Food allergens affect the intestinal tight junction permeability in inducing intestinal food allergy in rats

Tao Chen,[1,2,#] Xiaoyu Liu,[1,#] Li Ma,[1,2,#] Weiyi He,[1] Weizhong Li,[1] Yanjuan Cao[1] and Zhigang Liu[1,*]

Summary

Background: **The intestinal tract plays an important role in food allergy and the intestinal mucosa barrier is critical for maintenance of its function. The underlying mechanisms of how food allergens modulate the intestinal permeability in inducing intestinal food allergy remain elusive.**

Objective: **The aim of this study was to explore the mechanism of how food allergens influence the function of intestinal barrier and induce intestinal food allergy.**

Methods: **Ovalbumin (OVA) was chosen to establish intestinal food allergy models in juvenile and adult rats that were confirmed by IgE and IgG assay. Intestinal tissue morphology was analyzed by HE staining. Intestinal permeability was dynamically monitored using a Lactulose (L)-Mannitol (M) assay. The morphology of the tight junctions in the intestinal mucosa barrier were analyzed under TEM. The expression of key molecules in tight junction regulation was evaluated by Real-time PCR.**

Results: **We found: 1) The sensitization rate in juvenile rats was higher than in adult rats; 2) Intestine fluff erosion was more serious in juvenile rats than in adult rats in the duodenum and ileum; 3) Intestinal permeability was severely damaged, according to the results of the Lactulose (L)-Mannitol (M) assay; 4) Tight junction damage on the mucosal barrier was observed; Real-time PCR results showed that the expression of some key molecules that are involved in tight junction regulation was also affected. Conclusions: Our data suggested that the allergy sensitization rate of Ovalbumin (OVA) in the juvenile group is higher than in adults and food allergens may increase intestinal mucosal permeability through intestinal tight junction regulation in inducing intestinal food allergy.** ***(Asian Pac J Allergy Immunol 2014;32:345-53)***

Keywords: *Food allergen, ovalbumin, allergy, intestinal permeability, tight junction*

Introduction

Food allergy is a serious public health problem,[1,2] which cause severe symptoms in the gastrointestinal tract, skin, respiratory tract and other organ systems, and even anaphylactic shock and death.[3-5] Epidemiological studies have reported that the incidence of food allergies (FA) has been increasing. Previous research has contributed a lot of information about food allergy. Its incidence is higher in children (6% to 10%) than in adults (just 1% to 2%)[5] and its pathogenesis is still not well understood.

As the mainly organ involved in digestion, the intestinal tract is considered to be a barrier to food allergens in cases of food allergy. The intestinal barrier, which can be divided into the mechanical barrier, microbial barrier, immunological barrier and chemical barrier etc., plays an important role in maintaining intestinal function to prevent bacteria, endotoxins and other toxic substances passing into the underlying tissues.[6,7] Studies suggested that abnormal intestinal permeability is also asociated with diabetes, celiac disease, inflammatory bowel disease and other diseases besides food allergy.[8,9] Increased intestinal permeability is also believed to

# Authors equally contributed to this work.
From 1. State Key Laboratory of Respiratory Disease for Allergy at Shengzhen University; School of Medicine, Shenzhen University, 3688 Nanhai Ave., Shenzhen, Guangdong, P. R. China, 518060.
2. Shenzhen ENT Institute, Longgang Central Hospital, Shenzhen, Guangdong, P. R. China, 518116.
Corresponding author: Zhigang Liu
E-mail: lzg195910@126.com
Submitted date: 24/9/2013
Accepted date: 3/3/2014

be significantly related to the pathogenesis of these diseases and more and more studies are focusing on this relationship.

It seems that the food allergy morbidity varies in different age groups, which is possibly associated with the development and functional status of the intestinal mucosal barrier.[10] Permeability increase and functional damage can be found in the intestinal barrier when food allergy occurs.[7,11, 12] It is reported that intestinal permeability increases in the early stages of food allergy.[12,13] Yet the molecular basis of the structural changes in the intestinal barrier associated with food allergy remain unknown. The aim of our study was to explore the molecular mechanisms of how food allergens affect and damage the morphology and function of the intestinal mucosal barrier to induce intestinal food allergy, through OVA induced food allergy models in juvenile or adult rats.

Methods

Animals

4 week (considered Juvenile[14]) and 12 week old (considered Adult[14]) BN (Brown Norway) rats were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) and raised in SPF facilities. The animal studies were approved by the School of Medicine of Shenzhen University, and carried out as previously reported.[15,16] Briefly, the juvenile and adult rats were randomly divided into a sensitization group and a control group. The sensitization group was administrated 1mg/mL OVA (in PBS, phosphate-buffered saline) (Sigma/Flu/Ald, USA) orally everyday for 48 days, while the control group was administrated1 mL PBS. All the animals were challenged on the 49th day with 100mg/mL OVA after the last sensitization and sacrificed 8hrs later (Figure 1). Blood and intestinal tissue samples were collected for further study.

