Mechanisms of Food Allergy/Intolerance and Oral Desensitization Therapy

YOSHIKAZU OHTSUKA*

*Department of Pediatrics and Adolescent Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan

Most infants are immunologically active to develop a tolerance to oligoclonal antigens by producing IgA and introducing regulatory T cells in early infancy. Cytokines and their signalling molecules are important mediators in the intestine regulating both oral tolerance and mucosal inflammation. This system works efficiently in most individuals, but for an undefined reason, some people react to food and other proteins as though they were pathogens and induce chronic inflammation as food allergy/intolerance.

The adverse reaction caused by ingested foods is defined as food intolerance. The clinical features of food intolerance include vomiting, diarrhea, bloody stool, eczema, failure to thrive, and a protean range of other symptoms. Intolerance can be divided into two categories depending on whether they are immunologically mediated. The one immunologically mediated is known as “Food allergy”. Food allergy and mucosal inflammation are deeply related since tolerance cannot be introduced when there is an inflammation in the intestinal mucosa. Mast cells, eosinophils, mucosal lymphocytes, and epithelial cells are deeply involved and related each other to cause mucosal inflammation.

Meanwhile, it is known that tolerance can be introduced by keep eating antigenic foods, such as oral desensitization therapy. Feeding lower doses of antigen introduce “active suppression” by inducing TGF- β , IL-4 and IL-10 producing regulatory T (Th3) cells. At higher doses, “clonal anergy” occurs by deleting antigen-specific Th1 and Th2 cells by apoptosis, although TGF- β producing Th3 cells seen spared. These mechanisms are very important for infants to prevent/treat food allergy.

Key words: atopic dermatitis (AD), IgA, mucosal damage, active suppression, clonal anergy, neonatal transient enterocolitis (NTEC)

Introduction

The physiological needs of nutrient absorption require that the gut has a large surface area, lined by a single layer of columnar epithelium. This means that the immune system of the gut is constantly bombarded by foreign proteins (food), while at the same time the immune system is specifically designed to react to the foreign proteins of pathogens. To get around this problem, the gut immune system has acquired mechanisms to avoid excessive reactions to foods, yet at the same time

retain the ability to react to infectious agents. This system works efficiently in most individuals, but for an undefined reason, some people react to food and other proteins as though they were pathogens and induce chronic inflammation in the mucosa. Since food antigens are needed for nutrition, disease persists until the offending antigen is identified and eliminated from the diet.

The clinical features of inappropriate responses to food include vomiting, mal-absorption, diarrhea, bloody stool, eczema and a protean range of other symptoms. The adverse reactions caused by

Yoshikazu Ohtsuka

Department of Pediatrics and Adolescent Medicine, Juntendo University Graduate School of Medicine

2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

TEL: +81-3-3813-3111 FAX: +81-3-5800-0216 E-mail: yohtsuka@juntendo.ac.jp

45th Health Topics for Tokyoites: Atopic Dermatitis: The Latest Clinical Trials and Treatments

[Received Apr. 13, 2020] [Accepted Apr. 28, 2020]

Copyright © 2020 The Juntendo Medical Society. This is an open access article distributed under the terms of Creative Commons Attribution License (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original source is properly credited.
doi: 10.14789/jmj.2020.66.JMJ20-R06

ingested foods are defined as food intolerance. Intolerance can be divided into two categories depending on whether they are immunologically mediated. The one immunologically mediated is called as "Food allergy". This serves to exclude diseases where the pharmacological effects of foods or additives cause adverse reactions.

Non-immunologically mediated food intolerance includes the pharmacological effect of foods containing caffeine, alcohol, and toxins. It also includes primary and secondary metabolic disorders. Lack of lactase due to loss of microvilli during inflammation of intestine is included here. Idiosyncratic reactions, such as irritable bowel syndrome, may also be included but the mechanism of intolerance, if present, is obscure.

There are cases where immunological reactions overlap with non-immunological processes, for instance lactose intolerance in cow's milk allergy or inflammatory bowel disease. When we consider treatments, therefore, we have to be aware that both mechanisms may co-exist and exacerbate symptoms in a patient suffering from food intolerance.

Historical background of food allergy

Adverse reaction to foods, such as food allergy, was first reported more than 2000 years ago by Hippocrates. He had noted that gastric upset and urticaria could be induced by ingesting cow's milk. In modern times, Samuel Gee first described celiac disease in 1888, although it was a further 65 years before Dicke discovered the disease was induced by gluten.¹⁾ Early in the 20th century, as the number of babies raised on formula milk increased, an increased incidence of eczema²⁾ and mortality due to gastroenteritis and pneumonia was seen.^{3) 4)}

There is a need to prove that a certain food protein actually causes the adverse reactions, as opposed to another co-incident event. Thus, challenge tests were introduced in cow's milk allergy patients.⁵⁾ This procedure of eliminating and giving suspected food protein directly to patients in a blinded fashion allows accurate definition of the actual food antigen that introduces clinical symp-

toms. Similar criteria were also used in soya protein allergy.⁶⁾

The discovery of immunoglobulin E (IgE) provided the mechanistic basis for immediate type hypersensitivity reactions to antigens, including foods.⁷⁾ The subsequent discovery of the role of T cells in delayed type hypersensitivity gave another pathway by which the immune system could react to food antigens and causes disease, exemplified by the T cell responses to wheat which causes celiac disease.

