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Peanut allergen induces more serious allergic reactions than other allergens involving MAPK signaling pathways†

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There is no universally accepted uniform research to classify the severity of allergic reactions triggered by different food allergens. We established a food allergy model based on repeated intragastric administrations of proteins from peanut, egg, milk, or soybean mixed with cholera toxin followed by oral food challenges with a high dose of the sensitizing proteins. Increased specific IgE, specific IgG1, allergic symptom scores, histamine, murine mast cell proteases-1, vascular leakage, Th2 cytokines, and mast cell infiltration in the lungs and intestine were found in the allergic groups *via* enzyme-linked immunosorbent assay, hematoxylin–eosin, and toluidine blue staining. Each sensitized group showed a decrease in body temperature and Th1 cytokines after oral food challenge. The increased levels of Th2 cytokines, IL-25, IL-33, and TSLP, and related asthma genes ARG1, DCN, LTB4R1 and NFKBIA as well as the activation of MAPK signaling pathways were also revealed by quantitative real-time PCR and western blotting. In terms of the severity of food allergies, peanut allergy was the most serious followed by egg and milk, and soybean allergy was the least severe. Compared to other allergic groups, asthma genes were regulated through the MAPK signaling pathways to produce related Th2 cytokines in peanut allergy; consequently, mice in the peanut group exhibited more severe allergic reactions. Comparison of the severity of food allergies is required for the development of milder prevention for severe food allergies.

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1. Introduction

Food allergies have emerged as a major public health issue, affecting up to 10% of the population in westernized countries.¹ Approximately 90% of allergic reactions are generally thought to be caused by eight major food allergens, namely milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soybean.² It has been reported that many children will outgrow their allergies to soy, eggs, wheat and cow's milk before school age, but peanut allergy is usually lifelong and potentially fatal.^{3,4} However, there is no universal research to classify the severity of allergic reactions depending on the food allergen considered.

Allergic reactions are triggered when allergenic proteins induce the cross-linking of specific IgE bound to their high affinity receptor (FcεRI) on the surface of mast cells and baso-

phils, inducing inflammatory reactions by secreting chemical mediators and synthesizing leukotrienes and cytokines.^{5,6} This causes clinical symptoms including gastrointestinal tract issues (stomach cramps, nausea, vomiting, diarrhea), circulatory problems (hypotension, tachycardia, arrhythmia), neurological issues (agitation, loss of consciousness) and problems with the respiratory system (bronchospasm/whooping, respiratory arrest).⁷ Allergic reactions are more serious when they involve respiratory and cardiovascular diseases.⁸ At present, research on allergies caused by various food proteins mainly focuses on the mechanism of food allergies.^{9,10} The difference and severity of allergy symptoms caused by these food proteins is still not well understood. Interestingly, Mondoulet *et al.* studied CD62L⁺ Tregs isolated from mice treated with milk-epicutaneous immunotherapy (EPIT), and found that mice with CD62L⁺ Treg transfer before peanut sensitization were protected from anaphylaxis as compared with an allergic group. The data suggested that CD62L⁺ Tregs induced by milk can prevent peanut allergic reactions.¹¹ Mondoulet *et al.* in another paper also showed that the protective effect of EPIT is observed only when sensitization to additional allergens (pollen, house dust mite, or peanut) occurs subsequent to EPIT treatment for the first allergen (to cow's milk or house dust mite).¹² Moreover, another study suggested that immuniz-

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ation with egg lysozyme after tolerance induced by an unrelated antigen OVA did not induce a primary Th2 response to lysozyme.¹³ These results imply that immune tolerance induction with mild allergens can treat other severe allergic reactions. Therefore, comparison of the severity of food allergies is required for the development of milder prevention for severe food allergies.

In this study, a food allergy model in BALB/c mice was established to compare the severity of peanut, egg, milk, and soybean allergies. We studied detailed allergic indicators, investigated gastrointestinal and respiratory tract-related indicators and detected related signaling pathways.

2. Experimental

2.1. Preparation of proteins

Defatted peanut flour and defatted soybean flour were obtained from Juxinlong Bio-technology Co., Ltd, Shandong, China. Peanut extract and soybean extract were prepared by agitating defatted peanut flour and soybean flour with Tris buffer (20 mM, pH 7.2) and Tris buffer (30 mM, pH 8.0, 10 mM β -mercaptoethanol), and they were centrifuged at 3000g for 30 minutes (min) and 10 000g for 30 min, respectively.^{14,15} The egg whites were separated from fresh eggs and were then mixed with phosphate-buffered saline (PBS). Egg extract was prepared by agitation and centrifugation (3000g, 30 min). Cow's milk protein extract was prepared from skimmed dry milk (Sanyuan, Beijing) with PBS and centrifugation (3000g, 30 min).^{16,17} The protein was lyophilized and dissolved in PBS to use for the experiments.

2.2. Mice experiments

Six-week-old female BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). The mice were treated in accordance with the Guidelines for Care and Use of Laboratory Animals of the China Agricultural University, and the experiments were approved by the Animal Ethics Committee of China Agricultural University. The mice were acclimated for 1 week before beginning stimulation. Then, the mice (12 per group) were intragastrically administered 10 mg peanut, egg, milk, or soybean protein/mouse mixed with 20 μ g of cholera toxin/mouse on days 0, 7, 14, 21 and 28. The control group was treated with PBS. Blood samples were collected from the posterior orbital venous plexus on day 35 for antibody assays. On day 42, the mice were challenged intragastrically with 50 mg of peanut, egg, milk, or soybean protein. After the intragastric challenges on day 42, the anaphylactic symptom scores and body temperature levels were measured. On day 42, the mice were sacrificed by cervical dislocation and their blood and organs were collected.

