DOI: 10.26599/FSHW.2026.9251030

Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

Allergy Off Switch: Targeted Immune Tolerance Peptide for Alpha-Lactalbumin Allergy

Jincheng Han[a,b], Wenhan Kang[b], Yang Wan[b], Xiwen Miao[a,b], Jiukai Zhang[b], Ning Yu[b,*], Ying Chen[b,*]

[a] *College of Food Science and Engineering, Jilin Agricultural University, Changchun 130118, China*

[b] *Chinese Academy of Quality and Inspection & Testing, Beijing 100176, China*

ABSTRACT: Alpha-lactalbumin (ALA), a significant allergen in cow's milk, requires urgent preventative and therapeutic techniques. Peptide immunotherapy may modify allergic reactivity without cross-linking IgE, making it crucial to milk allergy treatment. In the present study, tolerogenic peptides of α-lactalbumin have been identified by constructing immune cells (MLN cells, splenocytes) from a mouse model of cow's milk allergy and co-culturing them in vitro with candidate peptides. The allergy model demonstrated significant alterations in physiological and biochemical indices within the experimental group. Subsequent co-culture of immune cells with candidate peptides revealed that peptide ALA AA (1-19) induced the expansion of Treg cells and promoted a tolerogenic phenotype in DCs. Taken together, these results led to the successful screening of ALA-tolerant peptides, which inhibited the release of allergy-related cytokines and induced the differentiation of immune cells toward the tolerant phenotype. This study provides a food-compatible peptide strategy for CMA management, with potential applications in hypoallergenic dairy products. Meanwhile, these findings laid the foundation for further validation in subsequent in vivo experiments.

Keywords: Functional food; Prevention of cow's milk allergy; Peptide-based immunotherapy; Oral tolerance; Mouse model of cow's milk allergy; dietary therapy

1. Introduction

Cow's milk allergy (CMA) is one of the most common food allergies due to frequent exposure to cow's milk early in life[1]. Avoidance of allergens remains the best treatment for food allergies. However, cow's milk contains more than 25 different types of proteins, of which the most important allergens are casein, α-lactalbumin (ALA, Bos d 4), and β-lactoglobulin[2]. ALA is a globular calcium-binding protein with a molecular weight of 14.4 kDa and four disulfide bonds to maintain its structural stability[3]. It is difficult to be hydrolyzed by digestive proteolytic enzymes and therefore prone to allergy[4]. According to statistics, about 35% of milk allergy patients worldwide have allergic reactions triggered by ALA[5]. The vast majority of these are food allergies caused by unintentional contamination during food processing and accidental ingestion[6]. In the food industry, ALA is a vital additive in infant formulas because it contains a wide range of essential amino acids and has a variety of physiological functions[7], such as inhibition of

***Corresponding author**
yu962925@126.com, chenyingcaiq@163.com

Received 2 August 2025
Received in revised from 18 October 2025
Accepted 12 November 2025

colon carcinogenesis and anti-inflammatory activity[8,9]. “Avoiding allergenic foods is not a ”cure" for food allergies, and it also reduces the quality of life of allergic people. The fundamental solution to food allergy is for the body to develop an immune tolerance and become truly “immune” to the allergen.

The induction of antigen-specific immune tolerance represents the fundamental therapeutic goal for managing food allergy. Although oral immunotherapy (OIT) with intact allergens can promote the generation of regulatory T cells (Tregs) and alleviate allergic symptoms [10,11], its clinical application is limited by a significant incidence of treatment-related adverse events [12–15]. Consequently, peptide-based immunotherapy (PIT) has emerged as a promising alternative strategy. By employing short, linear epitopes that are incapable of cross-linking IgE antibodies on effector cells, PIT is postulated to induce tolerance primarily through T-cell mechanisms, such as anergy, deletion, or the induction of allergen-specific Tregs, thereby presenting a improved safety profile [16]. The efficacy of this approach is supported by evidence demonstrating that oral administration of immunodominant T-cell epitopes can suppress allergic responses in murine models of egg allergy [10]. Immunodominant T-cell epitopes have also been reported to induce oral tolerance to pollen allergy in mice[17] and humans[18]. Furthermore, the progression of a tolerogenic peptide product for peanut allergy into phase II clinical trials underscores its translational potential [19]. Despite these advancements, the investigation of tolerogenic peptides derived from α-lactalbumin (ALA)—a major cow's milk allergen implicated in approximately 35% of cases globally [5]—remains unreported, highlighting a critical gap in the current research landscape.

In this study, we investigated all currently reported peptides against ALA (one of the major milk proteins) and finally retained 16 peptides under the principle of covering the entire ALA protein sequence as much as possible. First, an ALA allergy model was constructed. This model's primary immune cells (splenocytes, mesenteric lymph node cells) were isolated, and these peptides were co-cultured with these two types of immune cells, respectively (Fig. 1）. Subsequently, the tolerance response was assessed by antibodies, cytokines, Treg populations and DC surface markers in order to screen the key peptides that mediate ALA allergic tolerance and provide new ideas and targets for developing novel anti-allergy therapies and drugs.

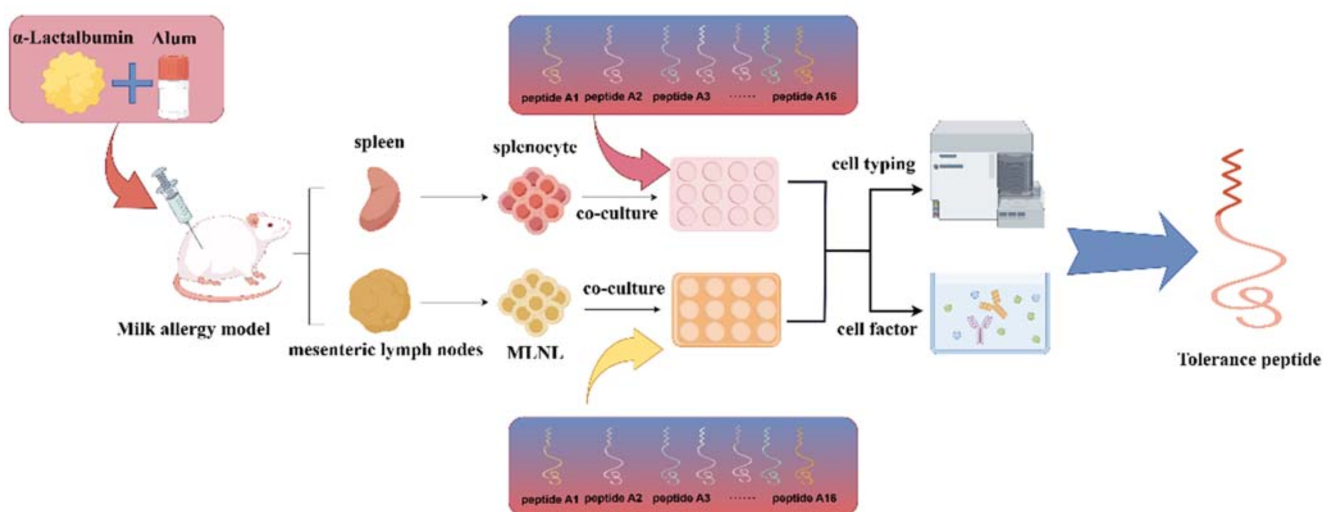

Fig. 1. Immunotolerance peptide screening methods.

2. Methods

6-8-week-old SPF-grade male and female BALB/c mice were purchased from Charles River Laboratories (Beijing, China) and housed under normal conditions at the Laboratory Animal Center of the Chinese Academy of Quality and Inspection & Testing. Animal Experiment under the supervision and assessment by the Laboratory Animal Welfare and Ethics Committee of Chinese Academy of Quality and Inspection & Testing (NO. 2025-7-D-03-1). The requirements for the care and handling of laboratory animals are in accordance with the EU Directive 2010/63 for the protection of animals used for scientific purposes.