Immunological mechanisms of food allergy

The immune system of the gut is continually exposed to benign foreign proteins, including foods, and yet, at the same time, the immune system must be able to react to pathogenic foreign proteins. To address this dichotomy, the gut immune system has acquired mechanisms to avoid excessive reactions to foods, known as "tolerance". TGF- β is an important cytokine which mediates active suppression against orally administered antigen. It is released by regulatory T cells and macrophages. In studies of multiple sclerosis patients, feeding myelin basic protein (MBP) slightly ameliorated disease activity.⁸⁾ Short term cultures of blood lymphocytes from these patients showed an increased frequency of MBP specific T cells that secreted TGF- β 1.⁹⁾

The immunological mechanisms of food tolerance/intolerance are well studied in ovalbumin (OVA)-specific T-cell receptor transgenic mice. Oral administration of OVA introduces tolerance into these mice. Feeding lower doses of OVA causes "active suppression" by inducing TGF- β , IL-4 and IL-10 producing regulatory T cells. At higher doses, "clonal anergy" occurs by deleting Th1 and Th2 cells by apoptosis, although TGF- β producing Th3 cells seen spared.¹⁰⁾ IFN- γ producing cells are also seen in Peyer's patches (PP) however this is transient. After feeding a high dose of OVA, systemic administrations of anti-IL-12 antibody inhibits IFN- γ production and enhances production of TGF- β in PP. IL-4 is not required for this TGF- β production, however it is able to enhance TGF- β

production.¹¹⁾ In addition, administration of OVA with anti-IL-12 antibody induced Fas-mediated apoptosis of antigen-specific T cells in local and systemic lymphoid tissue.¹²⁾ These studies demonstrate that orally administered food antigens can evoke TGF- β producing T cells, mostly CD4⁺CD25⁺ regulatory T cells, that can actively suppresses immunological reactions, and inhibit antigen-specific Th1 cell responses in the PP. High doses of antigen also induces tolerance by clonal deletion of antigen-specific T cells via Fas-mediated apoptosis. These immunological reactions are only introduced daily, but not intermittent, administration of food antigens. In infants, it is considered that “active suppression” can be introduced by breast feeding since very small amount of food antigen with TGF- β is contained in the maternal milk, where “clonal anergy” can be introduced through weaning foods.¹³⁾

The mucosal barrier in infants

Ingested foods are normally digested into peptides and amino acids by digestive enzymes before being absorbed by gut epithelial cells. Amino acids do not induce immunological reactions because they are too small to be recognized by antigen presenting cells (APC). However, some proteins are poorly digested in the intestinal lumen, and indeed intact macromolecules can be detected as allergic reactions after feeding protein antigens. Immunological and non-immunological mechanisms control the penetration of these undigested proteins. For instance, mucus provides the intestinal epithelium with a mechanical (covering) and a chemical (washing) protection as well as an immunological protection involving secretory IgA (s-IgA). s-IgA is the predominant Ig class found in human maternal milk and maternal milk IgA compensates for the IgA deficiency of the newborn infant. s-IgA binds to antigens in the mucus layer and also regulates immune response to dietary antigens. Despite this however, macromolecules do cross the gut epithelium and can be recognized by the immune system.

In infants, non-specific defenses in the gut may be compromised.¹⁴⁾ First, both intracellular and paracellular transport of macromolecules through epithelium is increased.¹⁵⁾ Second, insufficient

secretion of digestive enzymes causes reduced digestion and increases the amounts of undigested proteins in the intestinal lumen. Third, the production and secretion of IgA is low. Fourth, infants often suffer gastrointestinal infections, which damage the epithelium and allow permeation of proteins into the lamina propria. Along these same lines, frequent infections activate mucosal immune cells and induce further reactions, such as induction of Th1 and Th2 type cells and enhances the expression of MHC class-II molecules on intestinal epithelial cells. Hence, food intolerance can be a feature of the post-enteritis syndrome.