2.3. Assessment of hypersensitivity indicators

The allergic symptom scores were evaluated for 30 min after challenge on day 42 using a previously described scoring

system.¹⁸ The scoring criteria were as follows: 0, no symptoms; 1, nose, lip and eye puffiness; 2, decreased activity; 3, dyspnea, cyanosis, pale around the mouth and tail; 4, convulsions or no response after challenge; 5, death. Rectal temperature was monitored for 30 min after challenge using a WI88375 probe (Beijing Science and Technology, Beijing, China). Additionally, specific IgE and specific IgG1 were measured by ELISA as described in ref. 19 with minor modifications. Briefly, ELISA plates were coated with 10 μ g mL⁻¹ protein extract in 0.1 mol L⁻¹ carbonate buffer, pH 9.5, overnight at 4 °C. The plates were blocked with 200 μ L PBS containing 1% BSA for 2 hours at 37 °C. Serum samples were then added at 1 : 10 dilution for IgE and 1 : 8000 for IgG1, and the plates were incubated for 2 h at 37 °C. After washing, the plates were incubated with HRP-conjugated anti-mouse IgE diluted 1 : 2000 or anti-mouse IgG1 diluted 1 : 6000 (Abcam, USA) for 1 hour at 37 °C. After washing, peroxidase substrate was added, and after 15 minutes, the reaction was stopped with 2 mol L⁻¹ H₂SO₄. The concentrations of IL-4, IL-5, IL-13, and IFN- γ in the serum were determined using commercial ELISA kits (Elabscience Biotechnology Co., Ltd, Wuhan, China) according to the manufacturer's instructions.

2.4. Measurement of inflammatory mediators

The allergen-induced vascular permeability changes were determined using Evans blue dye. Sensitized and control mice were injected through the tail vein with 100 μ L Evans blue solution on day 42; the vascular permeability change was measured 30 min after the last intragastric provocation. Plasma histamine levels and murine mast cell protease 1 (mMCP-1) concentrations were determined using enzyme-linked immunoassays according to the manufacturer's instructions.

2.5. Assessment of histology

The paraffin-embedded lung and jejunum tissue sections were used to analyze the histological structures and cellular infiltration using hematoxylin and eosin staining (H&E). For the detection of mast cells, sections of jejunum and lung tissue were stained in an aqueous toluidine blue solution. Tissue slices were observed under a light microscope (Leica Microsystems Wetzlar GmbH).

2.6. Quantitative real-time PCR

The total RNA from lung tissues was extracted using Trizol Reagent (Biorigin (Beijing) Inc., China). First-strand cDNA was synthesized from 2 μ g RNA using TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). Quantitative real-time PCR was performed on a CFX96 Touch™ Real-time PCR Detection System (Bio-Rad) in a total reaction volume of 50 μ L using the SuperReal PreMix Plus (Tiangen Biotech, Beijing, China). The PCR conditions included an initial step at 95 °C for 15 min, followed by 40 cycles of amplification and quantification (95 °C for 10 seconds (sec), 60 °C for 20 sec, and 72 °C for 20 sec). The sequences of the primers (Arg1, Dcn, LTB4R1, NFKBIA, IL-25,

Table 1 Sequence of primers for quantitative real-time PCR

Primers		Sequence (5' to 3')	Primers		Sequence (5' to 3')
Arg1	Forward	GCAGTTGGAAGCATCTCTGG	IL-25	Forward	ACAGGGACTTGAATCGGGTC
	Reverse	GAGAAAGGACACAGGTTGCC		Reverse	TGGTAAAGTGGGACGAGTTG
Dcn	Forward	TTCTACTCGGCTGTGAGTC	IL-33	Forward	TATCCACGGGATTCTAGGAAGAG
	Reverse	AAGTTGAATGGCAGAACGCA		Reverse	CTCATAGTAGCGTAGTAGCACCT
LTB4R1	Forward	TACTAAGGCCTTTGCCCGAT	TSLP	Forward	ACGGATGGGGCTAACTTACAA
	Reverse	ATAGTTCGGAGCGCAGATCA		Reverse	AGTCCTCGATTTGCTCGAACT
NFKBIA	Forward	TTGGTCAGGTGAAGGGAGAC	β -actin	Forward	GATTACTGCTCTGGCTCCTAGC
	Reverse	GGATCACAGCCAGCTTTCAG		Reverse	GACTCATCGTACTCTCTGCTTGC

IL-33 and TSLP) and the β -actin internal control are listed in Table 1. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

