DOI 10.1007/s10517-021-05226-z

Effective Model of Food Allergy in Mice Sensitized with Ovalbumin and Freud's Adjuvant

Y. Zhang, J. Y. Liu, J. W. Shao, Q. Q. Luo, Y. Q. Zhang, G. Song, C. Y. Wang, S. Y. Zhao, C. Wan, X. H. Du, and L. Z. Xu

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 171, No. 3, pp. 333-338, March, 2021
Original article submitted November 27, 2020

To better explore the pathophysiology of FA and its therapy, we aimed to establish a simple and practicable FA model with Freund's adjuvant and introduce an easy and reliable laboratory evaluation method for assessment of inflammation in intestinal segments at different anatomical locations. BALB/c mice were sensitized with ovalbumin combined with Freund's adjuvant. Complete Freund's adjuvant was chosen for the first sensitization and two weeks later incomplete Freund's adjuvant was used for a second sensitization. Two weeks later, the sensitized mice were challenged with 50 mg ovalbumin every other day. After the 6 challenge, all mice were assessed for systemic anaphylaxis, and then sacrificed for sample collection. All sensitized mice showed anaphylactic symptoms and markedly increased levels of serum ovalbumin-specific IgE and IgG1. The activity of mast cell protease-1 (mMCPT-1) was significantly increased in the serum and interstitial fluid of the duodenum, jejunum, ileum, and colon. A successful FA model was established, of which inflammation occurred in the duodenum, jejunum, ileum, and colon. This model provides a reliable and simple tool for analysis of the mechanism of FA and methods of immunotherapy. Moreover, combined detection of ovalbumin-specific antibody and local mMCPT-1 levels could potentially be used as the major indicator for assessment of food allergy.

Key Words: *food allergy; ovalbumin; Freund's adjuvant; IgE; mouse mast cell protease-1*

The incidence of food allergy (FA) in the world has been increasing over the past few decades, which have badly influenced the quality of life of patients and their families [13]. FA is a specific immune response to certain foods, *e.g.* peanut, nut, egg, or milk accompanied by severe allergic disorders: urticaria, gastrointestinal hypersensitivity, diarrhea, or anaphylaxis [12]. FA is mainly caused by IgE-mediated type I hypersensitivity involving activation of T helper cells (Th) type-2 cell secreting Th2 cytokines such as IL-4, IL-5, IL-10, and IL-13. Th2 cytokines induce B cells to produce antigen-specific IgE and IgG1 and promote proliferation of eosinophils. Then, IgE binds to human IgE receptor (FcεRI) expressed on mast cells (MC) and basophils, where it can induce MC and basophil expansion in affected mucosae [1,7].

Animal models of FA, the most commonly used in the research, have been adopted by various investigations. Inbred BALB/c mice characterized by domination of Th2 response, are sensitive to food allergens and can intensively produce IgE when challenged by allergens, therefore, they are good candidates used in FA research [10]. The adjuvant-dependent sensitization models helped us to better understand the type 2 immune responses [7]. Adjuvants such as complete Freund's adjuvant (CFA) [12], alum ($AlK(SO_4)_2$-$12H_2O$) [4], cholera toxin, *etc*., are widely used in the experimental FA models. When alum is used as immune adjuvant, only about 40% ovalbumin (OVA) can be adsorbed by alum, whereas 90% OVA can be adsorbed by CFA [3], suggesting that using CFA as an immune adjuvant can be more effective in developing animal models of FA.

Key Lab for Immunology, Universities of Shandong Province, School of Basic Medical Sciences, Weifang Medical University, Weifang, China. ***Address for correspondence:*** xxllzz2006@126.com. L. Z. Xu

In this work, we chose CFA and incomplete Freund's adjuvant (IFA) to sensitize mice. Overall, we aimed to establish a simple and practicable FA model with Freund's adjuvant and introduce an easy and reliable laboratory evaluation method for assessment of inflammation of the intestine at different anatomical locations.