Transcutaneous sensitization

It has been well established in animal studies that transcutaneous sensitization can occur to food allergens and give rise to IgE-mediated hypersensitivity reactions. Elevation of allergic reactions have been confirmed in many studies by applying antigenic foods on the surface of abraded/stripped skin but not through normal skin.¹⁶⁾ In clinical trials, the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort study demonstrated that infants with peanut allergy by the age of 5 years were more likely to have had severe AD in the first 6 months of life, and to have been treated with peanut oil for dry skin. It is considered that increased skin barrier permeability facilitates allergen penetration and subsequent sensitization leading to severe AD as well as FA.¹⁷⁾ Further, several studies have suggested that the regular application of emollients from birth reduces the risk of AD as well as FA development.¹⁸⁾

Mechanisms of food allergy (Figure-1)

1. Type I allergy - Mast cells

There is an increase in the number of IgE-bearing mast cells in the intestinal mucosa and submucosa of food allergic patients^{19) 20)} and in mouse models.²¹⁾ The specific IgE bound to high-affinity IgE receptors on the surface of mast cells becomes cross-linked upon exposure to allergens, and activates mast cells. These mast cells release mediators such as histamine, leukotriene (LT)B₄, LTC₄, tumor necrosis factor (TNF)- α , IL-4, and other cytokines. Significant elevation of serum

Figure-1 Mechanisms of food allergy in the intestine

Antigen and its specific IgE bound to high-affinity IgE receptors activate mast cells. Histamine, eotaxin, leukotriene (LT) B₄, LTC₄, tumor necrosis factor (TNF)-α, IL-4, and other chemical mediators are released and induce a local increase in vascular permeability and mucus production and elicit secretory diarrhea.

Eosinophils migrate into the gut, responding to eosinophil chemotactic factors, such as eotaxin, platelet activating factor, histamine, LTB₄ etc. Eosinophils release major basic protein, eosinophil cationic protein, and eosinophil peroxidase, which are highly cytotoxic pro-inflammatory mediators and may disrupt mucosal surface and contribute to the later phase of reactions.

TNF-α released from mast cells enhances the expression of adhesion molecules on endothelial cells and leads lymphocyte infiltration into the mucosa. This may promote further cell-mediated reactions against food antigen in the intestine.

As a result of an uncontrolled activation of Th1 cells, increased synthesis of Th1 cytokines stimulates macrophages, and stromal cells. They may contribute to mucosal damage, such as villous atrophy and crypt hyperplasia, by releasing keratinocyte growth factor and matrix metalloproteinases. Meanwhile activated Th2 cells promote antigen-specific IgE and induce further allergic reactions to the antigen.

Abbreviations: eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), high-affinity IgE receptors (FcεRIα), intercellular adhesion molecule-1 (ICAM-1), keratinocyte growth factor (KGF), leukotriene (LT), major basic protein (MBP), matrix metalloproteinases (MMP), mucosal addressing cell adhesion molecule-1 (MAdCAM-1), platelet-activating factor (PAF), tumor necrosis factor (TNF)

histamine,^{22) 23)} LTB₄, LTC₄,²¹⁾ TNF-α concentrations are regularly observed after antigen challenge.^{24) 25)} It is likely that these mediators induce a local increase in vascular permeability,²⁶⁾ lymph duct dilatation and increased mucus production. They also cause watery diarrhea, resulting in blood hypocirculation and anaphylactic shock.

2. Type I allergy - Eosinophils

Eosinophils are also important in Type I allergy. Eosinophil migration into the mucosa is often seen

in patient with food allergic colitis, and typically presents in children with an atopic family history, before the age of two years. Eosinophils migrate into the gut, responding to eosinophil chemotactic factors, such as eotaxin, platelet activating factor, histamine, and LTB₄, mostly derived from mast cells. The eosinophils also participate in IgE-mediated inflammation directly since they have low-affinity receptors for IgE.²⁷⁾ Eosinophils liberate an abundance of highly cytotoxic and pro-inflammatory mediators, such as major basic

protein, eosinophil cationic protein, and eosinophil peroxidase.^{23) 28)} These mediators may disrupt mucosal surface and contribute to the later phase of reactions.

3. Type IV allergy – Cell mediated reactions

Mucosal damage, such as villous atrophy and crypt hyperplasia is seen in biopsy specimens taken from patients with food protein-induced enteropathy. Similar pathological findings are also seen in patients suffering from persistent diarrhea, dehydration, malnutrition, and failure to thrive.^{29) 30)} Since the mucosal infiltrate consists mainly of intraepithelial and lamina propria lymphocytes, but not of mast cells and eosinophils, cell-mediated responses are considered to be involved in mucosal damage in these patients. The relationship between cell-mediated reactions and their effects on intestinal morphology have been investigated in detail during graft versus host reactions,^{31) 32)} which shows a striking morphological similarity to the mucosal damage seen in food protein-induced enteropathy. Direct evidence for an important T-cell mediated process in the pathogenesis of the flat mucosal lesion has been shown in other *ex vivo* model systems.³³⁾ The same group also reported that as a result of an uncontrolled activation of T-cells, increased synthesis of matrix metalloproteinases (MMP)³⁴⁾ and keratinocyte growth factor (KGF)³⁵⁾ contribute to the gut damage. It is considered that MMPs and KGF may be directly involved in mucosal damage since they cause the degradation of mucosa and the crypt hyperplasia by stimulating epithelial cell proliferation.