2.7. Western blotting

Total proteins were extracted using cell lysis buffer for western and IP (Beyotime). Protein lysates were analyzed by standard western blotting. 50 mg of lung tissue samples were studied using a Tissue Lyser instrument (Hoder, China) with an oscillation frequency of 60 Hz for 1 min. Lung tissue was lysed with 450 μ L of lysate buffer for 30 min on ice. The mixture was collected and centrifuged for 10 min at 14 000g at 4 °C. Protein concentrations of the tissue extracts were determined using a BCA Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Equal amounts of proteins (20 μ g) were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. After being blocked in Tris-buffered saline-Tween containing 5% nonfat dried milk for 1 h at room temperature, the membranes were probed with specific antibodies against phospho-ERK 1/2 (1 : 1000, ab31324, Multisciences), phospho-p38 (1 : 1000, ab30359, Multisciences), phospho-JNK1/2/3 (1 : 1000, ab30314, Multisciences), MAPK (1 : 1000, ab38476, Multisciences), and NF- κ B (1 : 1000, ab86299, Abcam) overnight at 4 °C. Immunoreactive bands were detected using horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibodies (Beijing BioDee Bio-technology Co. Ltd, Beijing, China) and visualized by an enhanced chemiluminescence (ECL) detection system (ChemiScope 3600 Mini, CLINX,

Shanghai, China). Quantitative changes in the band intensities were evaluated using the ImageJ software (NIH, Bethesda, MD, USA).

2.8. Statistical analysis

All numerical data are presented as the mean $\pm$ SEM. Statistical analysis was performed using the SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA). All variables were compared between the groups using the ANOVA-Tukey's HSD. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. Comparison of allergic symptoms in peanut-, egg-, milk-, and soybean-induced food allergic mice

A mouse model of food allergy was established to evaluate the allergic symptoms (Fig. 1). Increased levels of specific IgE and IgG1 are characteristic allergic markers. A drop in temperature and allergic symptom score can be used as correlative parameters for anaphylaxis.^{20,21} Therefore, we determined them to assess whether the food allergy mouse model was established successfully. Specific IgE and specific IgG1 were significantly elevated in the protein-sensitized mice (Fig. 2a and b). The allergic symptom score was elevated after the final allergen challenge (Fig. 2c). In addition, the groups sensitized with peanut, egg, milk, or soybean showed a severe drop in body temperature (Fig. 2d). In particular, the peanut group showed more severe allergic symptoms.

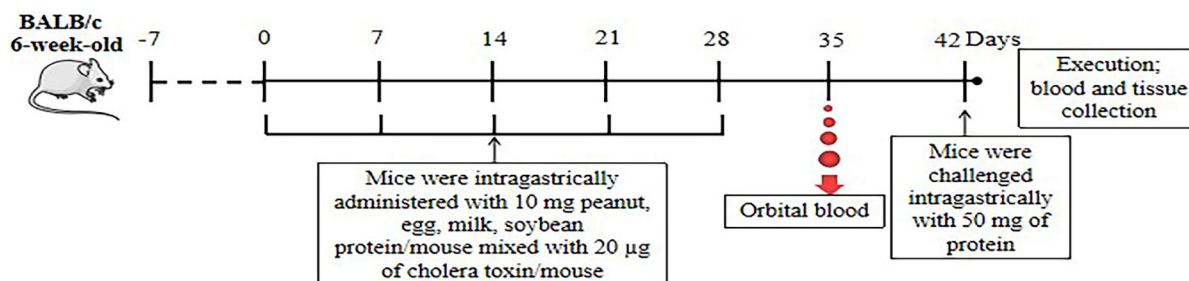


Fig. 1 Establishment of the food allergy mouse model. BALB/c allergic mice ($n = 12/\text{group}$) were intragastrically sensitized five times with 10 mg of protein in cholera toxin and challenged with five doses of peanut, egg, milk, or soybean *via* intragastric administration on day 42. The control group ($n = 12$) was treated with PBS. Blood was taken on the 35th day for antibody analysis. After the allergen challenge, the mice were sacrificed and their blood and organs were collected.