MATERIALS AND METHODS

The experiments were performed on 5-6-week-old female BALB/c mice purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The mice were housed under the specific pathogen-free conditions. The experimental protocols were approved by the Animal Ethic Committee at Weifang Medical University (No. WFMUAE2017061).

The mice were randomly divided into the food allergy group (FA) and the control group (without FA). FA was modelled as described previously [4] with modifications. On day 0, the mice were injected subcutaneously in each groin with 50 μg OVA (Sigma-Aldrich) or PBS mixed with 50 μl CFA followed by booster immunization with 50 μg OVA or PBS mixed with 50 μl IFA on day 14; then, gavage challenge with 50 mg OVA in 300 μl PBS or 300 μl PBS was performed (Fig. 1, *a*). In this study, anaphylactic symptoms were assessed over 1 h after the 6th oral challenge according to the scoring system [11]. Body weight was determined daily during the challenge period and rectal temperature was measured within 1 h after challenge using a mouse rectal thermometer [1].

Serum OVA-specific IgE and IgG1 were detected by indirect ELISA using an Epoch microplate (BioTek) precoated with 20 μg/ml OVA at 4°C overnight. Serum samples were added to the wells and incubated at 37°C for 2 h. The plates were washed four times with PBS with Tween and incubated with secondary antibodies labeled with horse radish peroxidase: rat anti-mouse IgE (#1130-05) or goat anti-mouse IgG1 (#1070-05; NeoBioscience) at 37°C for 2 h. For measuring OVA-specific IgE, the serum was diluted 1:5; for measuring OVA-specific IgG1, the serum was diluted 1:10 in the control group and 1:15,000 in the FA group. After color development, the absorbance was measured at 450 nm on an Epoch microplate reader (BioTek).

Serum and the intestinal tissues of duodenum, jejunum, ileum, and colon were collected after sacrifice. The tissue samples were cut into pieces in sterile PBS (400 μl per 20 mg tissue) and then centrifuged at 12,000*g* for 5 min at 4°C to collect intestinal interstitial fluids. mMCPT-1 levels in the serum and interstitial fluids of different intestines were measured using ELISA Kits (MultiSciences Biotech Co.) according to manufacturer's instruction. After the enzyme reaction, the absorbance was measured at 450 nm on an Epoch2 microplate reader.

The jejunum tissue samples were fixed in 4% formaldehyde for 24 h, washed in running water for 12 h, dehydrated in gradient alcohol, vitrified in dimethylbenzene, and then embedded in paraffin. Histological sections (5 μm) were stained with hematoxylin and eosin [2]. In addition, 5-μm sections were stained with 1% acid toluidine blue (pH=1) for 10 min [9]. Eosinophils and MC were counted under an Olympus BX53 light microscope at high-power field area at ×200 or at ×1000. At least 10 fields of view were examined, and the mean value of cells per 10 high-power fields was determined.

The data were analyzed using GraphPad Prism 5.0 software. The results are presented as the $M \pm SEM$. Unpaired Student's t test, Welch's correction, Mann—Whitney test, and two-way ANOVA were used for statistical analysis. The differences were considered significant at $p<0.05$, $p<0.01$, or $p<0.001$.

RESULTS

The systemic anaphylactic responses were assessed after the last OVA challenge. Symptoms of FA (no activity, piloerection, lose luster, and other allergic symptoms) were observed in all mice of the experimental group (Fig. 1, *b*). Weight loss was observed starting from day 28 in both FA group and control group and the difference between the groups reached significance on day 32 (Fig. 1, *d*). After challenge, FA mice exhibited significant decrease in rectal temperature (Fig.1, *c*). This is consistent with previous data obtained on an OVA-induced FA model [4] on severe hypothermia in mice intragastrically challenged with OVA in comparison with that in mice challenged with saline. In our previous experiments, some FA mice even experienced a significant drop in body temperature after a second or third challenge, which was not even detectable and subsequently died of shock (unpublished data). All the data suggested that the mice sensitized with CFA and IFA in our model can better mimic clinical manifestations of FA.