2.1. Animal models, allergy sensitization, and immune response analysis

The sensitization and elicitation protocol for mice was referred to the method of Tomoaki et al. with appropriate modifications[20–22]. Thirty SPF-grade BALB/c mice, half male and half female, 6-8 weeks old, were selected and randomly divided into three groups（Fig.S1）. Mice arrived and acclimatized to the feeding environment for 1 week (water with chow (no cow's milk protein) was provided ad libitum). Gavage of 0.2 mL saline was performed on days 1, 2, 3, 8, 9, and 10 to provide the basis for subsequent mouse tolerance model construction. After that, the mice were sensitized by intraperitoneal injection on days 15, 22, 29, and 36. Group A was sensitized and stimulated with 0.9% saline intraperitoneally as a negative control group; group B was injected intraperitoneally with 0.2 mL of aluminum adjuvant solution (containing 2 mg of aluminum adjuvant) as an adjuvant control group; and group C was injected intraperitoneally with saline containing 2 mg of aluminum adjuvant and 50 μg of ALA (0.2 mL) was injected intraperitoneally. Finally, on days 43, 45, and 47, groups B and C were sensitized three times by gavage of mice with 20 mg ALA protein dissolved in 0.2 mL saline. Moreover, body weight and body temperature were monitored before each challenge. Body temperature was measured 5, 10, 15, and 20 minutes after challenge, and symptoms were observed between 20 and 50 minutes. Finally, mice were anesthetized with isoflurane and then executed by cervical dislocation on day 48, and biological tissues such as serum, spleen, jejunum, and lymph nodes were collected for further analysis.

2.1.1. Assessment of clinical symptoms

Seven days after the last sensitization, mice were injected intragastrically with 20 mg of ALA and were observed by two independent observers in a blinded manner for the next 20-60 min. The models were clinically scored according to the Allergy Symptoms Scale （which evaluated key dimensions including scratching, swelling around the eyes and snout, decreased activity, and respiratory distress; for detailed criteria, see Table S1）. The body temperature of the mice was recorded using an anal thermometer, and weekly changes in body weight were recorded.

2.1.2. ELISA detection of serum ALA specific immunoglobulins

The collected mouse blood was centrifuged in 1.5 mL centrifuge tubes, tilted, and left to stand at 4 °C, and the next day, the supernatant was gently aspirated by centrifugation at 4000 rpm for 20 min and dispensed

and stored at -20 °C for spare use. Serum was tested for ALA-specific antibodies IgE, IgG, IgG1, IgG2, IgA, and HIS using previously reported methods[23].

2.1.3. Detection of cytokines in mouse splenocytes

Spleens were excised, and cell suspensions were prepared in RPMI medium containing 10% fetal bovine serum and antibiotics. 1 mL of cells per well was taken in a 48-well cell culture plate, and 40 μL of ALA protein (10 mg/mL) filtered through 0.22 μm filter membrane was added correspondingly to stimulate the cells cultured in a cell incubator for 72 h. Concentrations of cytokines IFN-γ (JL10967; Jianglai biology, Shanghai), IL-4 (JL20266; Jianglai biology, Shanghai), IL-5 (JL20267; Jianglai biology, Shanghai), IL-13 (JL20247; Jianglai biology, Shanghai), IL-10 (JL20242; Jianglai biology, Shanghai), in the supernatant were determined using an ELISA cytokine kit, following the manufacturer's instructions.

2.1.4. Mouse intestinal tissue H&E staining

After the mice were executed, the jejunum of mice was isolated in a sterile environment and fixed in fixative solution, followed by paraffin section deparaffinization, hematoxylin and eosin staining, dehydration sealing, and image acquisition and analysis under a microscope.

2.1.5. Expression of mast cells in the lamina propria of the mouse intestine

Small intestinal cells were resuspended in phosphate-buffered saline (PBS)-1 % bovine serum albumin (BSA). A blank control was set up; required flow-through antibody information: FVS780-APC-cy7, Mouse BD Fc Block TM, FITC-Lin, PE-cy7-CD45, PE-IgE, PerepCy5.5-CD117 (all from Biolegend). To exclude dead cells, experimental wells were added with 50 μL of wash solution containing dead and live dyes (50 μL PBS+0.1 μL FVD), incubated at room temperature for 15 min before adding 1 μL of Fc blocking agent (50 μL PBS+1 μL Fc blocking), and incubated for another 15 min, 100 μL of staining buffer containing surface antibody was added to each well, and incubated for 30 min at 4°C away from light. Incubate for 30 min; after incubation, add 0.9 mL TF Per/wash, shake at 400 g, centrifuge to remove supernatant, and add 0.3 mL of wash solution to be on the machine. The results were collected by FACSCanto flow cytometer (BD Biosciences, San Jose, CA, USA） and analyzed by Flowjo.v10 software（BD, USA）.

2.2. In vitro immune cell screening for tolerogenic peptides

2.2.1. Peptide

We thoroughly gathered the published ALA epitopes using the keywords "food allergy," "ALA," and "peptide" throughout "Web of Science," "PubMed," and other literature search platforms. Sixteen candidate peptides were integrated and selected for subsequent experiments based on the criteria of maintaining a length of 17-20 amino acids and maximizing coverage of the entire protein sequence (Table S2). The selected peptide segments are derived from linear T-cell epitopes reported in the literature, consistent with the T-cell targeting strategy employed in peptide immunotherapy. The peptides mentioned were synthesized by Anhui Guoping Pharmaceutical Company.

2.2.2. Isolation of cells from spleen, MLN

Cells from the spleen and mesenteric lymph nodes (MLN) were extracted by homogenising the tissue using 70 μm cell strainers. Red blood cells in splenocyte suspensions were eliminated by a 4-minute incubation on ice with lysis buffer (8.3 g/L NH4Cl, 1 g/L KHCO3, and 37.2 mg/L EDTA). To isolate cells from the mesenteric lymph nodes (MLN), rinsed in cold PBS. Following an initial wash in Hank's Balanced Salt Solution enriched with 15 mM HEPES at pH 7.2, fragments were incubated for 60 minutes in HBSS containing 15 mM HEPES, five mM Na_2-EDTA, 10% fetal bovine serum (FBS), and penicillin (100 U/mL)/streptomycin (100 μg/mL), pH 7.2. Samples were rinsed in RPMI 1640 enriched with 5% FBS and penicillin (100 U/mL)/streptomycin (100 μg/mL) and incubated for two intervals of 45 minutes in RPMI 1640 containing 5% FBS, penicillin (100 U/mL)/streptomycin (100 μg/mL), and 0.5 mg/mL collagenase type VIII (Sigma-Aldrich). Tissue pieces were vortexed for 10 seconds after each treatment and then poured over a 70 μm cell strainer to isolate cells. Digestion was halted by adding 10% FBS and subsequent washing of cells in HBSS/15 mM HEPES solution. Cells were then purified using Percoll gradient centrifugation. All splenocytes and MLN cells used for the subsequent in vitro co-culture assays were isolated from ALA-sensitized mice (Group C, α-ALA) to ensure the immune cells were primed and responsive to ALA-derived peptide stimulation.

2.2.3. Co-culture of immune cells with peptides and cytokine assays

For the co-culture experiments, splenocytes and MLN cells from all mice within the same experimental group (α-ALA) were pooled to obtain sufficient cell numbers for the high-throughput peptide screening. Penicillin (100 U/mL) and streptomycin (100 μg/mL) were added to RPMI 1640, which was supplemented with 10% FBS. Spleen and MLN cells were then resuspended in this particular medium. Then, 6×10^5 splenocytes or 4×10^5 MLN cells (200 μL) per well were cultured in pointed-bottomed petri dishes with buffer or 1 mg/mL synthetic peptide at 37 °C, 5% CO2 for 72 h. The supernatant was centrifuged to remove cell debris, collected, and stored at -20 °C until further analysis. The concentrations of cytokines IL-4, IL-5, IL-10, IL-13 and IFN-γ in the supernatant were determined by ELISA according to the manufacturer's protocol (Jianglai biology, Shanghai). Absorbance was measured at 450 nm on an enzyme labeling instrument (Varioskan LUX, Thermo Fisher). To determine the peptide-induced cytokine response, the cytokine concentration measured in the medium-stimulated wells was subtracted from the cytokine concentration measured in the blank wells to reflect the level of cytokines induced by the peptide.

2.2.4. Treg cells, DC surface markers measured by flow cytometry

Cells from the spleen and MLN were resuspended in PBS containing 1% BSA. In the detection of DC surface markers, Non-specific binding sites were obstructed by treating the cells for 15 minutes with anti-mouse CD16/CD32 (Mouse BD Fc Block; Biolegend) in a PBS-1% BSA-5% FBS buffer. Dendritic cells were labeled extracellularly for 30 minutes on ice with CD11c-PE, MHC class II (I-A/I-E)-BV421, CD86-FITC, and CD103-APC (all sourced from Biolegend). In the assay of Treg cells, T cell subsets were analyzed by first extracellularly staining the cells with CD4-FITC and CD25-APC (both from BioLegend). Fixable viability dye Zombie Aqua-BV510 was used to exclude dead cells. To identify the Foxp3 transcription

factor, cells were first fixed and permeabilized using the Foxp3 Staining Buffer Set per the manufacturer's instructions, followed by incubation with the Foxp3-PE antibody. Data were acquired using the FACSCanto flow cytometer (BD Biosciences, San Jose, CA, USA） and analyzed by Flowjo.v10 software（BD, USA）.