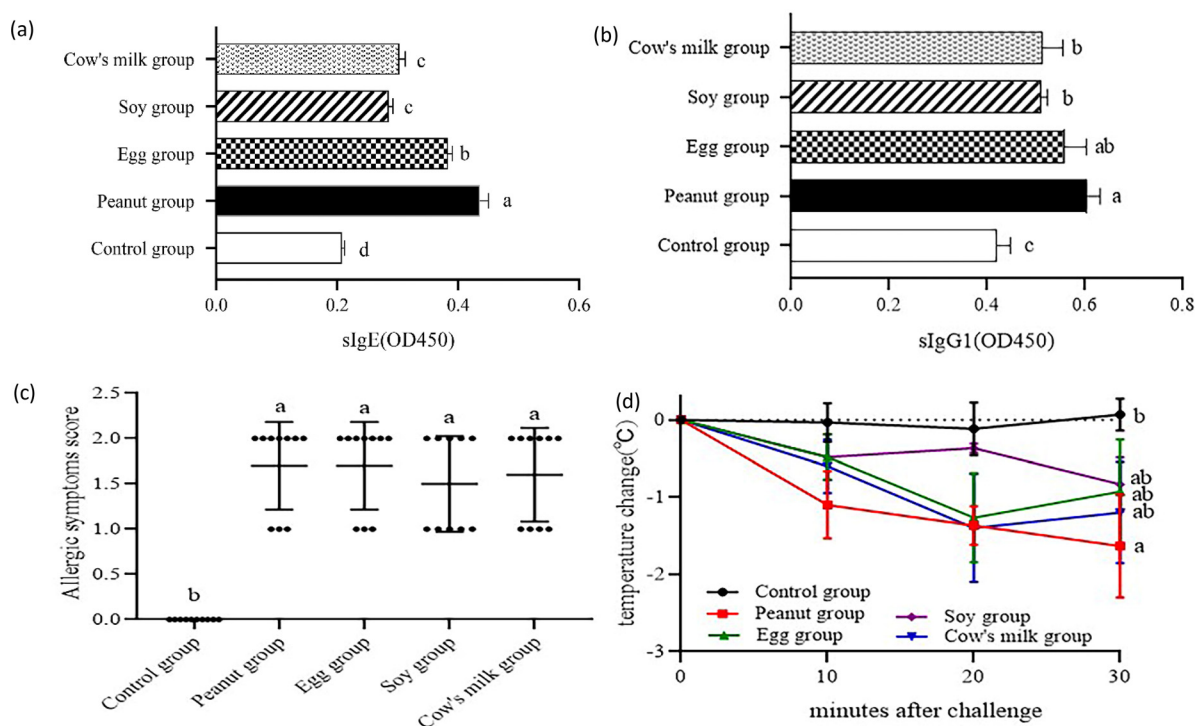


Fig. 2 Evaluation of the food allergy mouse model. (a) Specific IgE in serum and (b) IgG1 in serum, detected by ELISA. (c) Allergic symptoms score and (d) body temperature levels, measured after intragastric challenge on day 42. Different lowercase letters depict significant differences ($P < 0.05$ by ANOVA-Tukey's HSD). Different lowercase letters in the figures represent significant differences between the groups ($P < 0.05$ by ANOVA-Tukey's HSD).

3.2. Peanut allergy induces more serious hypersensitivity reactions

In order to further confirm that the peanut allergen exhibited more severe hypersensitivity, we next assessed the inflammatory mediators, cytokine content, lung histopathology, and jejunum histopathology. mMCP-1, a mast cell protease released from mucosal mast cells following allergen-dependent IgE cross-linking, was used as a serum marker of experimental food allergy severity.²² Histamine release is a major factor in food allergies.²³ Anaphylactic release of histamine and mMCP-1 were significantly elevated in the serum of allergen-sensitized mice compared with that of the control group (Fig. 3a and b). An immediate-type allergic reaction was induced by the intragastrically administered allergen protein. Intravenous injections of Evans blue were used to assess the vascular permeability in acute reactions.²⁴ Mice in the allergen-treated group showed increased vascular permeability (Fig. 3c). Particularly, mice in the peanut group exhibited more severe inflammatory mediators than those in the other groups (Fig. 3). Because allergic reactions are associated with Th1/Th2 balance, we examined the Th1 cytokine IFN- γ and the Th2 cytokines IL-4, IL-5, and IL-13. The allergen-treated mice, as compared with those in the control group, exhibited a decrease in Th1 cytokine (IFN- γ) production and an increase in Th2 cytokine (IL-4, IL-5, and IL-13) production (Fig. 4). Next, the histology of the lung and jejunum was evaluated *via* H&E and toluidine blue staining. The results show that mast cell infiltra-

tion in the lungs increased significantly in the allergen-treated mice. Reduced intestinal mast cells were also observed in the jejunum (Fig. 5a). In addition, the hematoxylin and eosin staining of the lungs revealed broadening of the pulmonary alveolar wall and the recruitment of inflammatory cells. The allergens disrupted the integrity of the jejunum crypt and villus (Fig. 5b).

3.3. Expression of IL-25, IL-33, TSLP, and related asthma genes in lung tissues

IL-25, IL-33, and TSLP promote type 2 inflammation and activate innate lymphocytes to produce IL-5 and IL-13 cytokines to participate in the allergic asthma reaction.^{25,26} The results show that the mRNA level of IL-25, IL-33, and TSLP detected by qPCR were significantly higher in the lung tissue of the allergic groups than those in the lung tissue of the control group, and the content was the highest in the peanut group (Fig. 6a). Given that the proteins ARG1, DCN, LTB4R1 and NFKBIA are associated with asthma,^{27–29} we further compared the expression levels of ARG1, DCN, LTB4R1, and NFKBIA in lung allergic tissues with control lung tissues. The results show that the lung allergic tissue showed a higher expression of ARG1, DCN, LTB4R1 and NFKBIA than that of the control lung tissue. We observed a significant increase in ARG1, DCN, LTB4R1, and NFKBIA expression in the peanut lung tissues with increasing allergy severity (Fig. 6b).