In this study, the serum levels of OVA-specific IgE and IgG1 were measured after the final challenge (Fig. 2, *a*, *b*). Both OVA-specific IgE and IgG1 were markedly elevated in the FA group in comparison with the control. In IgE-dependent FA, IgE bind to high affinity IgE receptors FcεRI expressed on MC and basophils [1] and evoke allergy attacks. Allergen re-entry into the body results in crosslinking of FcεRI-bound IgE to induce activation of MC, the latter release many allergic mediators to induce clinical allergic symptoms [5].

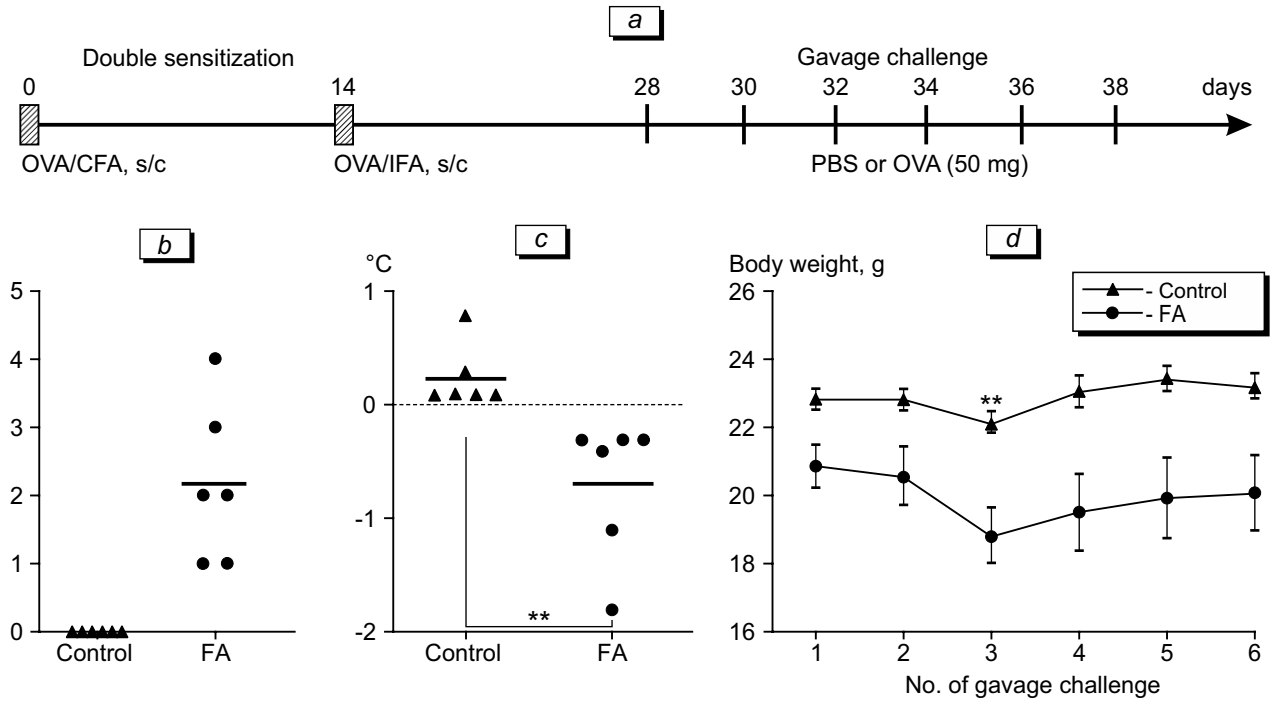

Fig. 1. Protocol of sensitization and gavage challenges (*a*), severity of anaphylactic reaction (*b*), changes in core temperature (*c*), and body weight (*d*) before and after allergic tests. s/c: subcutaneously. Here and in Figs. 2 and 3: the results are presented as *m*±*SE* (*n*=6) for 3 independent experiments.