2.3. Statistical analysis

All data were analyzed using the GraphPad Prism 10.0 program for Windows. One-way ANOVA, accompanied by Bonferroni's multiple comparison post hoc test, was used for data analysis. When data were not normally distributed or when there was high variation between the groups, a suitable data transformation was implemented prior to the one-way ANOVA analysis. When the assumptions of ANOVA were not satisfied post-transformation, the nonparametric Kruskal-Wallis test, accompanied by Dunn's post hoc test, was used. Data are expressed as mean ±S.E.M. for 10 animals per group, and immunoglobulin levels are shown in the Chart of columns. $P < 0.05$ is deemed statistically significant.

3. Results and Discussion

3.1. Symptom scores and changes in body temperature and weight in a mouse model of allergy

Mouse allergy symptoms, body temperature, and weight are the key indicators for testing the success of constructing mouse allergy models, and only when these physiological factors have changed in some way can the next step of biochemical indicators be carried out. In order to demonstrate that the ALA allergy model was indeed successful we first examined physiological changes. Group A (BLANK) served as a blank control with saline used in both the sensitization and excitation phases; group B (α-alum) served as an adjuvant control with only adjuvant and no α-lactalbumin in the sensitization phase, and only in the excitation phase; and group C (α-ALA) served as an experimental group with adjuvant plus α-lactalbumin in the sensitization phase, and a high dose of protein in the excitation phase as well. Clinical scores were determined 20-60 minutes after oral administration (Fig. 2A). It can be seen that α-ALA showed highly significant sensitization symptoms compared to α-alum, BLANK ($P < 0.05$), while α-alum showed no significant difference compared to BLANK ($P > 0.05$), indicating that adjuvant by itself could not cause anaphylactic symptoms in mice. We simultaneously recorded the body weight changes of the mice throughout the experimental cycle by setting the initial body weight, the first excitation, the second series of excitation, and the third excitation to 0,1,2,3, respectively (Fig. 2B); As can be seen from the data changes, there was no difference in the initial body weights of the three groups of mice ($P > 0.05$), and the trend of increasing body weights from the first excitation onward in the α-ALA compared with the α-alum, BLANK group slowed down significantly, and the body weights measured in the three excitations in the α-ALA group were all highly significantly different from the remaining two groups ($P < 0.05$).We also performed tests of body temperature, which were detected and recorded before and after each excitation(Fig. 2C, D, E). All three measurements were extremely significantly lower in the α-ALA group after excitation than in the α-ALA group before excitation ($P < 0.05$), and also extremely significantly lower in the α-ALA group after excitation than in the α-alum, BLANK group

after excitation ($P < 0.05$), but there was no significant difference between the two groups of α-ALA and BLANK either before or after excitation ($P > 0.05$).

When performing animal sensitization tests, the selection of the appropriate strain of animal is essential for the scientific results of the experimental study. Previously, researchers have conducted experiments on different strains of mice and compared the responses of several strains of mice to the same or different sensitizing proteins [24–27]. The comparative analyses showed that the immune responses of BALB/c mice after ingesting sensitizing proteins are more similar to those produced by human beings after ingesting sensitizing foods, which makes them more suitable for use in food sensitization studies. Therefore, we chose BALB/c mice to optimize and construct an allergy model. The symptom scores revealed that the scores of the α-ALA group were extremely significantly higher than those of the BLANK group and the α-alum group ($P < 0.05$), indicating the manifestation of obvious allergic symptoms. Meanwhile, the weight of the mice in the α-ALA group was significantly lower than that of the BLANK and α-alum groups ($P < 0.05$), indicating that the mice in the allergy group had damaged their digestive system, thus affecting the absorption of food, which is reflected in the fact that the α-ALA group is the only group that has a lower body weight for the same condition. It was also found that the body temperature of the α-ALA group decreased significantly before and after the three excitations, whereas it remained in a relatively stable range with no significant difference between the control groups ($P > 0.05$). All of these reactions revealed are typical clinical symptoms of allergic reactions, based on which we can further explore the changes related to humoral and cellular immunity caused by allergic reactions.

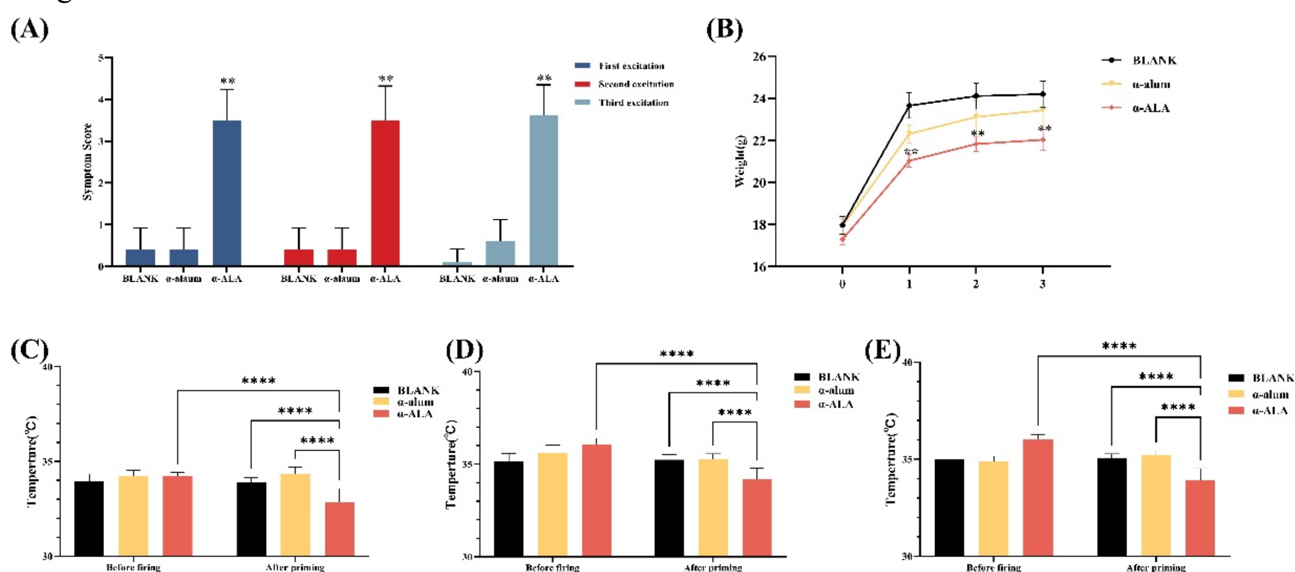

Fig. 2. Physiological indices of ALA allergy mouse model. (A), allergy symptom score; (B), body weight change; (C), body temperature change before and after the first stimulation; (D), body temperature change before and after the second stimulation; (E), body temperature change before and after the third stimulation. Statistically significant difference with one-way Anova followed by Bonferroni's test: **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

3.2. Detection of specific antibodies in mouse serum

In order to study the effect of ALA on the immune response in the allergy model, ALA-specific immunoglobulins were measured in serum collected 24 hours after oral administration of ALA. Changes in IgE, IgA, IgG, IgG, IgG1, IgG2a antibodies and HIS (Fig. 3) were detected separately. The data showed a

highly significant increase in specific IgE, IgA, IgG, IgG1, and IgG2a antibodies and HIS in the α-ALA group compared to the α-alum, BLAN (P < 0.05). In contrast, there was no significant difference in the α-alum compared to the BLAN group (P > 0.05), suggesting that the α-ALA group enabled the expression of specific antibodies by B cells significantly. In addition, increased HIS can also indicate the expression of clinical symptoms.

