



Contents lists available at ScienceDirect

Allergology International

journal homepage: <http://www.elsevier.com/locate/alit>

Original Article

Immune suppression of food allergy by maternal IgG in murine models

Hiroataka Yamashita^{a,*}, Tadamasa Hayashi^a, Kenichi Saneyasu^a, Hiroki Matsuhara^a, Teruaki Matsui^{a,b}, Hiroyuki Tanaka^a, Naoki Inagaki^a^a Laboratory of Pharmacology, Department of Bioactive Molecules, Gifu Pharmaceutical University, Gifu, Japan^b Department of Allergy, Aichi Children's Health and Medical Center, Aichi, Japan

ARTICLE INFO

Article history:

Received 16 November 2017

Received in revised form

28 February 2018

Accepted 28 March 2018

Available online xxx

Keywords:

Allergic diseases

Food allergy

Immunoglobulins

Oral tolerance

Ovalbumin

Abbreviations:

ITAM Immunoreceptor tyrosine-based activation motifs

ITIM Immunoreceptor tyrosine-based inhibitory motifs

ABSTRACT

Background: Most of the patients develop food allergy early in life. The factors related to parental immune condition might be one of the conceivable causes.**Methods:** We reported murine models of food allergy and oral OVA tolerance. To investigate the influence of parental immune condition on infant food allergy, female and male mice with food allergy or oral tolerance were mated with each other.**Results:** Food allergy was suppressed by decreased IgE production in the offspring of mice with food allergy. On the contrary, anaphylaxis for OVA was induced in the offspring of mice with oral tolerance. The suppression of food allergy being dependent on a maternal factor was revealed in the offspring after cross-mating mice with food allergy and oral tolerance. Because OVA-specific IgG, presumed to be from the allergic mother, was detected in the serum of naïve infants from mothers allergic to food, we assumed that the suppression was dependent on a specific IgG. The serum IgG purified by a G-protein column was administered before OVA sensitization in the food allergy model, and OVA-specific IgE production was found to be diminished in the administered mice. However, OVA-specific monoclonal IgG₁ and IgG_{2a} administration could not suppress food allergy. Because we detected OVA-IgG immune complex in the serum of mothers allergic to food, it might be a cause of maternal immune suppression. **Conclusions:** We demonstrated that maternal specific IgG conjugated food antigen is an important factor related to the development of food allergy and acquiring tolerance.Copyright © 2018, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Owing to the change in environmental conditions, numerous allergic diseases, such as seasonal allergic rhinitis, pediatric asthma, and peanut allergy, have increased over the last 100 years.^{1,2} Especially, food allergy has increased dramatically in the past few decades.³ Based on the challenge to some foods, the prevalence of food allergy has increased from 1 to 10.8% as reported in a meta-analysis,⁴ and about 5% of the children in Europe have had one or more food allergies in their lifetime.^{5,6} In Japan, epidemiology of pediatric food allergy in Ogasawara Islands has shown lifetime food allergy prevalence of 8.9%.⁷ The causes of increasing food allergy are considered to be genetic factors,^{8–10} changes in gut microbiome,¹¹

sunlight and vitamin D,^{12,13} (high vitamin D level is a risk factor for the development of food allergy in early infancy,¹⁴ or infants with vitamin D insufficiency are more likely to have multiple food allergies¹⁵), and intake of food additives.¹⁶ Genetic predisposition and environmental allergen exposure may be associated with the increasing prevalence of food allergy; however, these factors cannot explain the mechanisms underlying the development of food allergy.¹⁷

Children from asthmatic parents tend to develop asthma.¹⁸ Similarly, parental allergy can induce allergic and non-allergic rhinitis in their offspring.¹⁹ There is a strong familial aggregation of food allergy among family members and twin.^{20,21} Maternal inflammatory cytokine levels during pregnancy were related to the cytokine levels in the offspring, and maternal IgE levels were associated with IgE levels in children.²² Additionally, Treg number, which decreases in atopic diseases, in the cord blood was related to the risk of development of atopic dermatitis and sensitization towards food allergens during the first year of life.²³ These data indicate that allergic factors in parents affect the disposition of their

* Corresponding author. Laboratory of Pharmacology, Department of Bioactive Molecules, Gifu Pharmaceutical University, Daigakunishi 1-25-4, Gifu 501-1196, Japan.

E-mail address: hyamashita@gifu-pu.ac.jp (H. Yamashita).

Peer review under responsibility of Japanese Society of Allergology.