4. Mixed reactions

Could IgE-mediated reactions be involved in prolonged symptoms by chronic antigen exposure? This may be highly relevant to the clinical situation, because even Type I allergic patients often suffer from frequent and prolonged allergic responses as a result of repeated antigen exposure. Hence, we have shown that not only mast cells but also eosinophils and lymphocytes infiltrate into the intestinal mucosa upon chronic antigen exposure in antigen-specific IgE-mediated mouse model.³⁶⁾ Cell adhesion molecule expression on endothelial cells is up-regulated as a result of continuous stimulation with chemical mediators derived from mast cells,

especially TNF- α .^{37) 38)} This enhancement leads to increased infiltration of lymphocytes into the mucosa. These lymphocytes that have migrated, especially Th1 cells, produce IFN- γ and up-regulate antigen presentation by promoting MHC-class II molecule expression on APC in the intestine.³⁹⁾ This further promotes cell-mediated reactions against food antigen.

Epithelial cells

The primary function of the intestinal epithelial cells is to absorb nutrients while providing a physical and immunological barrier to the external world. Nevertheless epithelial cells are in direct contact with the intestinal milieu and they produce several chemokines influenced by the luminal contents that include bacteria, bacterial products, and chemical stimuli.^{40)–42)} By using the transgenic technique, we have described that neutrophil and lymphocyte infiltration into the mucosa due to a single chemokine, macrophage inflammatory protein (MIP)-2, expressed by the epithelial cells.⁴³⁾ Furthermore, the finding of the greatly increased neutrophil recruitment in MIP-2 transgenic mice by stimulation with dextran sulfate sodium (DSS), which may produce more MIP-2 in the small intestine, than do wild-type mice (Figure-2) suggest that chemokine production from the epithelium is an important mechanism of DSS induced mucosal inflammation. DSS may therefore stimulate intestinal epithelial cells and enhance the chemokine secretion to further increase neutrophil invasion.⁴⁴⁾

Rectal bleeding in infancy

Eosinophilia is relatively common in newborns and is caused by an increased rate of hemocytopoiesis, especially in pre-term infants, although most cases are symptomless. However, a closer examination of infants with bloody stool can often reveal abnormally elevated eosinophil counts both in the blood and gut mucosa. Since we have experienced several cases of neonates who showed rectal bleeding with massive eosinophilia on the first day of life before enteral feeding, and since these changes are antigen non-specific and are only observed transiently in neonates, we have termed

Figure-2 Epithelial cells in the inflammation

A: Neutrophil recruitment in the small intestine from wild-type and macrophage inflammatory protein (MIP)-2 transgenic mice with or without 2.5% dextran sulfate sodium (DSS) treatment. Neutrophil recruitment was greater in MIP-2 transgenic mice than in wild-type mice after DSS treatment. Neutrophils were stained by myeloperoxidase (MPO) using the Hanker Yates reaction. Magnification $\times 100$.

B: Neutrophil recruitment was much greater in MIP-2 transgenic mice than in wild-type mice both in the small intestine and colon after 2.5% DSS treatment. Neutrophil recruitment was demonstrated by total MPO activity. MPO was extracted from the whole small intestine and colon with 0.5% hexadecyltrimethylammonium bromide. Open columns show total MPO activity per unit weight of tissue derived from wild-type mice, and closed columns show those of MIP-2 transgenic mice. Data are presented as the mean $\pm$ SD. * $p < 0.01$.

this disease “neonatal transient eosinophilic colitis (NTEC),” which represents a new category of eosinophilic gastroenteritis.⁴⁵⁾ In these infants, we performed rectosigmoidoscopy to confirm the cause of rectal bleeding and found nodular lymphoid hyperplasia with a pale mucosal surface and massive bleeding around the recto-sigmoid colon with diffuse eosinophil infiltration in the lamina propria, which is similar to the signs of food protein-induced proctocolitis (FPIP).

To investigate the outcome and pathogenesis of rectal bleeding in infancy, especially when concomitant with FPIP and NTEC, 22 neonates with rectal bleeding with FPIP and NTEC from January 2008 to June 2012 were enrolled and their clinical course and mechanisms of inflammation were examined by microarray analysis.⁴⁶⁾ FPIP in infancy and NTEC are similar diseases and that IL-6, C-C motif ligand 11 (CCL11 known as eotaxin-1), and C-X-C motif chemokine ligand 13 (CXCL13) may play a major role in the pathogenesis of rectal bleeding (Figure-

3). Although the involvement of allergic reaction is possible,⁴⁷⁾ cow’s milk allergy was not a common outcome after 1 year of follow-up.