Intestinal permeability evaluation (Lactulose/ Mannitol assay)

Intestinal permeability was evaluated by the ratio of lactulose and mannitol (M/L) in urine excretion as previously reported.[17, 18] Rats were put into metabolic cages and given (i.g.) 100mg lactulose and 50 mg mannitol (dissolving in 1mLPBS) orally once a week. Rat urine was collected during a 24hrs fasting period (but with water). 5% acetic acid was added to the urine samples after centrifugation. The samples were then boiled, centrifuged and the filtered through a 0.22μm Millipore filter to remove impurities (such as urine proteins). Afterwards the specimen was applied to HPLC analysis to determine the ratio of lactulose and mannitol (L/M) [20μL was taken for analysis (mobile phase: acetonitrile/water (80%/20%); flow rate: 1mL/min; column temperature: 35°C)

Serum OVA-IgE/OVA-IgG analyzing

Blood was collected from the rats' tail vein every week. OVA-sIgE and OVA-sIgG were assayed by ELISA as previously reported [19]. In brief, undiluted serum samples were detected using OVA pre-coated plates (10 μg/mL OVA of 100 μL/well) and HRP-labeled rat-anti-mouse IgE or IgG (1:1000) as the secondary antibody (Southern Biotechnology Associates Inc., Birmingham, AL). After the incubation and reaction with TMB (3,3',5,5'-Tetramethylbenzidine), the final OD value was detected at 450 nm wavelength using a RT-2100C Microplate reader (Rayto Life and Analytical Sciences Co., Ltd., China).

HE staining

Intestinal samples were collected immediately after rat sacrifice. The specimens were fixed with 4% paraformaldehyde for 24 hours and stained with Hematoxylln-Eosinstain after dehydration,

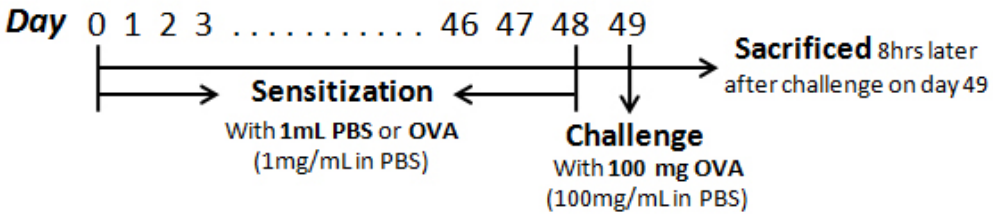

Figure 1. Protocol of OVA sensitization or challenge.
Control group rats were sensitized with PBS (1mL per rat, daily) intragastrically for 48 days; in the OVA treated group, rats were sensitized with OVA (1mg in 1mL PBS, daily per rat) intragastrically for 48 days. All rats in the two groups received challenges on day 49 with 100 mg/mL OVA (in PBS) and were sacrificed 8 hours later for further study.

embeding and slicing. The structural and morphological changes were observed and analyzed under a OLYMPUS BX51 microscope.

Electron microscope study

Rat intestinal samples were collected immediately after sacrifice. Samples were cut into 1.5mm×1.5mm×2 mm pieces and put into a fixation solution (provided by the electron microscopy room of the School of Medicine at Sun Yat-sen University). Samples then were kept at 4 degrees C overnight and sent to the electron microscopy room of the School of Medicine at Sun Yat-sen University for analysis. The structural and morphological changes (of epithelial cells, tight junction, etc.) were examined under the electron microscope.

Real-time PCR

50-100 mg of intestinal tissues were collected and ground in liquid nitrogen, then moved into a 1.5mL EP tube. The total RNA was extracted using a TRIzol Reagent (15596018, invitrogen/Gibco/MP) and reverse transcribed using a Reverse Transcriptase Kit (Fermentas). Real-time PCR was performed using C1000^{TM} Thermal Cyclers (Bio-Rad, USA) with SYBR green fluorescence. Samples were run in triplicate and the cycling parameters were: 95°C for 3 min, followed by 40 cycles of 95°C for 25s, 60°C for 20s, and a detection step at 72°C for 30 s. Human β-actin transcript was used as an internal control for results standardization ($2^{-\triangle\triangle CT}$) by eliminating variations in mRNA quantity and each RNA sample was arranged in triplicate. All the primers were synthesized by BGI Cooperation (Shenzhen, China) as list:

ZO-1: Forward, 5'-ACCCACGAAGTTATGAGCAAG-3'; Reverse, 5'-AGACTGTGGTTTCATTGCTGG-3';
Occludin: Forward, 5'-ATTCCTCTGACCTTGTCCGTG-3'; Reverse, 5'-CCTGTCGTGTAGTCGGTTTCA-3';
Claudin-2: Forward, 5'-ATTCCTCTGACCTTGTCCGTG-3'; Reverse, 5'-AGCCAACCGCCGTCACAATG-3';
Claudin-8: Forward, 5'-TGTCGTGTTTGAGAACCGCTGGG-3'; Reverse, 5'-ACGGACGCAGCACACATCAGTC-3';
Claudin-9: Forward, 5'-TTCCACTGGCCTTGAACTCCTCG-3'; Reverse, 5'-GCTGTTGCCAATGAAGGCGGT-3';
Claudin-15: Forward, 5'-AACTGCTGGGACTTCCCGTCCAT-3'; Reverse, 5'-TCGATGTTGCCCACGTTGGTGC-3';
β-actin: Forward, 5'-GTCTCACCACTGGCATTGTG-3'; Reverse, 5'-TCTCAGCTGTGGTGGTGAAG-3'.