<https://doi.org/10.1016/j.alit.2018.04.001>

1323-8930/Copyright © 2018, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

offspring. Maternal factors, such as the overall health condition during pregnancy and lactation, are attributable to the development of allergies early in life. However, reports on the association of maternal consumption of peanut, milk, and wheat during pregnancy and lactation with food allergy,²⁴ or with reduced odds of allergies,²⁵ responsible for the development of allergic diseases, are inconsistent. In this study, we revealed the association between parental (maternal) food allergy and the food allergy that developed in their offspring using murine models.

Methods

Animals

Male and female BALB/c mice, or female BALB/c-*nu/nu* mice used for obtaining monoclonal antibodies, were purchased from SLC, Inc. (Hamamatsu, Japan). The mice were housed in an air-conditioned animal room at $23 \pm 1^\circ\text{C}$ temperature and $50 \pm 10\%$ humidity. Experiments were conducted following the guidelines of the Animal Experiment Committee of Gifu Pharmaceutical University.

Murine models of food allergy and oral tolerance

Murine models of food allergy and oral tolerance to food antigens²⁶ are shown in Figure 1B, C. In the protocol of food allergy, male and female BALB/c mice were sensitized intraperitoneally with $1\ \mu\text{g}$ OVA (Sigma–Aldrich, St. Louis, MO) or PBS mixed with Imject Alum (Thermo Scientific, Rockford, IL). The mice were administered 10 mg/mouse OVA dissolved in PBS orally four times every other day to induce food allergy from 1 week after the second sensitization of OVA injection. Anaphylaxis was elicited by administration of 50 mg/mouse OVA. For the induction of oral

tolerance, mice were treated with 1 mg/mouse OVA orally for 5 successive days, 1 week before the first sensitization with OVA injection. Naïve male and female mice were used as controls (Fig. 1A–C).

In murine model, a scoring system for anaphylactic symptoms such as scratching, puffiness around the eyes and mouth, diarrhea, wheezing, and death,^{27,28} and changes in the rectal temperatures²⁹ were usually used. We evaluated food allergy by a decrease in the rectal temperature and allergic diarrhea induced by OVA intake according to our previous report.²⁶ The decrease in body temperature was measured as a change in 1 h from each oral OVA administration using a thermometer (KN-91, Natsume, Tokyo, Japan). Allergic diarrhea was evaluated for determining the condition of feces for 1 h after OVA administration. Severity of diarrhea was assessed by a score from 0 to 3: 0 = solid state; 1 = semi-solid form (solid containing a little liquid); 2 = slurry, and 3 = watery state.

Mating between mice allergic to food and mice with oral tolerance

Food allergy or oral tolerance to OVA was induced in five female mice, who were mated with two male mice with the same treatment. As controls, naïve male and female mice were mated. Offspring of each pair were weaned at the age of four weeks. Following weaning, food allergy was induced in female infant mice (Fig. 1D). In a cross-mating study, food allergy was induced in the female progeny from female mice with food allergy or oral tolerance or naïve mice mated with male mice with other treatment.

ELISA measurements

OVA-specific IgE, IgG₁, IgG_{2a}, and IgA levels in the blood were measured by ELISA to modify previously described reports.^{30,31}