1. CXCL13

As explained before, synthesizing IgA molecules targeting multiple antigens is an important task for the mucosal immune systems of neonates. IgA is mainly synthesized in the lymphoid follicles of the intestine, and the enhanced CXCL13 and CXCR5 (receptor for CXCL13) expression observed in neonates is convenient for IgA synthesis because CXCL13 is a chemokine related to lymphoid follicle formation.⁴⁸⁾ The immunohistochemical analysis revealed that infiltration by IgA-bearing cells was pronounced in FPIP (Figure-4) and NTEC, possibly suggesting that the enhanced immunological reaction against food antigen, including producing IgA, is one of the major elements of the pathogenesis of rectal bleeding in infants (RBI). Because lymphoid hyperplasia is one of the characteristic

Figure-3 Rectal bleeding in infancy

Rectosigmoidoscopy revealed nodular lymphoid hyperplasia with a pale mucosal surface and massive bleeding around the recto-sigmoid colon with diffuse eosinophil infiltration in the lamina propria. Microarray analysis revealed the enhanced expression of C-C motif ligand 11 (CCL11 known as eotaxin-1) and C-X-C motif chemokine ligand 13 (CXCL13) in these mucosae. Expression of CCL11 was more dominant in neonatal transient eosinophilic colitis (NTEC) where the expression of CXCL13 was more dominant in food protein-induced proctocolitis (FPIP).

Figure-4 Histological image of a representative sample from infants with food-protein induced proctocolitis (FPIP)

The immunohistochemical analysis was performed using anti-immunoglobulin (Ig) A antibody. IgA-positive cells were present in the lamina propria. Magnification $\times 400$.

findings in RBI⁴⁹⁾ and because IgA synthesis is predominant after birth, these changes can be favorable immunological findings in infants, including those with RBI.

Symptoms

Although food antigens are encountered through the gut, the symptoms of food intolerance are often seen in skin and mucosa of eyes, nose, respiratory system, causing eczema, erythema, itchiness,

wheezing, and respiratory distress. The common digestive symptoms of food intolerance are diarrhea, bloody stool, vomiting, and nausea. Edema and ulceration of oral cavity as well as constipation are seen in some cases.^{50) 51)} Digestive symptoms are often severe in infants and young children. Respiratory and dermal symptoms are also seen but are more frequent in older children.

These symptoms, observed within minutes to an hour after antigen ingestion, are due to antigen specific IgE-mediated reactions. These symptoms often start from the location where antigen is encountered, and sometimes develop to severe systemic reactions. In the intestine, biopsy specimens reveal mucosal edema and mast cell and eosinophil infiltration.⁵²⁾ IgE-mediated reaction in intestine cause secretory diarrhea and bloody diarrhea in patients with food allergic colitis. Diagnosis can be ascertained by the patient's past history, skin tests, and the presence of food-specific serum IgE antibodies, and oral food challenge test. Oral food challenge is necessary because the accuracy of antigen specific-IgE level and skin tests is generally unsatisfactory. However, challenge tests can be dangerous and induce shock, therefore should be performed under intensive surveillance.

On the other hand, cell-mediated reactions are involved in the prolonged symptoms, such as persistent diarrhea, dehydration, and failure to thrive. These symptoms are caused by mucosal

damage, evident as villous atrophy and crypt hyperplasia.^{26) 27)} Patients who suffered from cell-mediated reactions therefore show persistent diarrhea with dehydration, malnutrition, and failure to thrive after antigen exposure. Prolonged exposure to antigen may eventually result in anemia, hypo-proteinemia and hypo-circulation.

Treatment/oral desensitization therapy

Identification and elimination of the proteins that cause the adverse reaction improves the condition of these patients. In immunological food intolerance, hydrolyzed cow's milk, soya, and gluten, are available. In Type I allergy, nutritional status and the level of hydration have to be considered. Elimination of the offending food from the diet should prevent further malnutrition and dehydration. Hypolactasia may be seen in prolonged diarrhea with brush border damage, so that substituting lactose with another sugar such as glucose is necessary. However continuing elimination diet is not the fundamental treatment. Eliminating antigenic food for a few weeks can retain proper digestive function and by administering antigenic foods from small amount may be able to induce tolerance against antigenic food proteins, which is known as "oral desensitization therapy"⁵³⁾. "Oral desensitization therapy" is a useful tool to induce tolerance to food antigens in food allergy patients, thus there are few things that people have to be aware about. It must be conducted under the careful observation by doctors since there are always some possibilities to induce critical events, such as anaphylactic shock caused by inappropriate amount and timing of administration of food ingestion. Host condition is also important that infections can activate immunological reactions and make easier to react with food antigens. Meanwhile, gastroenterocolitis may increase antigen invasion into the host by disrupting tight junctions among epithelial cells. These things have to be explained precisely and informed consent should be obtained before the therapy.