Statistical analysis

The data were analyzed using SPSS v13.0 software. All values in the study are presented as mean±SD from at least three independent experiments. Statistical analysis of experiments in cell lines was evaluated using Student's t test. Values of $P < 0.05$ were considered to be statistically significant.

Result

Juvenile rats were more prone to allergy to OVA induction compared with the adults.

Successful food allergy model induction was determined according to a significant serum IgE rise and behavior observations (e.g., diarrhea). We found that it was earier to induce allergy in juvenile rats (with a sensitization rate 69.23%) to OVA, compared with their adult counterparts (Table 1). The serum OVA-IgE began to rise significantly growing in both the juvenile and adult groups from the 4th week (Figure 2A); The serum OVA-IgE level of juvenile rats was higher than that in adult rats at the 6th week (Figure 2A). Also, the serum OVA-IgG increased from the 2nd week in both the juvenile and adult rats, compared with controls (Figure 3A), though there is no statistically significant difference between them (Figure 2B).

Further HE staining studies showed that the intestinal villi of all OVA sensitized rats were damaged at certain levels and the mucosa in juvenile rats was more seriously ulcerated (Figure 2C and 2D). These data together showed that it was easier to establish food allergy to OVA in juveniles, and they more prone to get intestinal damage.

Table 1. Rate of successfully induced intestinal food allergy by OVA in the juvenile and adults rats.

Group	Rat in Total	Rat got Allergy	Rate (%)
Juvenile	13	9	69.23
Adult	12	4	33.33

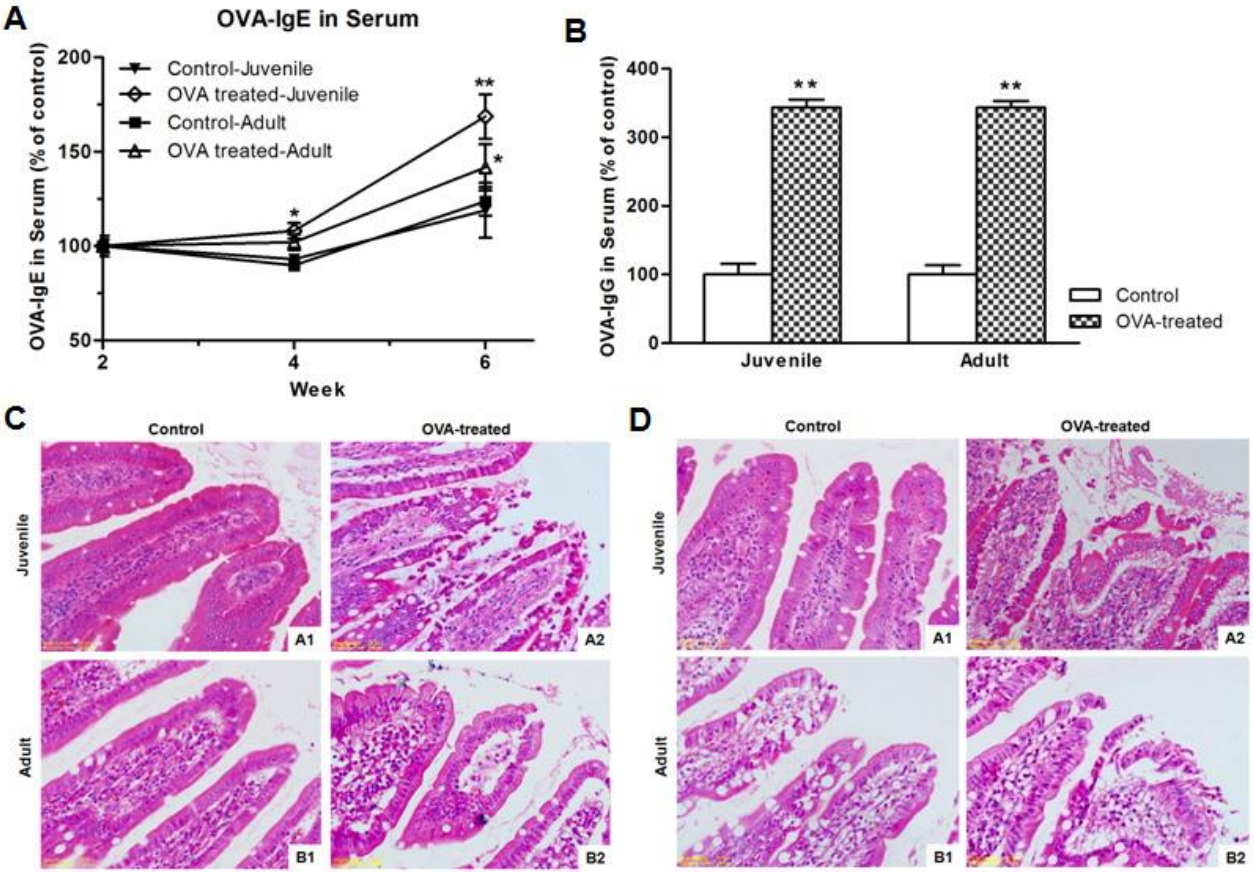

Figure 2. Juvenile rats were easier to get allergy under OVA induction compared with the adults.
A. Serum OVA-IgE levels rose in both juvenile and adult groups; B. Serum OVA-IgG level increased significantly, both in the juvenile and adult rats; C&D. HE staining of intestinal damage in the upper (C) or lower (D) jejunal villi of OVA sensitized rats. *, $p < 0.05$, **, $p < 0.01$ versus the control; respectively (Magnification: A1, A2, 400×; B1, B2, 400×).