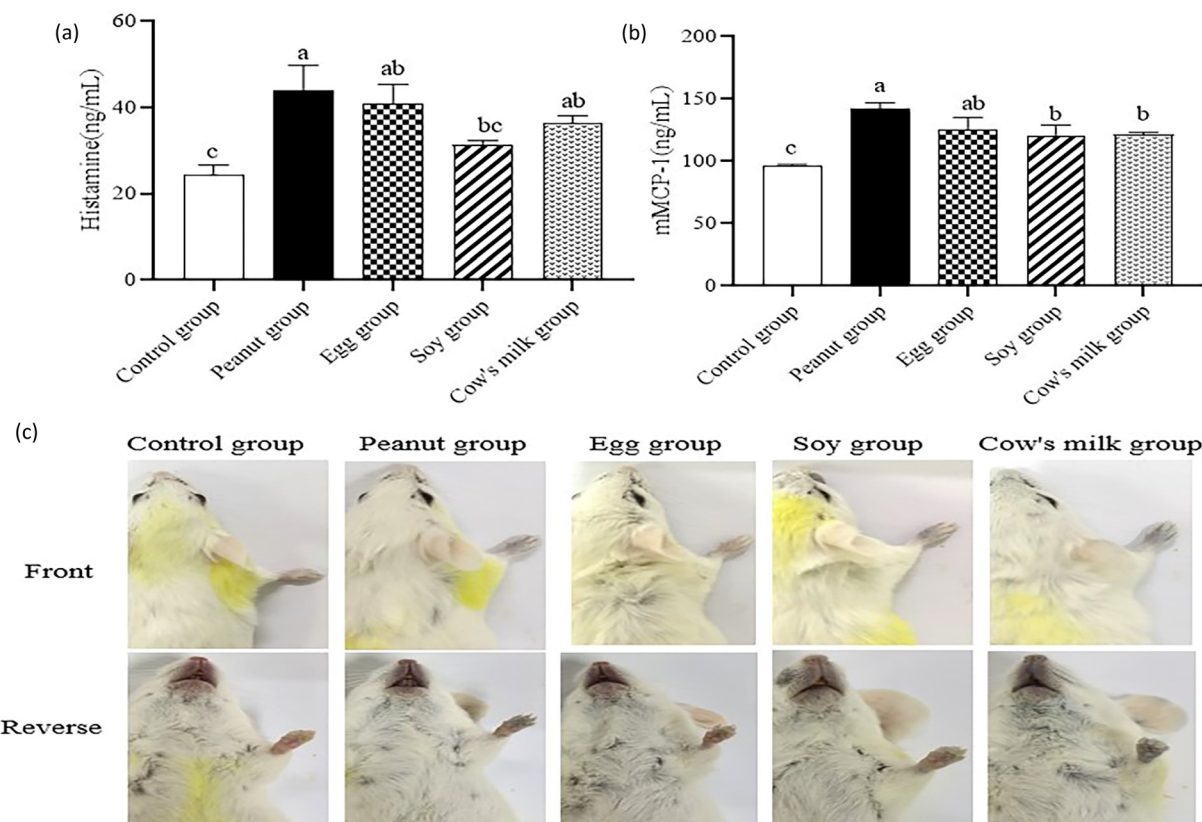


Fig. 3 Determination of allergen-induced histamine, mast cell protease and vascular permeability. (a) Serum levels of histamine and (b) serum levels of mast cell protease mMCP-1 levels, measured by ELISA. (c) Sensitized and control mice were injected through the tail vein with 100 μ L of Evans blue solution, and the vascular permeability change was measured 30 min after the last intragastric provocation. Different lowercase letters depict significant differences ($P < 0.05$ by ANOVA-Tukey's HSD).

3.4. Peanut-induced activation of MAPK (ERK, JNK, p38) pathways

MAPK pathways, which include the ERK, c-Jun N-terminal kinase (JNK) and p38 pathways, play significant roles in inflammatory responses. In addition, results in the literature have shown that differentially expressed genes that have been related to asthma such as ARG1, DCN and LTB4R1 are related to the MAPK and NF- κ B signaling pathways.³⁰ Thus, we investigated the MAPK signaling pathways using western blotting (Fig. 7). Analyses of the signaling pathways revealed phosphorylation and activation of JNK, ERK and p38 in the lungs of the peanut group. NF- κ B p65 was also upregulated in the peanut group.

4. Discussion

As is well-known, peanut, soybean, milk, and eggs are major allergenic foods. The structure and function of proteins have important implications on their allergenicity.^{31,32} To our knowledge, the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee have currently affirmed 18

peanut allergens (Ara h 1–Ara h 18), 10 hen's egg allergens (Gal d 1–Gal d 10), 8 soybean allergens (Gly m 1–Gly m 8) and 12 cow's milk allergens (Bos d 2–Bos d 13). A previous study performed a retrospective analysis of the published data from oral food challenges (OFCs) with peanut, milk, egg and soy allergic individuals. An integrated grading system for scoring the reaction severity of OFC reactions was established to assess the potential relationship between the minimal eliciting dose (MED) and reaction severities at the MED in individual patients. There were no significant differences in the threshold distributions for milk, egg, and soy by severity grading. Another retrospective study analyzed 1843 consecutive OFCs, which were performed for clinical purposes on children with 1 or more suspected food allergies based on their clinical history. Compared with challenges with cow's milk, hen's egg, soy, and wheat, respiratory symptoms were observed more often in peanut-allergic children upon challenge. However, these analyses have the following limitations: different studies used for data analysis may have different challenge protocols, different time intervals between doses, and different reporting of symptoms and eliciting doses. In addition, the number of individuals with allergies to peanut, milk, egg, and soy varied among the analyzed cases.^{33,34} In addition, for OFCs, the