Early induction is better or not

In order to prevent infants from food allergy, is it better to start weaning food earlier? There have

been several trials designing to confirm this important clinical question. Randomized trial of peanut consumption in infants at risk for peanut allergy was designed and published in 2015, as called LEAP study.⁵⁴⁾ 640 infants with severe eczema, egg allergy, or both were enrolled for this study, and consume or avoid peanuts from 4 to 60 months of age every day. As a result, prevalence of peanut allergy at 60 months of age was 13.7% in the avoidance group and 1.9% in the consumption group ($p < 0.001$). Then, EAT study was designed to confirm the early exposure to several allergenic foods on the subsequent development of food allergy.⁵⁵⁾ Among 1,303 exclusively breast-fed infants, cow's milk, peanut, hard-boiled egg, sesame, cod, and wheat were administered from 3 months of age and found that prevalence of peanut and egg allergy at 3 years of age was 2.5% and 5.5% in the avoidance group and 0% and 1.4% in the consumption group ($p = 0.003$ and $p = 0.009$, respectively). However, there was no significant difference was observed in the prevalence of allergy in other antigenic foods. Early consumption is also examined among Japanese population, as called PETIT study.⁵⁶⁾ Among 147 infants with eczema aged 4-5 months were enrolled and aggressively treated their eczema at entry and maintained control. They received 50 mg of heated egg powder per day from 6 to 9 months of age and 250 mg per day thereafter until 12 months of age. As a result, prevalence of egg allergy at 12 months of age was 38% in the avoidance group and 9% in the consumption group ($p = 0.0012$). It seems that the early introduction of weaning/antigenic foods significantly reduce the frequency of food allergy development among allergic children and can modulate immune responses to antigens. Larger studies and/or more rigorous design are required to determine whether early introduction is truly beneficial for preventing food allergy.

Conclusion

Studies of cytokine and their signaling network leads us deeper understand of the mechanisms of food allergy/intolerance and mucosal inflammation. Hopefully, these studies will give us an idea of how we can establish immunological tolerance in patients with food allergy, thereby bypassing the

need for eliminating diets.

Acknowledgement

Author is grateful to Professor Toshiaki Shimizu, Department of Pediatrics and Adolescence Medicine, and Emeritus Professor Yuichiro Yamashiro, Probiotics Research, Juntendo University Graduate School of Medicine, Tokyo, Japan for their tremendous support to my work.

Conflict of interest

The author has no conflicts of interest directly relevant to the content of this article.