OVA induces damage in duodenum, jejunum and ileum mucosal to affect intestinal permeability

Our results further suggested that in juvenile rats OVA induced damage exists not just in duodenum (Figure 3B), but also in the villi of jejunum (Figure 3C) and ileum (Figure 3D) in juvenile rats. Compared with the control group, damage and erosion could be found more frequently in the intestinal villi and mucosa of OVA-treated rats, with a certain amount leakage of mucus and fibrous protein (Figure 3B, 3C and 3D).

We then used the Lactulose/Mannitol assay to assess the intestinal permeability change. As shown in Figure 3E, the intestinal permeability was greatly increased, with the L/M value rising significantly from the 2nd week, indicating OVA induced damage in the duodenal, jejunal and ileal mucosa.

OVA causes damage to tight junctions of the intestinal villi epithelial cells

Compared with the control group, conspicuous damage can be seen in ileum villus in the OVA-treated group (Figure 4A, 4B and 4C). Moreover, we could see the gap expansion as well as tight junction damage in intestinal villus epithelial cells (marked with two black arrows) and swelling of mitochondria (Figure 4B and 4C). It's worth mentioning that the epithelial cells also swelled (Figure 4D) and we could see the increase and aggregation of eosinophils (Figure 4F), indicating that the basement membrane might be damaged causing osmosis, inflammation and aggregation of immune cells.