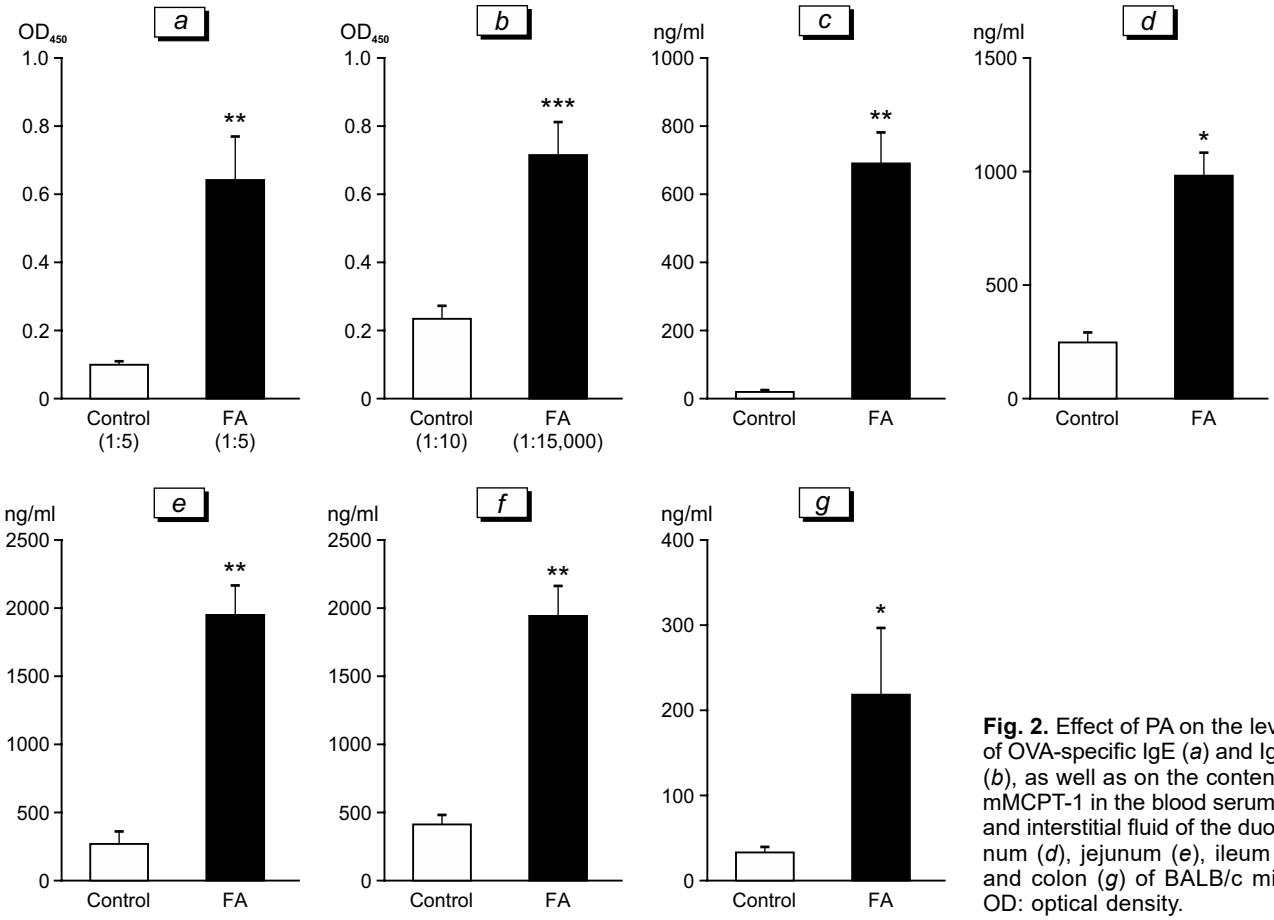

Fig. 2. Effect of PA on the levels of OVA-specific IgE (*a*) and IgG1 (*b*), as well as on the content of mMCPT-1 in the blood serum (*c*) and interstitial fluid of the duodenum (*d*), jejunum (*e*), ileum (*f*), and colon (*g*) of BALB/c mice. OD: optical density.