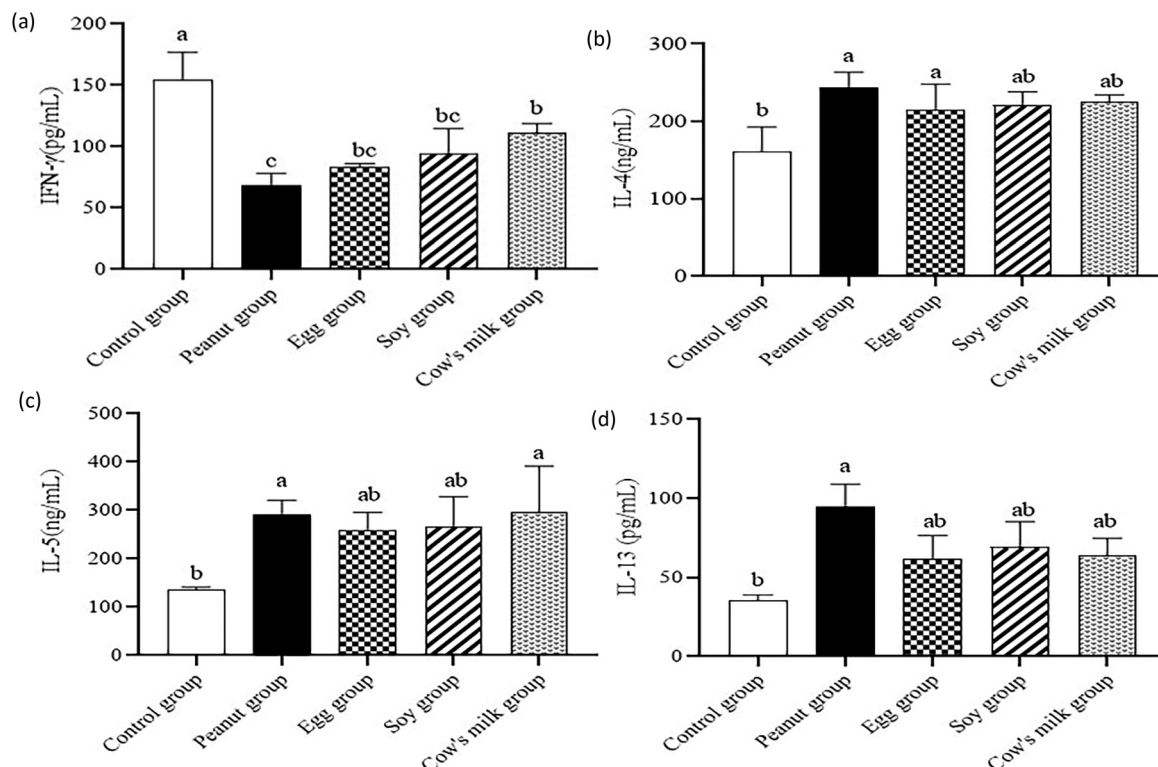


Fig. 4 The concentration of Th1- and Th2-associated cytokines in the serum of allergen-treated mice. The levels of (a) Th1 cytokines (IFN- γ) and (b–d) Th2 cytokines (IL-4, IL-5, and IL-13) were measured by ELISA.

thresholds for different foods were not comparable, and even when adjusted for protein content, the induction dose of foods was different.³⁵ Accordingly, there is no generally uniform method for discrimination in the allergenicity of these proteins. Therefore, we compared the allergenicity of these proteins in animal experiments at relatively quantitative levels. Compared to the control group, allergic indicators, increased specific IgE, IgG1, histamine, mMcp-1, and vascular leakage and decreased temperature drop were found in the allergic group. The results demonstrate that systemic anaphylaxis was more serious in peanut allergy. This is consistent with prior reports in the literature that have demonstrated that a peanut allergen reaction is often the most severe, and is the leading cause of deadly anaphylaxis.³⁶ Additionally, egg, soybean and milk allergies were relatively mild compared to peanut allergy. AllerCatPro (<https://allercatpro.bii.a-star.edu.sg/>) was used to predict the potential allergenicity of the proteins and it was found that allergenicity was associated with the epitopes (these data are provided in the ESI†). It was reported that the conformational epitope typically accounts for 90% of the total antibody binding to an antigen. Therefore, we speculate that the strength of protein sensitization is related to its conformational epitope.^{37–39} Interestingly, Mondoulet *et al.* reported that mice transferred with CD62L⁺ Tregs were protected from allergic reactions after peanut sensitization; the CD62L⁺ Treg cells were isolated from milk-epicutaneous immunotherapy-treated mice.¹¹ Therefore, severe food aller-

gies can be treated with gradual induction of immune tolerance with hypoallergenic foods.

It has been reported that deadly anaphylaxis is associated with asthma.⁴⁰ Accordingly, we hypothesized that, as compared with other food allergies, mice in the peanut group exhibited a more severe allergic reaction due to the induction of allergic asthma. It has been reported earlier that the signature cytokines of asthma are IL-4, IL-5, and IL-13, and inhibiting the production of these cytokines has resulted in clinical benefits in patients with asthma.^{41,42} Indeed, as we showed, the level of IL-4, IL-5 and IL-13 increased after peanut, egg, milk, and soybean allergen challenge. In addition, histamine and mast cell proteases, through their receptors, were putative mediators of asthma and histamine-mediated symptoms of asthma, including bronchospasms, edema, and mucus secretion.^{43,44}