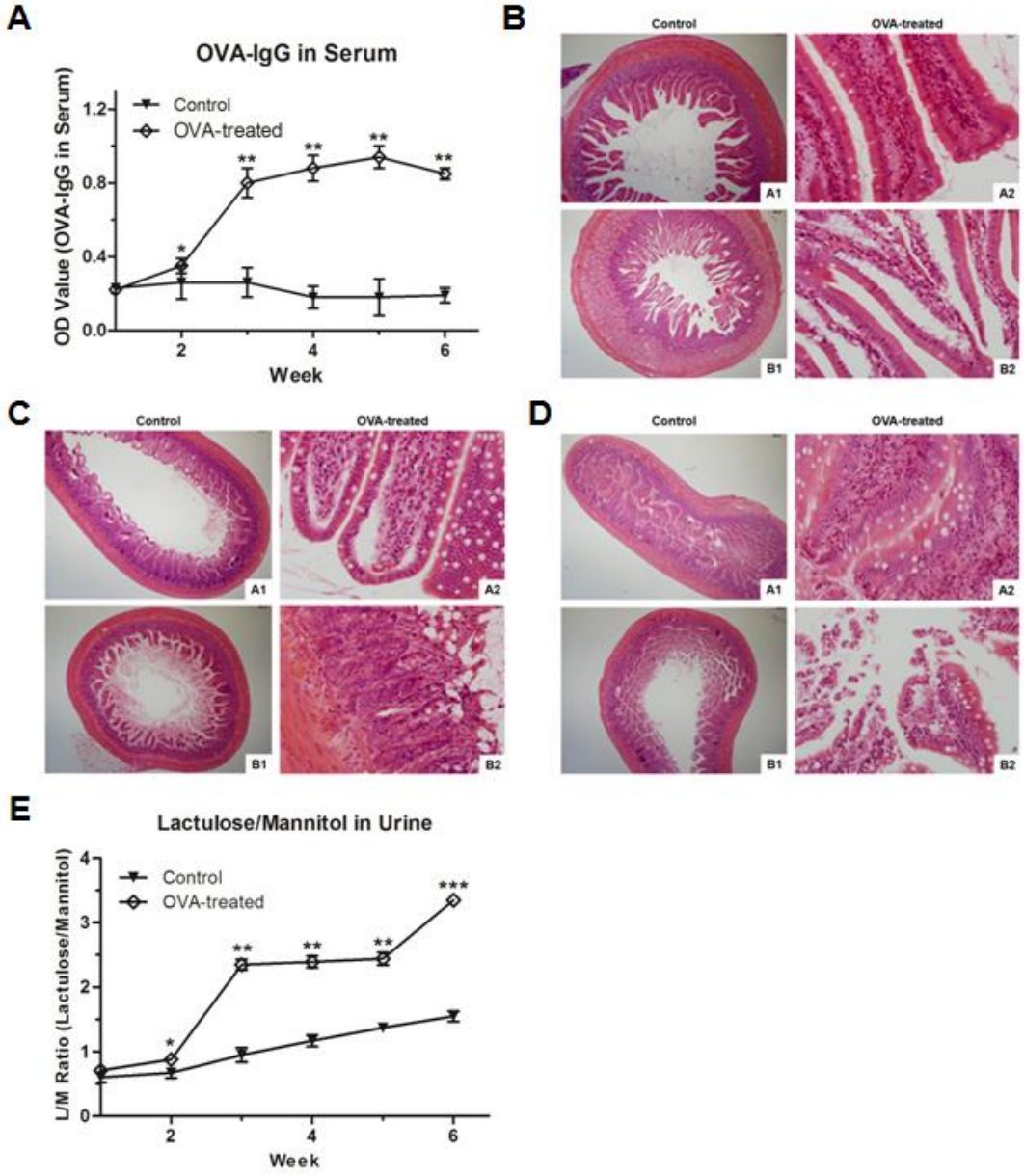

Figure 3. OVA induce damage in duodenum, jejunum and ileum mucosal affects intestinal permeability in Juvenile rats.

A. Serum OVA-IgE levels of OVA-sentitized juvenile rats; B. Serum OVA-IgG of OVA-sentitized juvenile rats; C, D&E. HE staining of intestinal damage in the duodenum (C), jejunum and ileum (E) in OVA sensitized juvenile rats; F. Intestinal permeability assay of Lactulose/Mannitol metabolism and absorption of OVA sensitized juvenile rats. *, $p < 0.05$, **, $p < 0.01$ versus the control; respectively (Magnification: A1, B1, 100×; A2, B2, 400×).

OVA regulates the expression of key proteins in tight junction.

Further studies with Real-time PCR indicated that OVA could regulate the expression of key proteins in tight junctions. As shown in Figure 5, tight junction regulating proteins such as ZO-1, and Claudin-2, Claudin-8, Claudin-15's expression were significantly down-regulated, while Claudin-9 appeared remarkably up-regulated (Figure 5A), indicating that OVA may be able to regulate the tight junction permeability.

Discussion

In the present study, by using the rat food allergy model we found that it is easier to induce sensitization to a food allergen (OVA) in juveniles