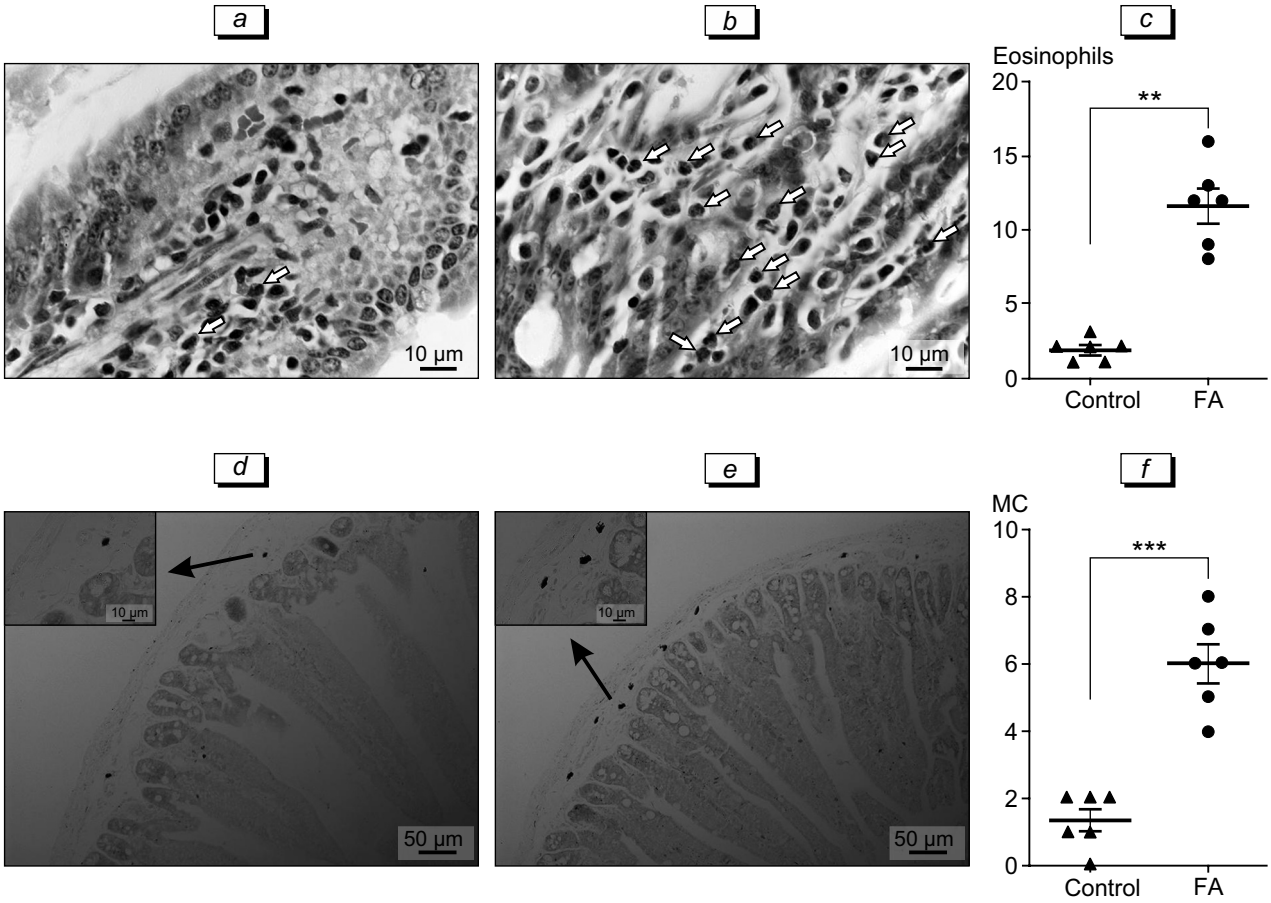

Fig. 3. Infiltration of the jejunum with eosinophils and MC. Immersion microscopy (*a*, *b*, *d*, *e*), ×200 (MC), ×1000 (eosinophils and MC). *a*, *d*) Control group; *b*, *d*) FA group; *c*, *f*) number of eosinophils (*c*) and MC (*f*) infiltrating the jejunum.

Next, we evaluated activation of MC in the intestinal tissues. mMCPT-1 is expressed in MC of the intestinal mucosa [6] and can be used as an indicator of MC activation. Therefore, we assessed the levels of mMCPT-1 in intestinal interstitial fluids of different anatomical sites to localize MC activation. The intestinal interstitial fluid and serum mMCPT-1 levels one day after challenge (day 38) were analyzed by ELISA (Fig. 2, *c*-*g*). The mean serum mMCPT-1 levels in the FA group were higher than in the control (Fig. 2, *c*). The results demonstrated significant increase in mMCP-1 in the duodenum (Fig. 2, *d*), jejunum (Fig. 2, *e*), ileum (Fig. 2, *f*), and colon (Fig. 2, *g*).

Moreover, we took jejunum tissue as an example to track the histological results in the intestinal allergy attacks. It was found that the jejunal tissue from mice of the FA group showed marked eosinophil infiltration of the lamina propria after OVA challenge (Fig. 3, *b*, *c*), while in control mice, only few eosinophils were seen (Fig. 3, *a*, *c*). Staining with toluidine blue showed that the content of MC increased significantly in the intestinal submucosa and notably in the jejunum of FA group mice (Fig. 3, *d*-*f*).

Both MC and eosinophils are the key effector cells in the allergy pathophysiology [8], which is confirmed in this study. In mice, MC of intestinal mucosa are commonly quantified by toluidine blue staining and serum mMCPT-1 is used as a systemic readout to show the activation of mucosal MC in response to antigen-specific IgE cross-linking [7,11]. Previously, we examined mMCPT-1 levels of colon interstitial fluid during the progression from chronic colitis to colon cancer to assess MC activation and involvement, and significant differences were revealed between the experimental group and the control group [14]. Consistently, in this study, we found that mMCPT-1 levels in the interstitial fluid of the duodenum, jejunum, ileum, and colon were significantly increased. This finding attests to significant activation of MC in the duodenum, jejunum, and ileum. Moreover, detection of mMCPT-1 levels in different intestinal interstitial fluid seems to be a good readout for the local MC activation in FA and it has not been reported in the previous studies.

In conclusion, we have established a successful FA model, in which the allergic inflammation mainly occurred in the duodenum, jejunum, ileum, and colon.

And we also found that activation of intestinal mucosal MC could be more easily and reliably identified by measuring mMCPT-1 levels of intestinal tissues, which can evaluate the anaphylaxis of different intestinal tissues in FA. We concluded that the combined detection of OVA-specific antibodies and local mMCPT-1 levels could potentially be used as the major indicator for assessment of FA.

This study was supported by the National Natural Science Foundation of China (grant No. 81701590), the Natural Science Foundation of Shandong Province (grant No. ZR2017BH071), the College Students Science and Technology Innovation Foundation of Shandong Province (grant No. S201910438018), the College Students Science and Technology Innovation Foundation of Weifang Medical University (grant No. KX2018061), and the National College Students Innovation Training Program (grant No. S202010438015).

The authors confirm no conflict of interests. Y. Zhang, J. Y. Liu, and J. W. Shao contributed equally to this work.