Enhanced histamine release and mast cell proteases were found in the allergic group, and the levels of histamine and mast cell proteases were significantly higher in the peanut group than in the other groups. As expected, the number of lung mast cells detected by toluidine blue staining increased in the allergic group, which was in line with previous reports.⁴⁵ Furthermore, IL-25, IL-33 and TSLP, also known as pro-Th2 cytokines, have been shown to participate in the induction of food allergies, and are also involved in the pathophysiology of allergic diseases, such as asthma and allergic rhinitis.⁴⁶ Our findings were in line with the previous literature

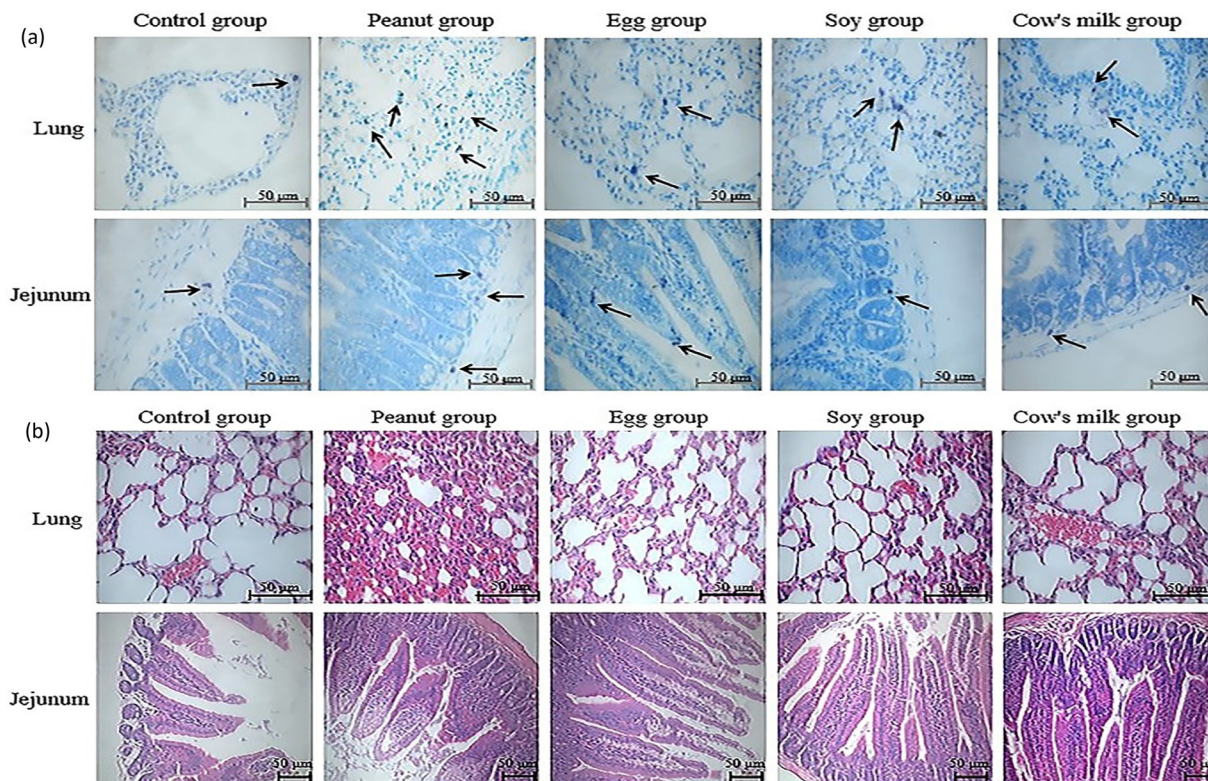


Fig. 5 Histological evaluation of the lung and jejunum in the allergic mice. Histological sections of the lung and jejunum from control mice and allergic mice (a) stained with aqueous toluidine blue and (b) stained with hematoxylin and eosin stain (scale bars = 50 μ m).

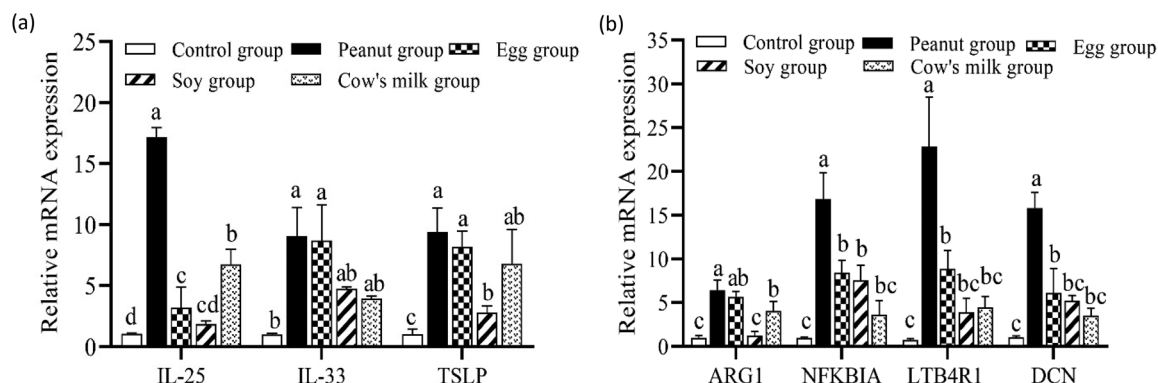


Fig. 6 Expression of IL-25, IL-33, TSLP and related asthma genes in lung tissues. (a) The levels of IL-25, IL-33 and TSLP transcription in the lung were detected by quantitative real-time PCR. (b) The related asthma genes including ARG1, DCN, LTB4R1 and NFKBIA were also detected by quantitative real-time PCR. Different lowercase letters in the figures represent significant differences between the groups ($P < 0.05$ by ANOVA-Tukey's HSD).