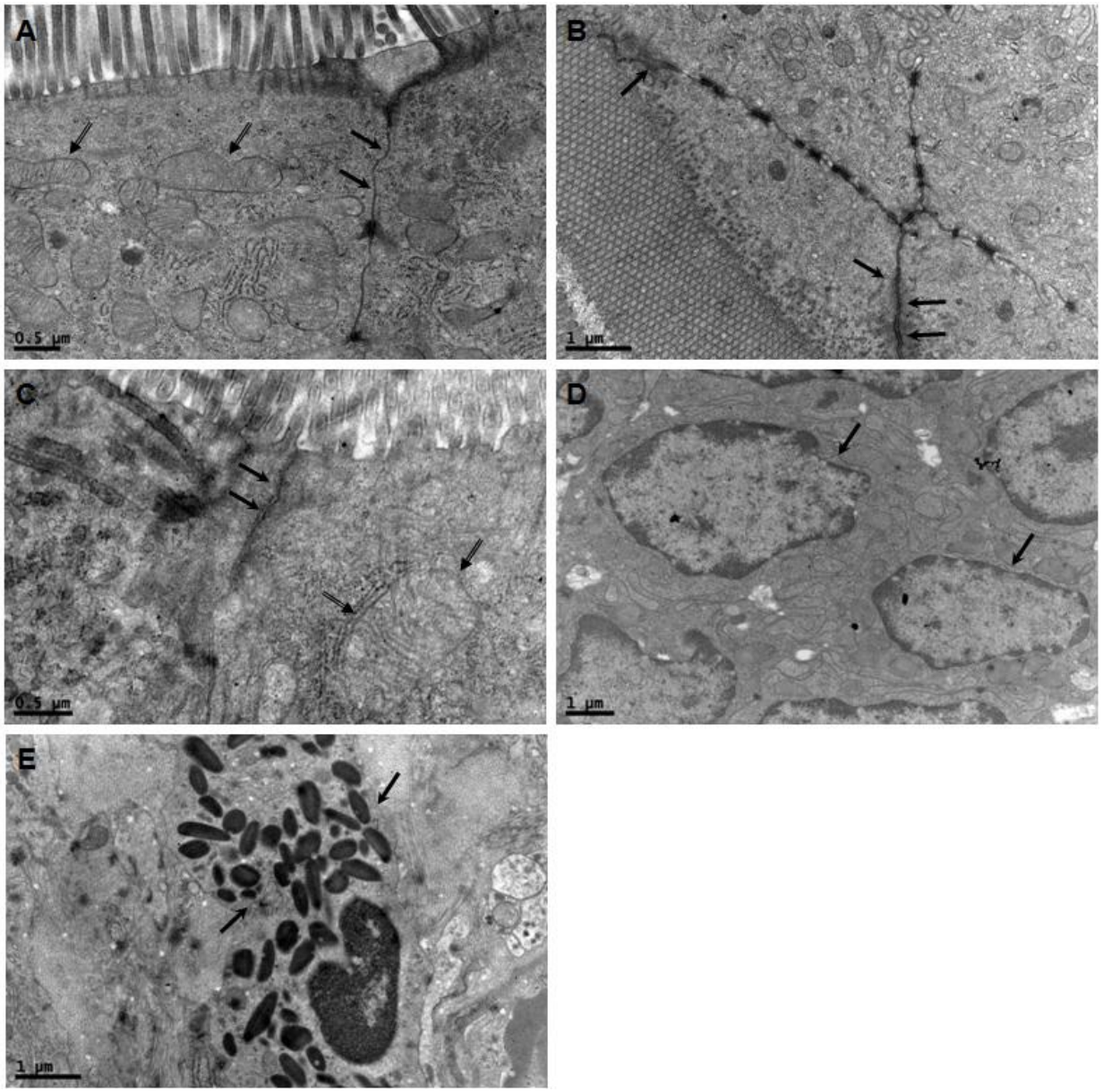

Figure 4. OVA induce intestinal damage in tight junction and inflammation in Juvenile rats. A&B&C. OVA causes damage to intestinal tight junctions (black arrows) and mitochondria (hollow arrows); D. Swollen intestinal epithelial cells following OVA sensitization in juvenile rats; E. Aggregation of eosinophils (indicative of inflammation) in intestinal mucosa of OVA-sensitized juvenile rats [(Magnification: A, 18500×; B, 13500×; C, 26500×; D, 9700×; E, 13500×); (A, control; B,C&D, OVA-treated)].

than in adults. Moreover, OVA can significantly influence the expression of key proteins of the tight junctions and in turn the intestinal permeability, to cause allergen absorption and induce food allergy.

Epidemiology studies have shown that the incidence of food allergy is higher in children than in adults.[20,21] We also found that juveniles were more susceptable to allergy, which is consistent with the epidemiology studies (Figure 2 and Table 1). Food allergy can be divided into IgE dependent (type I hypersensitivity), or non-IgE dependent, in which specific IgE plays a central role.[22] According to ELISA data, serum OVA-IgE increased weekly in our experiments. The OVA-IgE levels of the juvenile group had risen by the fourth week and reached a peak at the sixth week (Figure 2A). This is in agreement with a study from Taiwan which showed that the symptoms in skin, and the

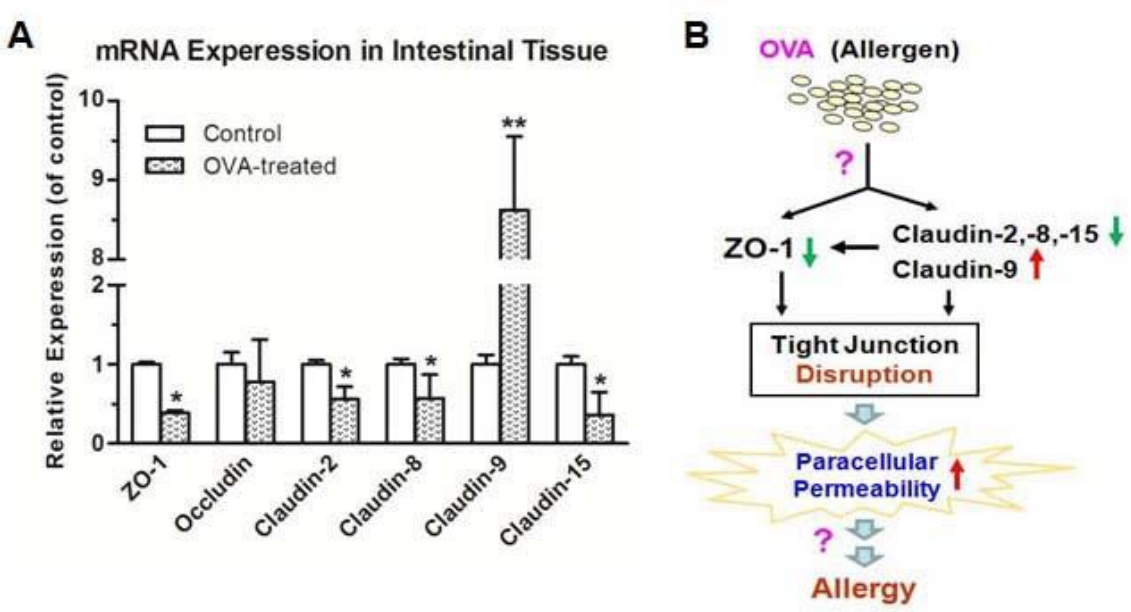

Figure 5. OVA increases intestinal permeability and induces food allergy through regulation of key proteins expression in tight junction.

A. mRNA expression of key proteins in controlling tight junctions under Realtime PCR assay; B. Scheme for possible mechanisms of action of OVA in inducing intestinal food allergy: regulates key proteins' expression in tight junctions to increase intestinal permeability and induce allergy.

respiratory and gastro-intestinal tracts are obviously different between adults and children (ie: more common in children than in adults) with allergy [20] and their sensitization rate was obviously higher than the adult group (Table 1). That might be related to the immaturity of the intestinal mucosa barrier and mucosal immunity in children (as well as in the the juvenile rats).

It is reported that food specific IgG levels may rise in food allergy patients.[23, 24] Our results also showed that OVA specific IgG rose at an early stage. Serum OVA-IgG increased both in juvenile and adults rats (Figure 2B). We hence verified that food allergy is not just type I IgE-mediated allergy, though the underlying mechanisms still need exploring.