Food allergic reaction is mainly manifested in the humoral immunity of the organism as an increase in the level of specific IgE, and the secretion of IgE from the immune cells of odontocetes is often accompanied by the secretion of IgG_1[28]. The IgG and IgA antibodies represent the degree to which the organism produces an immune response, so food allergic reaction is also an abnormal immune response. By detecting the level of specific antibodies in the serum of mice, there was a significant increase in particular antibodies IgE, IgG, IgG_1, IgG_{2a}, and IgA in the α-ALA group in the experiment (P < 0.05), which is consistent with the results of previous studies[29,30]. Food allergy produces an accumulation of mast cells in the lamina propria and basal lamina of the small intestine, which degranulate and release substances such as histamine and leukotriene when the body is stimulated by a high dose of the allergen, impairing the normal functioning of the body[31–33]. Therefore, we additionally examined the content of HIS in the serum and found that the content of histamine in the α-ALA group was extremely significantly higher than that in the control group (P < 0.05), which could explain why the mice in the α-ALA group showed apparent symptoms.

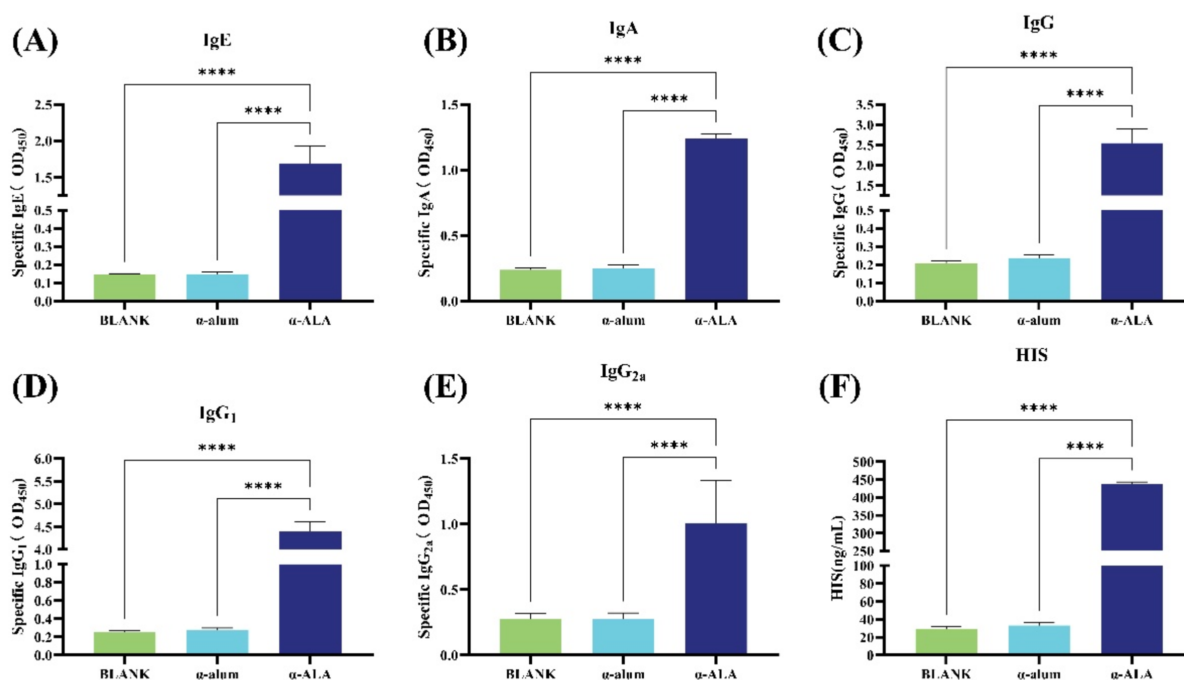

Fig. 3. Levels of ALA-specific antibodies in mouse allergy models. (A), IgE; (B), IgA; (C), IgG; (D), IgG1; (E), IgG2a; (F), HIS. Statistically significant difference with one-way Anova followed by Bonferroni's test: **** P < 0.0001, *** P < 0.001, ** P < 0.01, * P < 0.05.

3.3. Changes in cytokine secretion by mouse splenocytes

In order to test whether the ALA allergy model elicits a Th2-specific immune response, mouse splenic single-cell suspensions were taken, and the supernatants were stimulated with ALA for 72 h. Cytokine assays were performed on the supernatants. Among the Th2-specific cytokines, we found that IL-4, IL-5, and IL-13

were significantly increased in the α-ALA group compared with the α-alum, BLAN group ($P < 0.05$), whereas there was no significant difference between the two α-alum, BLAN groups ($P > 0.05$) (Fig. 4A, B, D). It was illustrated that Th2-mediated immune response was elicited in mice in the α-ALA group. In addition, there was also a significant decrease in Th1-related IFN-γ and IL-10 in the α-ALA group ($P < 0.05$) (Fig. 4C, E). These also further indicated that the Th1/Th2 balance was disrupted.

Food sensitization reactions are mainly the result of the presentation of antigenic determinants of sensitizing proteins to T cells after processing by antigen-presenting cells (APCs), which activate the T cells to become Th2-type cells[34], which secrete large quantities of cytokines such as IL-4, IL-5, and IL-10. As an antibody conversion stimulator, IL-4 can stimulate B cells to secrete high levels of specific IgE[30,35], and these antibodies bind to antigenic epitopes while binding to FcεRI on mast cells so that when the body is exposed to the same sensitizing agent again, a series of sensitization reactions will occur, and therefore, the induction of Th2 cells is the initiation of sensitization reactions. In addition to differentiating into Th2 cells, CD4+ T cells can also differentiate into Th1 cells, and the related cytokine is IFNγ[36,37]. Under normal conditions, Th1 and Th2 cells can constrain each other in order to maintain the balance of the body's immune status. In addition, some T cells also differentiate into Treg cells, and the related cytokine IL-10, which regulates the level of Th1 and Th2 cells, specifically suppresses the immune response to protect the body from damage[38]. The results of the present study showed that BALB/c mice in the α-ALA group produced significantly higher levels of Th2 cells than the other treated groups, in contrast to their Th1 and Treg cell levels, which were significantly lower than those of the other groups. It was hypothesized that oral sensitization by ALA caused BALB/c mice to produce high levels of Th cells and, at the same time, disrupted the induction and proliferation of Treg cells, resulting in a disproportionate ratio of Th1/Th2, which led to the eventual anaphylactic reaction of the organism.

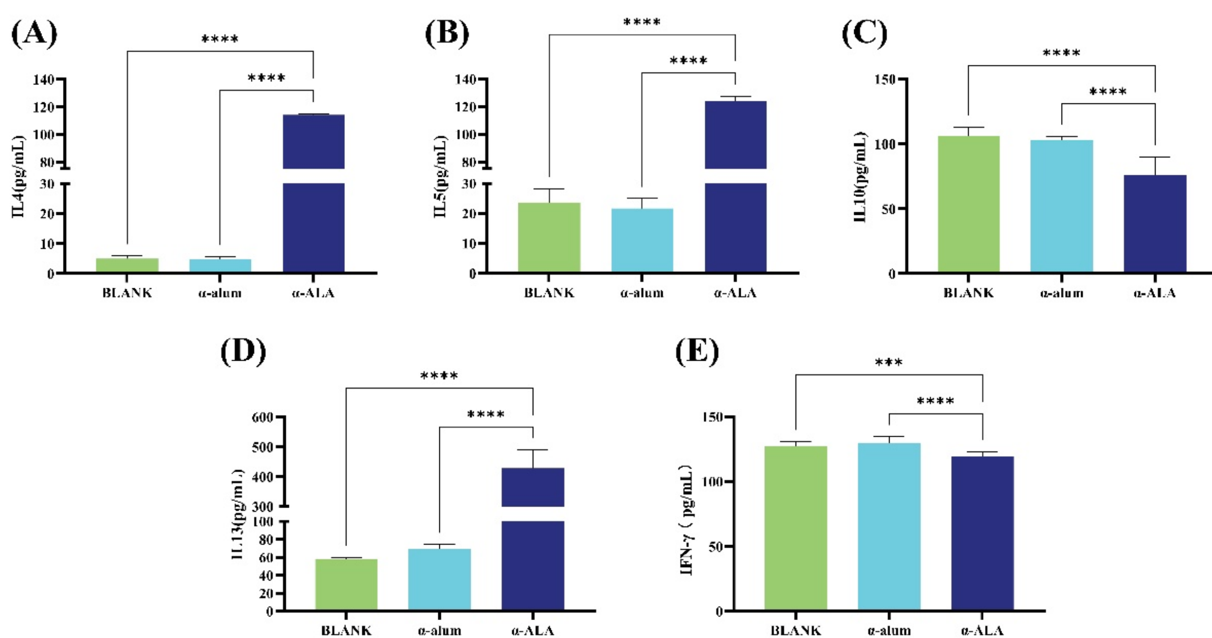

Fig. 4. Levels of cytokines secreted by splenocytes in a mouse model of allergy. (A), IL-4; (B), IL-5; (C), IL-10; (D), IL-13; (E), IFN-γ. Statistically significant difference with one-way Anova followed by Bonferroni's test: **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

3.4. Intestinal tissue changes in mice

In order to compare the histological changes in the three groups of mice, the small intestines of mice were selected for tissue sections (Fig. S2). α-ALA group allergic reaction resulted in damage to the jejunal tissue of the mice, which appeared to have irregularly aligned intestinal villi with breaks and breakage, as well as chaotic, loose and irregular structure, and crypts were absent whereas the intestinal villi of mice from the α-ALA group were regularly and neatly aligned with intestinal villi, and the structure of mice from the α-alum, BLAN group were complete. This all indicates that the allergy model caused intestinal damage.