References

- 1) Dicke WK, Weijers HA, Vav De Kamer JH: The presence in wheat of a factor having a deleterious effect in cases of coeliac disease. *Acta Paediatr*, 1953; 42: 34-42.
- 2) Grulee CG, Sanford NH: The influence of breast and artificial feeding on infantile eczema. *J Pediatr*, 1936; 9: 223-225.
- 3) Grulee CG, Sanford NH, Heron PH: Breast and artificial feeding. *JAMA*, 1934; 103: 735-739.
- 4) Woodbury RM: The relation between artificial feeding and infant mortality. *Am J Epidemiol*, 1922; 2: 668-687.
- 5) Goldman AS, Anderson DW Jr, Sellers WA, Saperstein S, Kniker WT, Halpern SR: Milk Allergy. I. Oral Challenge with Milk and Isolated Milk Proteins in Allergic Children. *Pediatrics*, 1963; 32: 425-443.
- 6) Ament ME, Rubin CE: Soy protein--another cause of the flat intestinal lesion. *Gastroenterology*, 1972; 62: 227-234.
- 7) Ishizaka K, Ishizaka T, Hornbrook MM: Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *J Immunol*, 1966; 97: 75-85.
- 8) Weiner HL, Mackin GA, Matsui M, *et al*: Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. *Science*, 1993; 259: 1321-1324.
- 9) Fukaura H, Kent SC, Pietrusewicz MJ, Khoury SJ, Weiner HL, Hafler DA: Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. *J Clin Invest*, 1996; 98: 70-77.
- 10) Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL: Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature*, 1995; 376: 177-180.
- 11) Marth T, Strober W, Seder RA, Kelsall BL: Regulation of transforming growth factor-beta production by interleukin-12. *Eur J Immunol*, 1997; 27: 1213-1220.
- 12) Marth T, Zeitz M, Ludviksson BR, Strober W, Kelsall BL: Extinction of IL-12 signaling promotes Fas-mediated apoptosis of antigen-specific T cells. *J Immunol*, 1999; 162: 7233-7240.
- 13) Verhasselt V: Neonatal tolerance under breastfeeding influence. *Curr Opin Immunol*, 2010; 22: 623-630.
- 14) Ohtsuka Y, Sanderson IR, MacDonald TT: Chapter 23, Food intolerance. In: Preedy V, Grimble G, Watson R, eds. *Nutrition in the Infant: Problems and Practical Procedures*. Greenwich Medical Media Ltd. London, 2001; 247-256.
- 15) Sanderson IR, Walker WA: Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology*, 1993; 104: 622-639.
- 16) Saloga J, Renz H, Larsen GL, Gelfand EW: Increased airways responsiveness in mice depends on local challenge with antigen. *Am J Respir Crit Care Med*, 1994; 149: 65-70.
- 17) Lack G, Fox D, Northstone K, Golding J; Avon Longitudinal Study of Parents and Children Study Team: Factors associated with the development of peanut allergy in childhood. *N Engl J Med*, 2003; 348: 977-985.
- 18) Simpson EL, Chalmers JR, Hanifin JM, *et al*: Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol*, 2014; 134: 818-823.
- 19) Rognum TO, Brandtzaeg P: IgE-positive cells in human intestinal mucosa are mainly mast cells. *Int Arch Allergy Appl Immunol*, 1989; 89: 256-260.
- 20) Caffarelli C, Romanini E, Caruana P, Street ME, de' Angelis G: Clinical food hypersensitivity: the relevance of duodenal immunoglobulin E-positive cells. *Pediatr Res*, 1998; 44: 485-490.
- 21) Ohtsuka Y, Naito K, Yamashiro Y, Yabuta K, Okumura K, Ra C: Induction of anaphylaxis in mouse intestine by orally administered antigen and its prevention with soluble high affinity receptor for IgE. *Pediatr Res*, 1999; 45: 300-305.
- 22) Nolte H, Schiøtz PO, Kruse A, Stahl Skov P: Comparison of intestinal mast cell and basophil histamine release in children with food allergic reactions. *Allergy*, 1989; 44: 554-565.
- 23) Bengtsson U, Knutson TW, Knutson L, Dannaeus A, Hållgren R, Ahlstedt S: Eosinophil cationic protein and histamine after intestinal challenge in patients with cow's milk intolerance. *J Allergy Clin Immunol*, 1997; 100: 216-221.
- 24) Heyman M, Darmon N, Dupont C, *et al*: Mononuclear cells from infants allergic to cow's milk secrete tumor necrosis factor alpha, altering intestinal function. *Gastroenterology*, 1994; 106: 1514-1523.
- 25) Majamaa H, Miettinen A, Laine S, Isolauri E: Intestinal inflammation in children with atopic eczema: faecal eosinophil cationic protein and tumour necrosis factor-alpha as non-invasive indicators of food allergy. *Clin Exp Allergy*, 1996; 26: 181-187.
- 26) Jalonen T: Identical intestinal permeability changes in children with different clinical manifestations of cow's milk allergy. *J Allergy Clin Immunol*, 1991; 88: 737-742.
- 27) Capron M, Capron A, Dessaint JP, Torpier G, Johansson SG, Prin L: Fc receptors for IgE on human and rat eosinophils. *J Immunol*, 1981; 126: 2087-2092.
- 28) Venge P, Håkansson L, Peterson CG: Eosinophil activation in allergic disease. *Int Arch Allergy Appl Immunol*, 1987; 82: 333-337.
- 29) Phillips AD, Rice SJ, France NE, Walker-Smith JA: Small intestinal intraepithelial lymphocyte levels in cow's milk protein intolerance. *Gut*, 1979; 20: 509-512.