The intestinal tract is the main digestion organ. Most patients have alimentary symptoms such as abdominal pain, distension, diarrhea, and other irritable bowel symptoms during food allergy.[2,9,13,25,26] The structure and function of the intestinal mucosa may change when allergy occurrs [9, 13, 26]. Intestinal villus cells of the duodenum or the lower ileum were swollen, damaged or even eroded after OVA sensitization in our study (Figure 2 and 3, Figure 4D). Moreover, inflammatory cell infiltration was observed under electron microscope, indicating inflammation in intestinal tract during allergy induction by OVA (Figure 4E), accompanied by swelling of mitochondrial (Figure 4C).

Intestinal mucosa is the first defense barrier to prevent the intake of harmful substances in cases of food allergy.[7,9] It is reported that the intestinal permeability may increase when the intestinal mucosal barrier function is damaged during allergy.[9,11,12] Our lactulose/mannitol assay showed that the intestinal permeability increased after OVA sensitization (Figure 3E), which was further confirmed by the electron microscopy which showed that the intercellular tight junctions of intestinal villus epithelial cells were damaged with expanded gaps (Figure 4B and 4C).

Tight junctions play important role in intestinal permeability maintenance, which is considered to be the barrier which determines selective cellular absorption.[8,27] Tight junctions consist of multiple proteins as a functional complex in which transmembrane proteins, the ZO protein family and cytoskeleton proteins are the most important.[28] Transmembrane proteins (including occludin, claudins, JAMs and CAR and etc.) are the structural proteins arranged in a linear structure, while the cytoplasmic adhesion proteins, ZO-1, ZO-2 and

ZO-3, are the foundations or supporting structure and are responsible for the connection of transmembrane proteins to the cytoskeleton.[28-30]

We further explored the underlying mechanisms that are responsible for intestinal barrier damage and tight junction permeability induced by OVA. Our Real-time PCR study showed that OVA could down-regulate the expression of ZO-1, Claudin-2, Claudin-8 and Claudin-15 (Figure 5A). ZO-1 plays an important role in the regulation of tight junction permeability.[27,31] Studies have shown that the inflammatory bowel disease, chylous diarrhea, is closely related to ZO-1 expression.[32] Celiac patients have been found to have reduced ZO-1 expression in the duodenal mucosa.[27,31] Claudins also play an important role in intestinal barrier function regulation.[32-35] Abnormal expression of Claudins (e.g., claudins-3, -5, and -8) can lead to Crohn's disease.[33] Transgenic Claudin-1 knockout mice may die rapidly because of a serious lack of transdermal water loss.[33] Our results suggested that OVA induces damage to the intestinal barrier and tight junction permeability was related to the expression and regulation of ZO-1, Claudins and other key tight junction proteins (Figure 5B). However, Claudin-9 appeared to be significantly up-regulated, which may be due to its differential expression style between the adults and juveniles[35] and there were no significant changes in Occluding expression (Figure 5A).

In summary, our study has demonstrated that juvenile rats were more easily sensitized by OVA induction; OVA could modulate the intestinal permeability through tight junction (key proteins expressions) regulation, which might cause intestinal mucosal leakage and induce intestinal food allergy (Figure 5B). Though the exact underlying molecular mechanism remains to be clarified, these findings to some extent add new knowledge to our understandings of intestinal food allergy and provide new insights for food allergy treatment or prevention.

Conflict of interest

The authors confirm that there are no conflicts of interest.

Acknowledgement

This study was supported by Grants from Natural Science Foundation of China (31101280, 81271950, 81300292, 81373176), Grants from International/HMT (Hong Kong, Macau, and Taiwan) Cooperation and Innovation Platform in Science and Technology of Guangdong Higher Education Institutions (2012gjhz0009), Grants of Science and Technology plan of Shenzhen (JCYJ20120613172559904, GJHZ20130408174112021), Key Laboratory Construction Program of Shenzhen (SW201110010), to Liu ZG; and by Grants from the China Postdoctoral Science Foundation (2012M521585, 2013T60791), to Chen T.

References

1. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the Diagnosis and Management of Food Allergy in the United States: Summary of the NIAID-Sponsored Expert Panel Report. J Allergy Clin Immunol 2010;126:1105-18.
2. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. Nutr Res 2011;31:61-75.
3. Macdougall CF, Cant AJ, Colver AF. How dangerous is food allergy in childhood? The incidence of severe and fatal allergic reactions across the UK and Ireland. Arch Dis Child 2002;86:236-9.
4. Sakaguchi M, Nakayama T, Inouye S. Food allergy to gelatin in children with systemic immediate-type reactions, including anaphylaxis, to vaccines. J Allergy Clin Immunol 1996;98:1058-61.
5. Sicherer SH, Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2008. J Allergy Clin Immunol 2009;123:319-27.
6. Farhadi A, Banan A, Fields J, Keshavarzian A. Intestinal barrier: an interface between health and disease. J Gastroenterol Hepatol 2003;18:479-97.
7. Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. J Allergy Clin Immunol 2009;124:3-20; quiz 1-2.
8. Arrieta MC, Bistritz L, Meddings JB. Alterations in intestinal permeability. Gut 2006;55:1512-20.
9. Secondulfo M, Iafusco D, Carratu R, deMagistris L, Sapone A, Generoso M, et al. Ultrastructural mucosal alterations and increased intestinal permeability in non-celiac, type I diabetic patients. Dig Liver Dis 2004;36:35-45.
10. Majamaa H, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. J Allergy Clin Immunol 1996;97:985-90.
11. Caffarelli C, Cavagni G, Menzies IS, Bertolini P, Atherton DJ. Elimination diet and intestinal permeability in atopic eczema: a preliminary study. Clin Exp Allergy 1993;23:28-31.
12. Laudat A, Arnaud P, Napoly A, Brion F. The intestinal permeability test applied to the diagnosis of food allergy in paediatrics. West Indian Med J 1994;43:87-8.