The intestinal tract efficiently absorbs nutrients, water, and electrolytes from food. At the same time, it constitutes a tight barrier against harmful substances from the external environment and pathogens and their toxins. Damage and dysfunction of the intestinal barrier are associated with susceptibility to and exacerbation of a variety of conditions, including food allergies[39]. In the present study, we determined the damage of small intestinal tissues through pathological sections of small intestinal tissues, and the results of H&E staining showed that the mice in the α-ALA group showed severe intestinal damage, villi atrophy, and infiltration of inflammatory cells compared with the mice in the BLANK and α-alum groups, whereas the intestinal tracts of the BLANK and α-alum groups were more intact, and the villi were stretched out. Inflammatory response occurred in the small intestine, and the severity of inflammation was closely related to its degree of degranulation. In conclusion, H&E staining of small intestinal tissues showed that the α-ALA group had the most severe damage to the intestinal barrier caused by allergic reactions. Whereas in the BLANK group, the damage was less severe in the α-alum group.

3.5. Expression of mast cells in the lamina propria of the mouse intestine

Mast cells play an important role in the effector phase of allergic reactions, and to further understand the mechanisms of their sensitization, we characterized gut-associated immune cells in an animal model of sensitization. The intestinal lamina propria was first isolated and the levels of FcεRI and IgE expressed on mast cells were examined (Fig. 5). We detected a significant elevation of $IgE^{+}c\text{-}kit^{+}$ expression in the α-ALA group compared to the α-alum, BLAN group ($P < 0.05$). Similarly, the expression level of $Fc\varepsilon RI^{+}$ $c\text{-}kit^{+}$ was significantly increased in the α-ALA group ($P < 0.05$). Thus, the success of constructing a mouse model of cow's milk allergy was further validated by detecting the levels of these major surface markers of mast cells in the intestinal lamina propria.

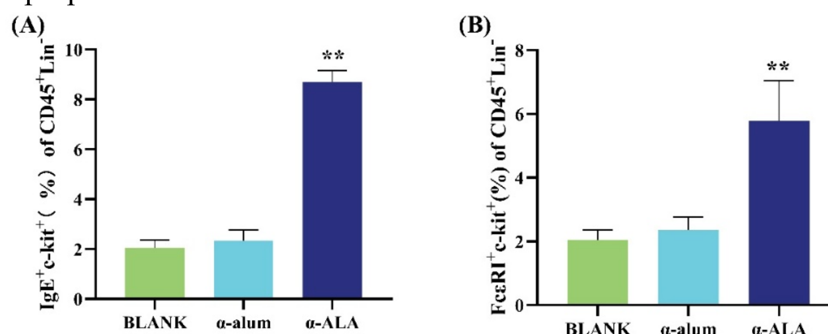

Fig. 5. Mast cell surface markers in the lamina propria of the mouse intestine. (A), IgE+c-kit+; (B), FcεRI+ c-kit+. Statistically significant difference with one-way Anova followed by Bonferroni's test: **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Food allergy-mediated type I hypersensitivity is caused by the binding of IgE antibodies to Fc receptors on the surface of effector cells, such as mast cells, and when the antigenic epitope re-enters the organism, it binds to IgE on the surface of the cell and releases biologically active mediators to produce an allergic reaction[39]. The expression of mast cells in food allergies is an effector cell that plays a key role in allergic responses[39]. Several experiments have been performed to investigate this. López et al. determined the effect of processed proteins on sensitization in mice by flow assaying the expression of basophils ($IgE^{+}CD49b^{+}$)[40]; similarly, Meng et al. found that irradiation reduces sensitization to ALA by the expression of mast cells ($Fc\varepsilon RI^{+}c\text{-}kit^{+}$). The present experiment was further judged to reflect the success of the allergy model by characterizing the expression of mast cells in the small intestine of mice[41]. Significantly, the expression of mast cells was significantly elevated in the sensitized group. Therefore, the multifaceted analysis all indicated that ALA caused the most intense rapid-onset sensitization reaction (type I sensitization) in the animals and could also indicate that our constructed mouse model of IgE-mediated type I hypersensitivity allergic reaction was successful.

3.6. Changes in cytokines after peptide stimulation of splenocytes and MLN cells

The spleen, as the largest peripheral immune organ, is also the site of T and B cell settlement and immune response[42]. After 72 hours of ALA stimulation, splenocytes were examined and supernatants were taken for cytokine detection. As shown in Fig. 6A, Th2-related cytokine IL-4 was significantly decreased in peptides 1, 6, 8, 12 and 14 compared with the control group (cells cultured with medium alone) ($P < 0.05$). IL-5 was significantly decreased in peptides 1, 4, 13, 14, 15 and 16 ($P < 0.05$) (Fig. 6B); IL-13 expression was significantly reduced in peptides 1, 3, 8, 10, and 15 ($P < 0.05$) (Fig. 6C). The Treg cell-associated cytokine IL-10 was significantly increased in peptides 1, 13, 14 and 15 ($P < 0.05$) (Fig. 6D). Th1 cell-associated cytokine IFNγ was significantly increased in peptides 1, 8, 10, 14 and 16 compared with the control group ($P < 0.05$) (Fig. 6E).

MLN single-cell suspensions from mice in the α-ALA group were taken and co-cultured with the peptides for 72 h, and the supernatants were taken to detect changes in cytokines. Effector T cells secrete a variety of cytokines, which are important bioactives that play different roles in cell induction. Fig. 6 demonstrates the changes of cytokines in cell supernatants. Compared with the control group, peptides 1, 6, 8, 13, and 14 could make IL-4 significantly decreased ($P < 0.05$) (Fig. 6F), IL-5 significantly decreased ($P < 0.05$) in peptides 1, 6, 8, 14, and 16 (Fig. 6G), and IL-13 significantly decreased ($P < 0.05$) in peptides 1, 6, 8, 14, and 16 (Fig. 6H). Based on these results, the above peptides significantly reduced Th2-related cytokines. As shown in Fig. 6D, peptides 6, 10, 12, and 13 significantly increased ($P < 0.05$) the Treg-associated cytokine IL-10, which is largely produced by Treg cells.

IL-4, IL-5, and IL-13 are Th2-associated cytokines that play an important role in the secretion of IgE and IgG1[43]. Studies have shown that IL-10 has several anti-inflammatory effects[44], such as down-regulating the level of IL-4 produced by Th2[37]. IL-10 can be produced by Treg cells[35]. In our results, peptides 1,6,8,14,15 stimulation of immune cells did not result in an increase in Th2 cytokines, while there was an

increase in Th1- and Treg-related factors, suggesting that these peptides may have the potential to induce tolerance, which may have a positive impact on the development of tolerance.

