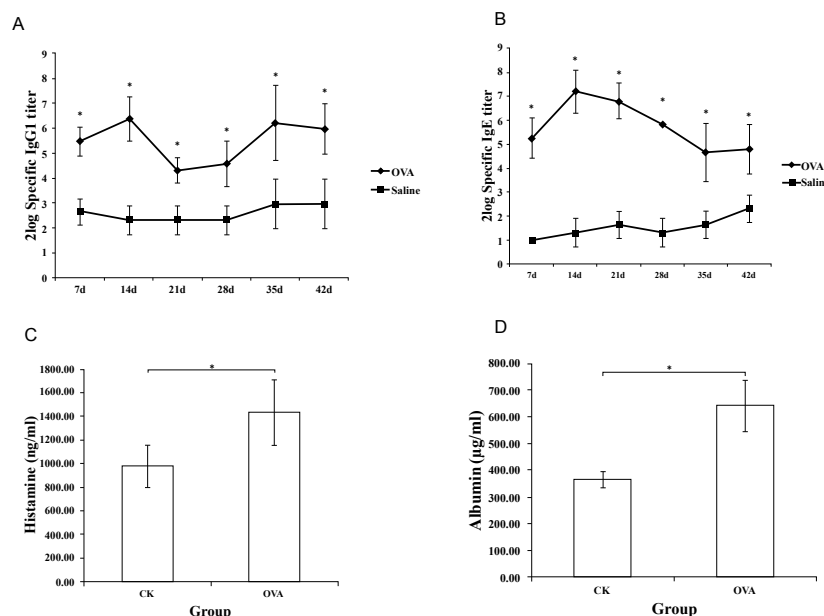


Figure 3: The results of specific antibodies, histamine and vascular permeability of OVA-sensitized group and negative group. **A** represents the results of specific IgG1. **B** represents the results of specific IgE. **C** represents the results of histamine. **D** represents the results of vascular permeability. All these four indexes show that Th2 response was induced in OVA-sensitized mice.

*represents the significant differences ($p < 0.05$).

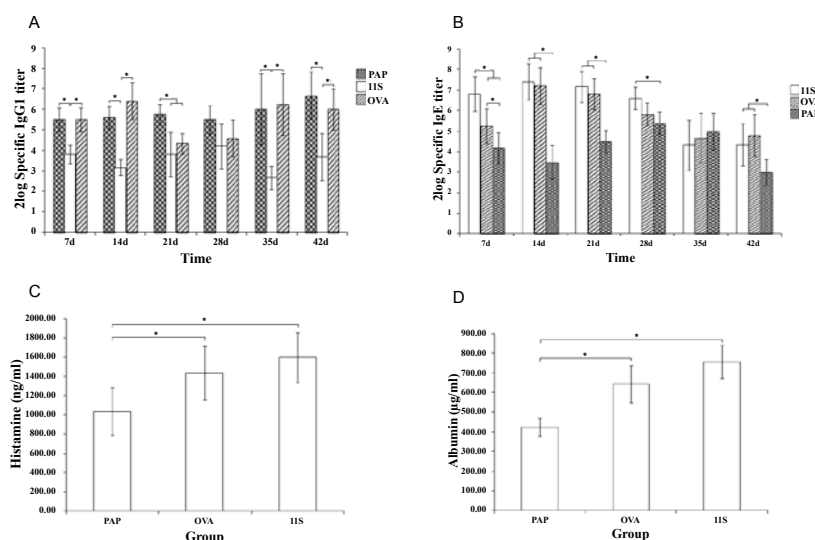


Figure 4: The results of specific antibodies, histamine and vascular permeability of PAP-, OVA- and 11S globulin-sensitized groups. **A** represents the results of specific IgG1. **B** represents the results of specific IgE. **C** represents the results of histamine. **D** represents the results of vascular permeability. Except of specific IgG antibodies, the results of specific IgE, histamine and vascular permeability are in accordance with the allergenicity of these three proteins (11S globulin>OVA>PAP).

* represents the significant differences ($p < 0.05$).

For studying the different immune responses in animal model, we established Balb/c mouse model. According to the results of specific IgG and IgE allergenicity, histamine and vascular permeability, Th2 response can be induced with food allergens (Figure 1). Moreover, three food proteins with different allergenicity were used to sensitize mice. Except of the protein-specific IgG₁ antibodies, the results of protein-specific IgE antibodies, histamine and vascular permeability distinguished the allergenicity of these three food proteins (11S globulin>OVA>PAP). A

study about C3H/HeJ mice model showed that C3H/HeJ can identify the allergenicity of food proteins through oral gavage with CT (cholera toxin) [11]. However, long-term use of CT seems to be responsible for the unselective immune response induced by this adjuvant [12]. The Balb/c mice model in our experimental condition demonstrated that sensitized mice by gavage without adjuvant could evaluate the allergenicity of food proteins. But the specific IgG₁ is not appropriate for this mice model, as the result of specific IgG₁ was opposite with the