REFERENCES

1. Adel-Patient K, Bernard H, Ah-Leung S, Créminon C, Wal JM. 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. 2005;60(5):658-664. doi: 10.1111/j.1398-9995.2005.00767.x
2. Batista NV, Pereira RV, Noviello ML, Dourado LP, Perez DA, Foureaux G, Ferreira AJ, Ferreira AV, Cara DC. Prolonged ingestion of ovalbumin diet by sensitized mice improves the metabolic consequences induced by experimental food allergy. Clin. Exp. Immunol. 2014;178(3):416-427. doi: 10.1111/cei.12435
3. Beck L, Spiegelberg HL. The polyclonal and antigen-specific IgE and IgG subclass response of mice injected with ovalbumin in alum or complete Freund's adjuvant. Cell Immunol. 1989;123(1):1-8. doi: 10.1016/0008-8749(89)90263-3
4. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, Zimmermann N, Finkelman FD, Rothenberg M.E. Mast cells are required for experimental oral allergen-induced diarrhea. J. Clin. Invest. 2003;112(11):1666-77. doi: 10.1172/JCI19785
5. Galli SJ, Tsai M. IgE and mast cells in allergic disease. Nat. Med. 2012;18(5):693-704. doi: 10.1038/nm.2755
6. Honjo A, Nakano N, Yamazaki S, Hara M, Uchida K, Kitaura J, Nishiyama C, Yagita H, Ohtsuka Y, Ogawa H, Okumura K, Shimizu T. Pharmacologic inhibition of Notch signaling suppresses food antigen-induced mucosal mast cell hyperplasia. J. Allergy Clin. Immunol. 2017;139(3):987-996.e10. doi: 10.1016/j.jaci.2016.05.046
7. Kanagaratham C, Sallis BF, Fiebiger E. Experimental Models for Studying Food Allergy. Cell. Mol. Gastroenterol. Hepatol. 2018;6(3):356-369.e1. doi: 10.1016/j.jcmgh.2018.05.010
8. Ko MT, Huang SC, Kang HY. Establishment and characterization of an experimental mouse model of allergic rhinitis. Eur. Arch. Otorhinolaryngol. 2015;272(5):1149-1155. doi: 10.1007/s00405-014-3176-2
9. Liu DR, Xu XJ, Yao SK. Increased intestinal mucosal leptin levels in patients with diarrhea-predominant irritable bowel syndrome. World J. Gastroenterol. 2018;24(1):46-57. doi: 10.3748/wjg.v24.i1.46
10. Shin HS, Eom JE, Shin DU, Yeon SH, Lim SI, Lee SY. Preventive Effects of a Probiotic Mixture in an Ovalbumin-Induced Food Allergy Model. J. Microbiol. Biotechnol. 2018;28(1):65-76. doi: 10.4014/jmb.1708.08051
11. Smarr CB, Hsu CL, Byrne AJ, Miller SD, Bryce PJ. Antigen-fixed leukocytes tolerize Th2 responses in mouse models of allergy. J. Immunol. 2011;187(10):5090-5098. doi: 10.4049/jimmunol.1100608
12. Toyoshima S, Wakamatsu E, Ishida Y, Obata Y, Kurashima Y, Kiyono H, Abe R. The spleen is the site where mast cells are induced in the development of food allergy. Int. Immunol. 2017;29(1):31-45. doi: 10.1093/intimm/dxx005
13. Walkner M, Warren C, Gupta RS. Quality of Life in Food Allergy Patients and Their Families. Pediatr. Clin. North Am. 2015;62(6):1453-1461. doi: 10.1016/j.pcl.2015.07.003
14. Xu L, Yi H.G, Wu Z, Han W, Chen K, Zang M, Wang D, Zhao X, Wang H, Qu C. Activation of mucosal mast cells promotes inflammation-related colon cancer development through recruiting and modulating inflammatory CD11b(+) Gr1(+) cells. Cancer Lett. 2015;364(2):173-80. doi: 10.1016/j.canlet.2015.05.014