- 30) Walker-Smith JA, Ford RP, Phillips AD: The spectrum of gastrointestinal allergies to food. *Ann Allergy*, 1984; 53 (6 Pt 2): 629-636.
- 31) MacDonald TT, Ferguson A: Hypersensitivity reactions in the small intestine. 2. Effects of allograft rejection on mucosal architecture and lymphoid cell infiltrate. *Gut*, 1976; 17: 81-91.
- 32) MacDonald TT, Ferguson A: Hypersensitivity reactions in the small intestine. III. The effects of allograft rejection and of graft-versus-host disease on epithelial cell kinetics. *Cell Tissue Kinet*, 1977; 10: 301-312.
- 33) MacDonald TT, Spencer J: Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *J Exp Med*, 1988; 167: 1341-1349.
- 34) Pender SL, Tickle SP, Docherty AJ, Howie D, Wathen NC, MacDonald TT: A major role for matrix metalloproteinases in T cell injury in the gut. *J Immunol*, 1997; 158: 1582-1590.
- 35) Bajaj-Elliott M, Breese E, Poulsom R, Fairclough PD, MacDonald TT: Keratinocyte growth factor in inflammatory bowel disease. Increased mRNA transcripts in ulcerative colitis compared with Crohn's disease in biopsies and isolated mucosal myofibroblasts. *Am J Pathol*, 1997; 151: 1469-1476.
- 36) Ohtsuka Y, Suzuki R, Nagata S, *et al*: Chronic oral antigen exposure induces lymphocyte migration in anaphylactic mouse intestine. *Pediatr Res*, 1998; 44: 791-797.
- 37) Wershil BK, Furuta GT, Wang ZS, Galli SJ: Mast cell-dependent neutrophil and mononuclear cell recruitment in immunoglobulin E-induced gastric reactions in mice. *Gastroenterology*, 1996; 110: 1482-1490.
- 38) Furuta GT, Schmidt-Choudhury A, Wang MY, *et al*: Mast cell-dependent tumor necrosis factor alpha production participates in allergic gastric inflammation in mice. *Gastroenterology*, 1997; 113: 1560-1569.
- 39) Steiniger B, Falk P, Lohmüller M, van der Meide PH: Class II MHC antigens in the rat digestive system. Normal distribution and induced expression after interferon-gamma treatment in vivo. *Immunology*, 1989; 68: 507-513.
- 40) McCormick BA, Parkos CA, Colgan SP, Carnes DK, Madara JL: Apical secretion of a pathogen-elicited epithelial chemoattractant activity in response to surface colonization of intestinal epithelia by *Salmonella typhimurium*. *J Immunol*, 1998; 160: 455-466.
- 41) Ohno Y, Lee J, Fusunyan RD, MacDermott RP, Sanderson IR: Macrophage inflammatory protein-2: chromosomal regulation in rat small intestinal epithelial cells. *Proc Natl Acad Sci U S A*, 1997; 94: 10279-10284.
- 42) Fusunyan RD, Quinn JJ, Ohno Y, MacDermott RP, Sanderson IR: Butyrate enhances interleukin (IL)-8 secretion by intestinal epithelial cells in response to IL-1beta and lipopolysaccharide. *Pediatr Res*, 1998; 43: 84-90.
- 43) Ohtsuka Y, Lee J, Stamm DS, Sanderson IR: MIP-2 secreted by epithelial cells increases neutrophil and lymphocyte recruitment in the mouse intestine. *Gut*, 2001; 49: 526-533.
- 44) Ohtsuka Y, Sanderson IR: Dextran sulfate sodium-induced inflammation is enhanced by intestinal epithelial cell chemokine expression in mice. *Pediatr Res*, 2003; 53: 143-147.
- 45) Ohtsuka Y, Shimizu T, Shoji H, *et al*: Neonatal transient eosinophilic colitis causes rectal bleeding in early infancy. *J Pediatr Gastroenterol Nutr*, 2007; 44: 501-505.
- 46) Mori M, Ohtsuka Y, Ishida A, *et al*: Outcome of infants presenting rectal bleeding: a retrospective study in a single institution. *Pediatr Int*, 2014; 56: 884-890.
- 47) Ohtsuka Y, Jimbo K, Inage E, *et al*: Microarray analysis of mucosal biopsy specimens in neonates with rectal bleeding: is it really an allergic disease? *J Allergy Clin Immunol*, 2012; 129: 1676-1678.
- 48) Carlsen HS, Baekkevold ES, Johansen FE, Haraldsen G, Brandtzaeg P: B cell attracting chemokine 1 (CXCL13) and its receptor CXCR5 are expressed in normal and aberrant gut associated lymphoid tissue. *Gut*, 2002; 51: 364-371.
- 49) Kaplan B, Benson J, Rothstein F, Dahms B, Halpin T: Lymphonodular hyperplasia of the colon as a pathologic finding in children with lower gastrointestinal bleeding. *J Pediatr Gastroenterol Nutr*, 1984; 3: 704-708.
- 50) O'Farrelly C, O'Mahony C, Graeme-Cook F, Feighery C, McCartan BE, Weir DG: Gliadin antibodies identify gluten-sensitive oral ulceration in the absence of villous atrophy. *J Oral Pathol Med*, 1991; 20: 476-478.
- 51) Iacono G, Cavataio F, Montalto G, *et al*: Intolerance of cow's milk and chronic constipation in children. *N Engl J Med*, 1998; 339: 1100-1104.
- 52) Kosnai I, Kuitunen P, Savilahti E, Sipponen P: Mast cells and eosinophils in the jejunal mucosa of patients with intestinal cow's milk allergy and celiac disease of childhood. *J Pediatr Gastroenterol Nutr*, 1984; 3: 368-372.
- 53) Skripak JM, Sampson HA: Towards a cure for food allergy. *Curr Opin Immunol*, 2008; 20: 690-696.
- 54) Du Toit G, Roberts G, Sayre PH, *et al*: Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med*, 2015; 372: 803-813.
- 55) Perkin MR, Logan K, Tseng A, *et al*: EAT Study Team: Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. *N Engl J Med*, 2016; 374: 1733-1743.
- 56) Natsume O, Kabashima S, Nakazato J, *et al*: PETIT Study Team: Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT): a randomised, double-blind, placebo-controlled trial. *Lancet*, 2017; 389: 276-286.