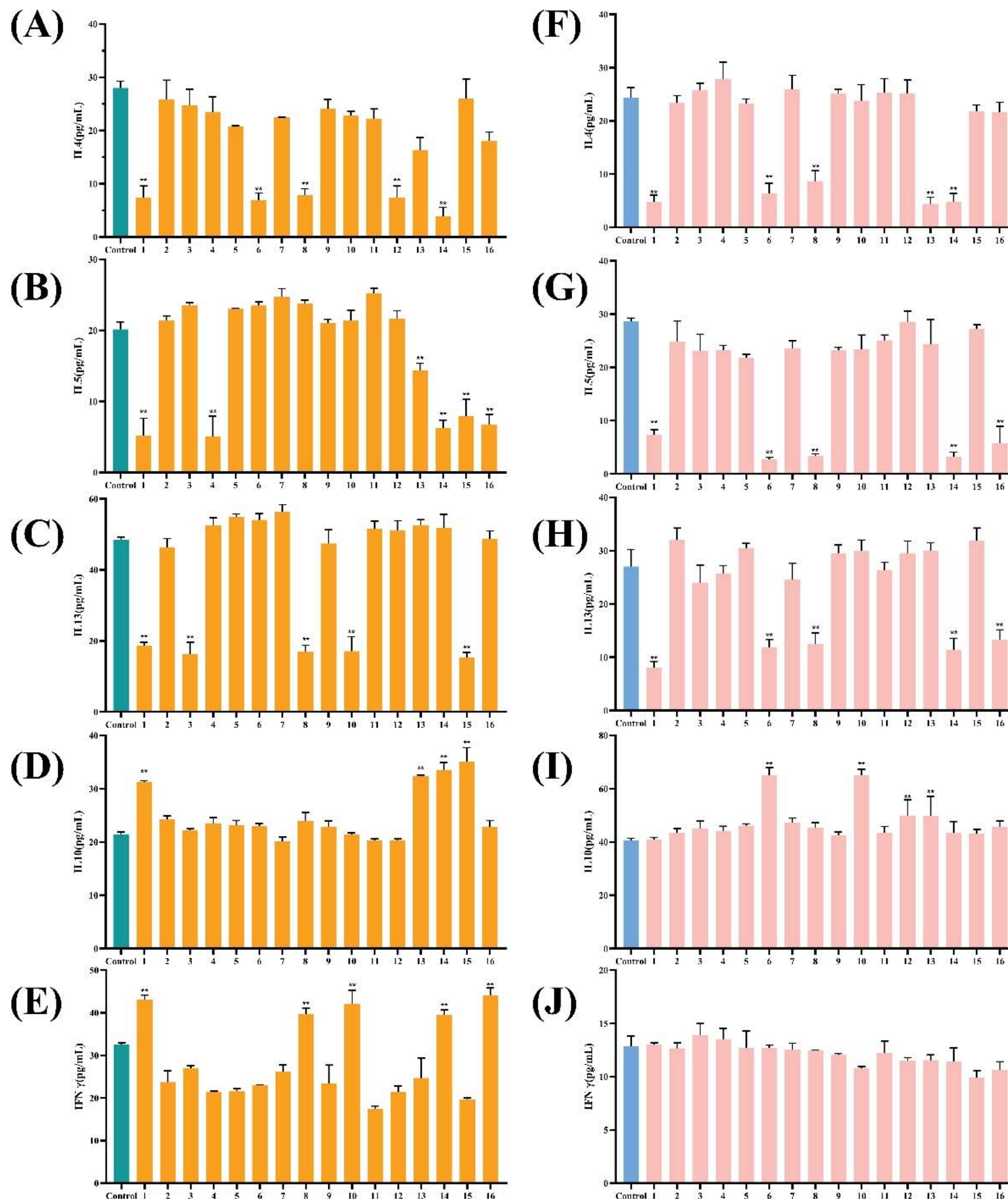

Fig. 6. Changes in cytokine levels in spleen cells and MLN cells after co-culture with ALA peptides. The 'Control' group represents unstimulated cells cultured with medium alone. Spleen cell: (A), IL-4; (B), IL-5; (C), IL-13; (D), IL-10; (E), IFN-γ. MLN cell: (F), IL-4; (G), IL-5; (H), IL-13; (I), IL-10; (J), IFN-γ. Statistically significant difference with one-way Anova followed by Bonferroni's test: **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

3.7. Changes in Treg and dendritic cells (DC) surface markers after co-culture of splenocytes and MLN cells with peptides

Treg cells play a very important role in the induction of tolerance and can inhibit the production of a wide range of cells. The expression of $CD4^+CD25^+Foxp3^+$Treg was detected in peptide-stimulated splenocytes after 72 h. The results showed that peptides 1,8,14 significantly increased the expression of $CD4^+CD25^+Foxp3^+$Treg compared to the control (Fig. 7A). In Fig. 7B, we detected the expression of the DC

surface marker CD103. Peptides 1, 4, 8, 11, and 16 significantly increased $CD11c^+CD103^+$DC compared with control, indicating that these peptides can induce the production of tolerogenic DCs.

Similarly, we also detected changes in the associated cells in MIN cells. The results of $CD4^+CD25^+Foxp3^+$Treg expression after peptide stimulation are shown in Fig. 7C. The results showed that peptides 1, 5, 11, 15, and 16 significantly increased the expression of $CD4^+CD25^+Foxp3^+$Treg, which is important for tolerance and maintenance of immune homeostasis. In Fig. 7D, the level of DC expression of CD103 is demonstrated. Peptides 1, 6, and 14 can significantly increase $CD11c^+CD103^+$DC expression compared to control.

Tolerance begins in the MLN, and DCs have been shown to be critical to this process[45]. Oral tolerance is initiated by CD103 DCs, which capture antigens in the lamina propria and migrate to the MLN, where they induce naïve T-cells to differentiate into Tregs through a mechanism that relies on TGF-β and retinoic acid and suppress Th2 responses to induce tolerance[38]. Therefore, in the present study, DC surface markers were detected and analyzed, and peptide 1,11 stimulated immune cells causing DC to differentiate towards a tolerogenic phenotype with the potential to induce tolerance compared to controls.

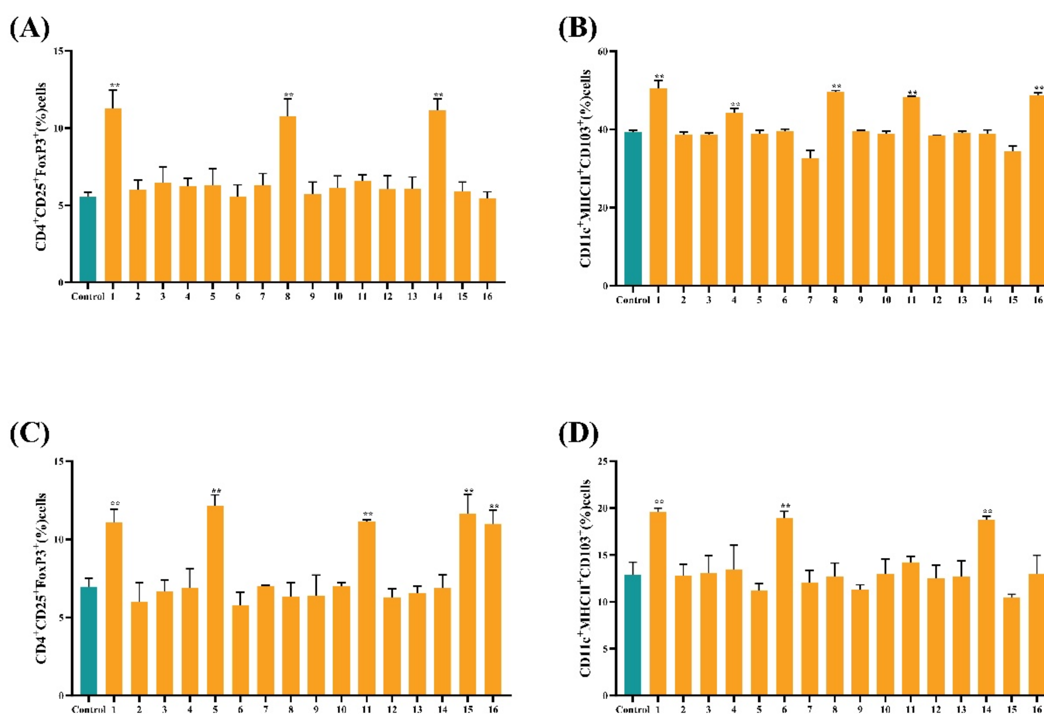

Fig. 7. Expression of Treg and DC surface markers in mouse splenocytes, MLN cells. The 'Control' group represents unstimulated cells cultured with medium alone. (A), splenocyte-Treg; (B), spleen cells -CD103+; (C), MLN-Treg; (D), MLN-CD103+. Statistically significant difference with one-way Anova followed by Bonferroni's test: **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

All results based on peptide stimulation of splenocytes and MLN cells are summarized in Table 1, and the scoring criteria were scored as conversion to the tolerant state was recorded as plus one point (decrease in Th2-related cytokines; increase in Th1-type-related cytokines; increase in Treg-type-related cytokines; and $CD103^+$ increase in tolerant DC cells), and the remaining cases were not scored. Peptides 1,8,14 most notably showed a decrease in Th2 cytokines after stimulation of the cells with a concomitant increase in Treg or Th1,

so these peptides may have the potential to induce tolerance, which may have a positive effect on the development of tolerance.