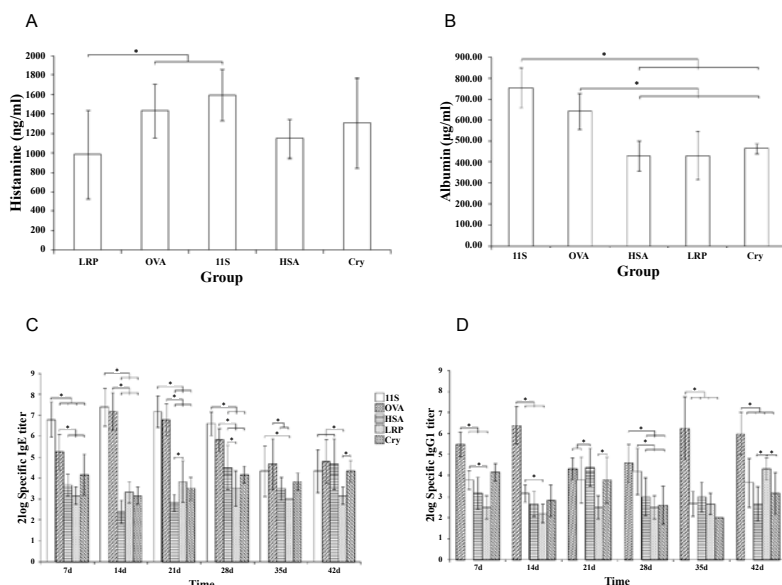


Figure 5: The results of specific antibodies, histamine and vascular permeability of 11S- globulin, OVA-, HSA-, LRP- and Cry 1Ab/Ac-sensitized groups. **A** represents the results of specific IgG1. **B** represents the results of specific IgE. **C** represents the results of histamine. **D** represents the results of vascular permeability.

* represents the significant differences ($p < 0.05$).

allergenicity of three known food proteins. Many studies have put focus on IgG-mediated allergy at present. Mouse FcγRIII is a low affinity receptor of IgG₁. In contrast to the IgE-mediated pathway, this pathway takes relatively longer to induce anaphylaxis, and requires high concentration of IgG₁, and antigen for elicitation [13,14]. Previous study has shown that IgG₁ antibodies can function as blocking reagents to inhibit IgE-mediated systemic anaphylaxis when antigen-specific IgE and IgG₁ coexist [13,15]. We have also measured the rectal temperature, but there was no significant differences among each groups (data not shown), which can be explained by the reports mentioned above.

We also used the Balb/c mouse model to analyze the different patterns of immune response induced by food proteins from different allergen families. It has been suggested sequence comparison of allergenic members of some protein families showed a wide range of the degree of sequence conservation. Furthermore, the high extent of IgE cross-reactivity observed within families may reflect this sequence conservation [16]. We hypothesize that food proteins from different allergen families can induce different patterns of immunological response.

From the analysis of bioinformatic, five food proteins (11S globulin, OVA, HSA, LRP and Cry 1Ab/Ac) used in this study belong to different allergen families (Cry 1Ab/Ac is not a potential food allergen proved by bioinformatic analysis). Serpin serine protease inhibitor is a cross-food allergen family, which covers both plant and animal food allergens. Most of allergens in this family share a multi-domain fold containing a bundle of 8 or 9 α-helices and a β-sandwich formed by 3 β-sheets. Cupin superfamily includes 24 food allergens and 26 isoallergens. Most of allergens are from legumes, nuts and seeds [10]. These proteins share two short conserved consensus sequence motifs and a β-barrel structure [17]. The members of this family mainly belong to seed storage globulins that can be divided into two subfamilies: 7/8S vicilins and mature 11S legumins.

Bet v 1-related protein includes 18 food allergens and 44

isoallergens, most of which are widely distributed among vascular plants, such as Rosaceae (apple, pear, stone fruits), Apiaceae (celery, carrot), and Fabaceae (soybean, peanut). Allergens from this family are formed by seven anti-parallel β-strands and three α-helices. Besides, they also share a C-terminal amphipathic helical motif, which consists of a helix [18]. This family is classified by sequence similarity into three subfamilies: pathogenesis-related (PR)-10, Bet v 1 major allergen and Ripening-Related (RRP) proteins [18]. PR-10 includes a majority of allergens in those three subfamilies [19]. Bet v 1 is the major birch pollen allergen. Many patients with pollen allergy are also allergic to vegetable and fruit, which suggests plant food allergens have IgE cross-reactivity with Bet v 1 [20].

Allergens in serum albumin can induce anaphylaxis by inhalation as well as ingestion [21]. Bos d 6 is capable of sensitizing by inhalation or by ingestion of beef or cow's milk [22]. The results of bioinformatics analysis indicated that mice sensitized by these five food proteins generated different patterns of immune responses. Specific IgG₁ antibodies induced by OVA from Serpin serine protease inhibitor represented downtrend at day 21 and 28. Specific IgE antibodies declined from day 28. 11S globulin from Cupin superfamily induced a strong IgE response, and it declined from day 35. The level of specific IgG₁ was relatively low. Three exogenous proteins all show weaker allergenicity than OVA and 11S globulin. However, they induced different patterns of immunological response as well. LRP shares a homology with Gly m 4, which belongs to Bet v 1-related protein. The level of LRP-specific IgG₁ tended to higher at day 42. HSA may belong to Serum albumin according to bioinformatic analysis. HSA-specific IgG₁ antibodies reached the maximum value at day 21, and the level of specific IgE increased from day 28. More information of family-specific patterns of immunological response needs further researches about allergen families and food proteins.

Conclusion

We have established a Balb/c mice model for evaluating the

allergenicity of food proteins. We demonstrated in this model that food proteins from different allergen families can induce different patterns of immunological response, which may have relationship with the family-specific sequence conservation.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content of this manuscript.

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