13. Perrier C, Corthesy B. Gut permeability and food allergies. Clin Exp Allergy 2011;41:20-8.
14. Iwata K, Yamada Y, Nakata A, Oghiso Y, Tani S, Doi K, et al. Co-operative effects of thoracic X-ray irradiation and N-nitrosobis(2-hydroxypropyl) amine administration on lung tumorigenesis in neonatal, juvenile and adult Wistar rats. Toxicol Appl Pharmacol 2013;267:266-75.
15. Knippels LM, Houben GF, Spanhaak S, Penninks AH. An oral sensitization model in Brown Norway rats to screen for potential allergenicity of food proteins. Methods 1999;19:78-82.
16. Pilegaard K, Madsen C. An oral Brown Norway rat model for food allergy: comparison of age, sex, dosing volume, and allergen preparation. Toxicology 2004;196:247-57.
17. Jarvinen KM, Konstantinou GN, Pilapil M, Arrieta MC, Noone S, Sampson HA, et al. Intestinal permeability in children with food allergy on specific elimination diets. Pediatr Allergy Immunol 2013;24:589-95.
18. Quadro L, Gamble MV, Vogel S, Lima AA, Piantedosi R, Moore SR, et al. Retinol and retinol-binding protein: gut integrity and circulating immunoglobulins. J Infect Dis 2000;182 Suppl 1:S97-S102.
19. Li F, Wang L, Jin XM, Yan CH, Jiang S, Shen XM. The immunologic effect of TGF-beta1 chitosan nanoparticle plasmids on ovalbumin-induced allergic BALB/c mice. Immunobiology 2009;214:87-99.
20. Hsin YC, Huang JL, Yeh KW. Clinical features of adult and pediatric anaphylaxis in Taiwan. Asian Pac J Allergy Immunol 2011;29:307-12.
21. Lyons AC, Forde EM. Food allergy in young adults: perceptions and psychological effects. J Health Psychol 2004;9:497-504.
22. Sicherer SH. Food allergy. Lancet 2002;360:701-10.
23. Dannaeus A, Inganas M. A follow-up study of children with food allergy. Clinical course in relation to serum IgE- and IgG-antibody levels to milk, egg and fish. Clin Allergy 1981;11:533-9.
24. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2010;125:S116-25.
25. Mansueto P, Montalto G, Pacor ML, Esposito-Pellitteri M, Ditta V, Lo Bianco C, et al. Food allergy in gastroenterologic diseases: Review of literature. World J Gastroenterol 2006;12:7744-52.
26. Piche T, Barbara G, Aubert P, Bruley des Varannes S, Dainese R, Nano JL, et al. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. Gut 2009;58:196-201.
27. Pizzuti D, Bortolami M, Mazzon E, Buda A, Guariso G, D'Odorico A, et al. Transcriptional downregulation of tight junction protein ZO-1 in active coeliac disease is reversed after a gluten-free diet. Dig Liver Dis 2004;36:337-41.
28. Gonzalez-Mariscal L, Betanzos A, Avila-Flores A. MAGUK proteins: structure and role in the tight junction. Semin Cell Dev Biol 2000;11:315-24.
29. Gonzalez-Mariscal L, Betanzos A, Nava P, Jaramillo BE. Tight junction proteins. Prog Biophys Mol Biol 2003;81:1-44.
30. Coyne CB, Bergelson JM. CAR: a virus receptor within the tight junction. Adv Drug Deliv Rev 2005;57(6):869-82.
31. Montalto M, Cuoco L, Ricci R, Maggiano N, Vecchio FM, Gasbarrini G. Immunohistochemical analysis of ZO-1 in the duodenal mucosa of patients with untreated and treated celiac disease. Digestion 2002;65:227-33.
32. Gassler N, Rohr C, Schneider A, Kartenbeck J, Bach A, Obermuller N, et al. Inflammatory bowel disease is associated with changes of enterocytic junctions. Am J Physiol Gastrointest Liver Physiol 2001;281:G216-28.
33. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 2002;156:1099-111.
34. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 1998;141:1539-50.
35. Abuazza G, Becker A, Williams SS, Chakravarty S, Truong HT, Lin F, et al. Claudins 6, 9, and 13 are developmentally expressed renal tight junction proteins. Am J Physiol Renal Physiol 2006;291:F1132-41.