Table. 1. Cytokine changes and cell expression after co-culture of immune cells and peptides from α-lactalbumin allergic mice. "↑" represents a significant increase compared to the negative control; "↓" represents a significant decrease compared to the negative control.

Peptide Number	Th2						Th1		Treg		Foxp3		CD103		Score (14)
	IL-4		IL-5		IL-13		IFNγ		IL-10						
	Spleen	MLN	Spleen	MLN	Spleen	MLN	Spleen	MLN	Spleen	MLN	Spleen	MLN	Spleen	MLN	
1	↓	↓	↓	↓	↓	↓	↑		↑		↑	↑	↑	↑	12
2															0
3					↓										1
4			↓										↑		2
5												↑			1
6	↓	↓		↓		↓				↑				↑	6
7															0
8	↓	↓		↓	↓	↓	↑				↑		↑		8
9															0
10					↓		↑			↑					3
11												↑	↑		2
12	↓									↑					2
13		↓	↓						↑	↑					4
14	↓	↓	↓	↓		↓	↑		↑		↑			↑	9
15			↓		↓				↑			↑			4
16			↓	↓		↓	↑					↑	↑		6

We note that the increased frequency of regulatory T cells (Tregs) and tolerogenic dendritic cells (DCs) observed in vitro likely reflects the activation and expansion of pre-existing, directionally differentiated cell populations rather than the de novo differentiation of precursor cells. To formally establish the mechanism of peptide-induced differentiation, future studies should employ purified precursor cells, such as using naive CD4+ T cells for Treg polarization studies or bone marrow-derived cells for DC differentiation studies

4. Conclusion

In this study, the main allergen of cow's milk, α-lactalbumin, was used as the research object. Firstly, an ALA allergy model was successfully constructed by mice, and the allergic effect of the model was verified in terms of physiological indexes, humoral immunity and cellular immunity. Subsequently, bioinformatics and in vitro cellular experiments were performed to screen for peptides (AA 1-19) that promoted a tolerogenic immune phenotype, characterized by the expansion of Tregs in spleen and MLN cells, which might lead to the development of tolerance. The identified tolerogenic peptides provide a basis for subsequent in vivo experiments and a practical and safe method for inducing tolerance and preventing sensitization. In addition, further more refined in vivo and ex vivo experiments are needed to investigate the complex mechanisms.

Acknowledgments

We are grateful for the financial support from the National Key Research and Development Program of China (2024YFF1105900).

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J.W. Gouw, J. Jo, L.A.P.M. Meulenbroek, T.S. Heijjer, E. Kremer, E. Sandalova, A.C. Knulst, P.V. Jeurink, J. Garssen, A. Rijnierse, L.M.J. Knippels, Identification of peptides with tolerogenic potential in a hydrolysed whey-based infant formula, Clin. Exp. Allergy 48 (2018) 1345–1353. https://doi.org/10.1111/cea.13223.

[2] L. Zhao, Proteolytic activities of combined fermentation with lactobacillus helveticus KLDS 1.8701 and lactobacillus plantarum KLDS 1.0386 reduce antigenic response to cow milk proteins, 104 (2021).

[3] Y. Qiu, M. Ye, H. Tan, X. Zhang, H. Shao, S. Chen, Y. Zhang, Y. Wu, J. Gao, X. Meng, X. Li, H. Chen, Screening human IgE epitopes on α-lactalbumin and developing specific detection tools for enhanced allergen detection in food matrices, J. Agric, Food Chem, (2025).

[4] X. Ge, α-lactalbumin: functional properties and potential health benefits, Food Biosci. (2024).

[5] X. Wang, Investigation on potential allergenic peptides and critical amino acids of the digestive products of glycated α-lactalbumin via allergenicity evaluation and molecular dynamic simulation, (2024).

[6] J. Hu, Rapid determination of α-lactalbumin in raw cow's milk and recombined milk using an immunochromatographic assay strip analysis method, (2024).

[7] D. Xu, Quantitative detection of hidden milk allergens in hypoallergenic formulas through bottom-up proteomics, high-resolution mass spectrometry and the mechanism of D-ribose in reducing the allergenicity in vitro, (2025).

[8] M. Yamaguchi, K. Yoshida, M. Uchida, Novel Functions of Bovine Milk-Derived a-Lactalbumin: Anti-nociceptive and Anti-inflammatory Activity Caused by Inhibiting Cyclooxygenase-2 and Phospholipase A2, 32 (2009).

[9] M. Yamaguchi, S. Takai, A. Hosono, T. Seki, Bovine milk-derived α-lactalbumin inhibits colon inflammation and carcinogenesis in azoxymethane and dextran sodium sulfate-treated mice, Biosci., Biotechnol., Biochem. 78 (2014) 672–679. https://doi.org/10.1080/09168451.2014.890034.

[10] P. Rupa, Y. Mine, Oral immunotherapy with immunodominant T-cell epitope peptides alleviates allergic reactions in a Balb/c mouse model of egg allergy, Allergy 67 (2012) 74–82. https://doi.org/10.1111/j.1398-9995.2011.02724.x.

[11] N.G. Cortes-Perez, D. Lozano-Ojalvo, M.A. Maiga, S. Hazebrouck, K. Adel-Patient, Intragastric administration of Lactobacillus casei BL23 induces regulatory FoxP3+RORγt+ T cells subset in mice, Benef. Microbes 8 (2017) 433–438. https://doi.org/10.3920/BM2016.0174.

[12] A.T. Clark, S. Islam, Y. King, J. Deighton, K. Anagnostou, P.W. Ewan, Successful oral tolerance induction in severe peanut allergy, Allergy 64 (2009) 1218–1220. https://doi.org/10.1111/j.1398-9995.2009.01982.x.

[13] N. Itoh, Y. Itagaki, K. Kurihara, Rush Specific Oral Tolerance Induction in School-Age Children with Severe Egg Allergy: One Year Follow Up, Allergol. Int. 59 (2010) 43–51. https://doi.org/10.2332/allergolint.09-OA-0107.

[14] P. Meglio, P.G. Giampietro, S. Gianni, E. Galli, Oral desensitization in children with immunoglobulin E-mediated cow's milk allergy – follow-up at 4 yr and 8 months, Pediatr. Allergy Immunol. 19 (2008) 412–419. https://doi.org/10.1111/j.1399-3038.2007.00670.x.

[15] P. Varshney, S.M. Jones, A.M. Scurlock, T.T. Perry, A. Kemper, P. Steele, A. Hiegel, J. Kamilaris, S. Carlisle, X. Yue, M. Kulis, L. Pons, B. Vickery, A.W. Burks, A randomized controlled study of peanut oral immunotherapy: Clinical desensitization and modulation of the allergic response, J. Allergy Clin. Immunol. 127 (2011) 654–660. https://doi.org/10.1016/j.jaci.2010.12.1111.

[16] A. Verhoef, C. Alexander, A.B. Kay, M. Larché, T Cell Epitope Immunotherapy Induces a CD4+ T Cell Population with Regulatory Activity, PLoS Med. 2 (2005) e78. https://doi.org/10.1371/journal.pmed.0020078.

[17] H. Takagi, T. Hiroi, L. Yang, Y. Tada, Y. Yuki, K. Takamura, R. Ishimitsu, H. Kawauchi, H. Kiyono, F. Takaiwa, A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses, Proc. Natl. Acad. Sci. 102 (2005) 17525–17530. https://doi.org/10.1073/pnas.0503428102.

[18] F. Takaiwa, A Rice-Based Edible Vaccine Expressing Multiple T-Cell Epitopes to Induce Oral Tolerance and Inhibit Allergy, Immunol. Allergy Clin. 27 (2007) 129–139. https://doi.org/10.1016/j.iac.2006.11.001.

[19] A.L. Voskamp, S. Khosa, T. Phan, H.A. DeBerg, J. Bingham, M. Hew, W. Smith, J. Abramovitch, J.M. Rolland, M. Moyle, K.C. Nadeau, G. Lack, M. Larché, E. Wambre, R.E. O'Hehir, P. Hickey, S.R. Prickett, Phase 1 trial supports safety and mechanism of action of peptide immunotherapy for peanut allergy, Allergy 79 (2024) 485–498. https://doi.org/10.1111/all.15966.