data, and suggest that secretions of IL-25, IL-33, and TSLP were increased by the induction of allergens.⁴⁷

We also observed related asthma genes exhibiting changes in expression during food allergen challenge (Fig. 6). One of the key genes was arginase I (ARG1), which increases in asthma and is strongly induced by Th2 cytokines, such as IL-4 and IL-13.⁴⁸ Another key gene was leukotriene B4 receptor 1 (LTB4R1), which is required for the development of the Th2-

type immune response; LTB4R1 recruits/activates T cells, which produce IL-13 in the lungs, and the dysregulation of LTB4R1 has been associated with asthma. LTB4R1 is also a key driver of peanut allergy.^{49–51} Decorin (Dcn), an extracellular matrix proteoglycan, has demonstrated importance in allergic models of asthma; a report also found that tissue inflammation and the mRNA expression of IL-13 significantly increased in OVA-challenged Dcn^{+/+} mice.⁵² NFKBIA, ARG1,

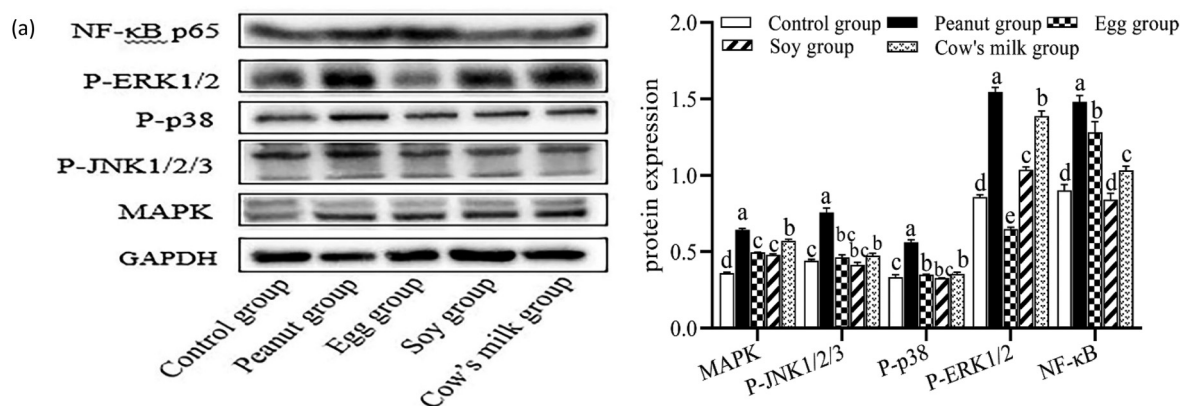


Fig. 7 The expression levels of MAPK (ERK, JNK, p38) and NF-κB proteins in the lungs. (a) Western blotting was used to detect the expression levels of MAPK, P-JNK1/2/3, P-p38, P-ERK1/2 and NF-κB proteins in the lungs. GAPDH was used as a loading control. (b) Protein quantification. Different lowercase letters in the figure represent significant differences between the groups ($P < 0.05$ by ANOVA-Tukey's HSD).

and DCN acted as hubs in the networks, promoted the differentiation of the Th2 lineage, and regulated the acute phase response.^{53,54} In addition, NFKBIA, ARG1, and DCN were all involved in allergic inflammation.^{55–57} Related asthma genes in various allergic groups were highly expressed, especially in the peanut-allergic group.

NFKBIA, ARG1, and DCN related to the genetic risk of asthma have also been associated with NF-κB and MAPK regulation.^{58–60} In addition, Yu *et al.* demonstrated that protease allergens induced IL-25 and TSLP via Erk and p38 MAPK signaling pathways.⁶¹ However, it is not known if these genes play a role in the MAPK pathways in food allergies. In this study, we evaluated the effect of allergens on markers of the MAPK signaling pathways in the lungs, including p-JNK, p-p38, p-ERK, and MAPK, using western blot analysis. Among the three MAPKs, MAPK/ERKs, MAPK/JNKs, and MAPK/p38 were activated by peanut allergens. Surprisingly, the expression of MAPK/ERKs was not found, which suggests that the egg allergen regulated the asthma-related genes through MAPK/JNKs and MAPK/p38.

5. Conclusions

In this study, we compared the severity of peanut, egg, soybean and milk allergies, for the first time, which indicated that mice in the peanut group exhibited more serious hypersensitivity reactions. Particularly, we demonstrated that peanut induced allergic asthma via the MAPKs pathway.

Author contributions

Junjuan Wang and Huilian Che: conceptualization, methodology, writing—original draft preparation, funding acquisition; Ruolin song and Ruoxi Lan: investigation, resources; Mengzhen hao, Guirong Liu and Manman Liu: software, vali-

dation, formal analysis; Shanfeng Sun and Cheng Chen: writing—review and editing.

Conflicts of interest

There are no conflicts to declare.

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