[20] S.M. Afify, I. Pali-Schöll, K. Hufnagl, G. Hofstetter, M.A.-R. El-Bassuoni, F. Roth-Walter, E. Jensen-Jarolim, Bovine Holo-Beta-Lactoglobulin Cross-Protects Against Pollen Allergies in an Innate Manner in BALB/c Mice: Potential Model for the Farm Effect, Front. Immunol. 12 (2021) 611474. https://doi.org/10.3389/fimmu.2021.611474.

[21] T. Ando, J. Kashiwakura, N. Itoh-Nagato, H. Yamashita, M. Baba, Y. Kawakami, S.H. Tsai, N. Inagaki, K. Takeda, T. Iwata, N. Shimojo, T. Fujisawa, M. Nagao, K. Matsumoto, Y. Kawakami, T. Kawakami, Histamine-releasing factor enhances food allergy, J. Clin. Invest. 127 (2017) 4541–4553. https://doi.org/10.1172/JCI96525.

[22] A.C. Gomes-Santos, R.C. Fonseca, L. Lemos, D.S. Reis, T.G. Moreira, A.L. Souza, M.R. Silva, M.P.C. Silvestre, D.C. Cara, A.M.C. Faria, Hydrolyzed whey protein prevents the development of food allergy to β-lactoglobulin in sensitized mice, Cell. Immunol. 298 (2015) 47–53. https://doi.org/10.1016/j.cellimm.2015.09.001.

[23] X. Yin, X. Guo, H. Hong, Y. Luo, Y. Tan, Whey protein hydrolysates alleviated food allergy in mice by balancing the Th1/Th2 pathway and increasing IgA antibody production, Food Sci. Anim. Prod. 1 (2023) 9240046. https://doi.org/10.26599/FSAP.2023.9240046.

[24] S. Kazemi, E. Danisman, M.M. Epstein, Animal models for the study of food allergies, Curr. Protocol. 3 (2023) e685. https://doi.org/10.1002/cpz1.685.

[25] P.S. Krenger, J. Sobczak, M. Paolucci, T.M. Kundig, P. Johansen, M. Vogel, M.F. Bachmann, BALB/c and C3H mice are both suitable as peanut allergy models, Clin. Exp. Allergy 53 (2023) 1310–1313. https://doi.org/10.1111/cea.14398.

[26] A. Levescot, N. Cerf-Bensussan, Loss of tolerance to dietary proteins: from mouse models to human model diseases, Immunol. Rev. 326 (2024) 173–190. https://doi.org/10.1111/imr.13395.

[27] J.J. O'Konek, Animal models in the study of food allergens: long-term maintenance of allergic reactivity in mouse models of food allergy, Methods Mol. Biol. (Clifton N.J.) 2717 (2024) 321–335. https://doi.org/10.1007/978-1-0716-3453-0_22.

[28] M.C. Berin, S. Sicherer, Food allergy: mechanisms and therapeutics, Curr. Opin. Immunol. 23 (2011) 794–800. https://doi.org/10.1016/j.coi.2011.08.010.

[29] L. Paparo, R. Nocerino, E. Ciaglia, C. Di Scala, C. De Caro, R. Russo, G. Trinchese, R. Aitoro, A. Amoroso, C. Bruno, M. Di Costanzo, A. Passariello, F. Messina, A. Agangi, M. Napolitano, L. Voto, G.D. Gatta, L. Pisapia, F. Montella, M.P. Mollica, A. Calignano, A. Puca, R. Berni Canani, Butyrate as a bioactive human milk protective component against food allergy, Allergy 76 (2021) 1398–1415. https://doi.org/10.1111/all.14625.

[30] N. Samadi, M. Klems, D. Heiden, R. Bauer, C. Kitzmüller, E. Weidmann, D. Ret, A.S. Ondracek, A. Duschl, J. Horejs-Hoeck, E. Untersmayr, Nitrated food proteins induce a regulatory immune response associated with allergy prevention after oral exposure in a Balb/c mouse food allergy model, Allergy 75 (2020) 412–422. https://doi.org/10.1111/all.14030.

[31] M. Curin, H.-J. Huang, T. Garmatiuk, S. Gutfreund, Y. Resch-Marat, K.-W. Chen, K. Fauland, W. Keller, P. Zieglmayer, R. Zieglmayer, P. Lemell, F. Horak, W. Hemmer, M. Focke-Tejkl, S. Flicker, S. Vrtala, R. Valenta, IgE Epitopes of the House Dust Mite Allergen Der p 7 Are Mainly Discontinuous and Conformational, Front. Immunol. 12 (2021). https://doi.org/10.3389/fimmu.2021.687294.

[32] C. Kanagaratham, Y.S. El Ansari, O.L. Lewis, H.C. Oettgen, IgE and IgG Antibodies as Regulators of Mast Cell and Basophil Functions in Food Allergy, Front. Immunol. 11 (2020) 603050. https://doi.org/10.3389/fimmu.2020.603050.

[33] D. Moldaver, M. Larché, Immunotherapy with peptides: Immunotherapy with peptides, Allergy 66 (2011) 784–791. https://doi.org/10.1111/j.1398-9995.2011.02610.x.

[34] T.B. Bertolini, M. Biswas, C. Terhorst, H. Daniell, R.W. Herzog, A.R. Piñeros, Role of orally induced regulatory T cells in immunotherapy and tolerance, Cell. Immunol. 359 (2021) 104251. https://doi.org/10.1016/j.cellimm.2020.104251.

[35] P. Satitsuksanoa, K. Jansen, A. Głobińska, W. Van De Veen, M. Akdis, Regulatory Immune Mechanisms in Tolerance to Food Allergy, Front. Immunol. 9 (2018) 2939. https://doi.org/10.3389/fimmu.2018.02939.

[36] K. Adel-Patient, H. Bernard, S. Ah-Leung, C. Créminon, J. -M. Wal, Peanut- and cow's milk-specific IgE, Th2 cells and local anaphylactic reaction are induced in Balb/c mice orally sensitized with cholera toxin, Allergy 60 (2005) 658–664. https://doi.org/10.1111/j.1398-9995.2005.00767.x.

[37] M. Jutel, M. Akdis, K. Blaser, C.A. Akdis, Mechanisms of allergen specific immunotherapy – T-cell tolerance and more, Allergy 61 (2006) 796–807. https://doi.org/10.1111/j.1398-9995.2006.01175.x.

[38] A. Carambia, B. Freund, D. Schwinge, M. Heine, A. Laschtowitz, S. Huber, D.C. Wraith, T. Korn, C. Schramm, A.W. Lohse, J. Heeren, J. Herkel, TGF-β-dependent induction of CD4+CD25+Foxp3+ Tregs by liver sinusoidal endothelial cells, J. Hepatol. 61 (2014) 594–599. https://doi.org/10.1016/j.jhep.2014.04.027.

[39] S.H. Sicherer, H.A. Sampson, Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management, J. Allergy Clin. Immunol. 141 (2018) 41–58. https://doi.org/10.1016/j.jaci.2017.11.003.

[40] I. López-Expósito, R. Chicón, J. Belloque, R. López-Fandiño, M.C. Berin, In vivo methods for testing allergenicity show that high hydrostatic pressure hydrolysates of β-lactoglobulin are immunologically inert, J. Dairy Sci. 95 (2012) 541–548. https://doi.org/10.3168/jds.2011-4646.

[41] X. Meng, X. Li, X. Wang, J. Gao, H. Yang, H. Chen, Potential allergenicity response to structural modification of irradiated bovine α-lactalbumin, Food Funct. 7 (2016) 3102–3110. https://doi.org/10.1039/C6FO00400H.

[42] O. Hemmings, G. Du Toit, S. Radulovic, G. Lack, A.F. Santos, Ara h 2 is the dominant peanut allergen despite similarities with Ara h 6, J. Allergy Clin. Immunol. 146 (2020) 621-630.e5. https://doi.org/10.1016/j.jaci.2020.03.026.

[43] R.J. Dearman, I. Kimber, Animal models of protein allergenicity: potential benefits, pitfalls and challenges, Clin. Exp. Allergy 39 (2009) 458–468. https://doi.org/10.1111/j.1365-2222.2008.03194.x.

[44] M. Saraiva, A. O'Garra, The regulation of IL-10 production by immune cells, Nat. Rev. Immunol. 10 (2010) 170–181. https://doi.org/10.1038/nri2711.

[45] G. Matteoli, E. Mazzini, I.D. Iliev, E. Mileti, F. Fallarino, P. Puccetti, M. Chieppa, M. Rescigno, Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction, Gut 59 (2010) 595–604. https://doi.org/10.1136/gut.2009.185108.