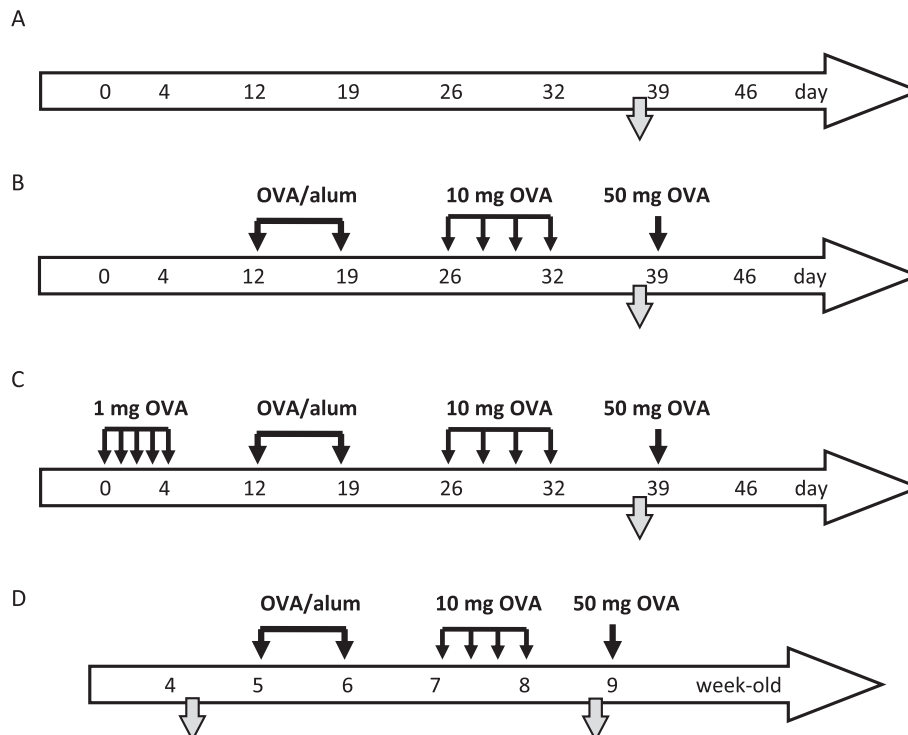


Fig. 1. Protocols for development of murine food allergy and oral tolerance models. Male and female mice were sensitized for OVA by administration of OVA/alum intraperitoneally. (A) Naïve female and male mice were used as controls. (B) Food allergy was induced in mice by oral administration of 10 mg OVA 4 times and elicited by 50 mg OVA. (C) Oral tolerance was induced by preliminary oral administration of 1 mg OVA for 5 successive days. (D) Offspring from each pair was induced for development of food allergy. (A–C) For measurements of immunoglobulin levels, the blood was collected from each parent at day 38, or (D) from offspring at 4 weeks and 8 weeks of age.

Table 1

Amounts of IgE, IgG1, and IgG2a antibodies in the injected samples.

Injection of IgG or serum	OVA-sIgG1 (μ g)	OVA-sIgG2a (μ g)	OVA-sIgE (ng)
Naïve serum (NS)	N.D.	N.D.	N.D.
Food allergy serum (FAS)	6.08	0.14	2.14
Food allergy serum through protein-G column (IgGs)	5.90	0.40	N.D.
Anti-OVA IgG1 monoclonal antibody (IgG1)	6.58	N.D.	N.D.
Anti-OVA IgG2a monoclonal antibody (IgG2a)	N.D.	0.18	N.D.
Anti-OVA monoclonal IgG1 and IgG2a (Mix)	6.58	0.18	N.D.

The samples were prepared from serum in food allergy mice, or ascites of mice injected with hybridoma cells producing OVA-specific IgG1 and IgG2a. N.D., not detected.

OVA-specific IgE, IgG1, or IgA were bound to an immune plate (Nunc, Roskilde, Denmark) coated with anti-mouse IgE antibody (MAC419, clone LO-ME-3; Serotec Ltd., Oxford, UK), anti-mouse IgG1 antibody (STAR81, Serotec Ltd.), or anti-mouse IgA antibody (STAR137, Serotec Ltd.), respectively, and detected by enzyme reaction after incubating with biotinylated antigen, and then with horseradish peroxidase-conjugated streptavidin (Dako, Glostrup, Denmark). In a measurement of OVA-specific IgG2a, OVA was directly caught on an immune plate, and specific IgG2a was detected by horseradish peroxidase conjugated anti-mouse IgG2a antibody (MCA421P, Serotec Ltd.). After the enzyme reaction, absorbance was measured at 492 nm (reference 690 nm).

IgG administration

The serum from mice allergic to food was collected 1 week after the last oral challenge with OVA. The serum was passed through protein G sepharose 4 fast flow column (GE healthcare, Little Chalfont, UK) to remove other immunoglobulins, except for IgG, such as OVA-specific IgE. Original serum contained 304.3 μ g/mL OVA-specific IgG1, 7.02 μ g/mL OVA-specific IgG2a, and 107.24 ng/mL OVA-specific IgE, and the serum through the column contained 118.0 μ g/mL OVA-specific IgG1 and 8.0 μ g/mL OVA-specific IgG2a. We diluted the original serum 10 times with PBS, and the serum through the column 4 times with PBS to refer to the concentration of OVA-specific IgG1 in offspring from food allergy parents, and 0.2 mL of the diluted sera were administered. Amounts of the administered IgG were shown in Table 1.

Hybridoma cells producing anti-OVA mouse IgG1 monoclonal antibody or anti-OVA mouse IgG2a monoclonal antibody were kindly provided by Dr. Kuniwa, (Kyushu University, Fukuoka, Japan). Ascetic fluid containing OVA-specific IgG1 or IgG2a was collected from BALB/c-*nu/nu* mice administered with hybridoma cells. Monoclonal IgGs were purified at salting-out followed by passing through the protein G sepharose 4 fast flow column (GE Healthcare). The monoclonal IgGs were diluted to refer to the amounts of IgG1 and IgG2a in the diluted food allergy serum indicated as FAS in Table 1.

Food allergy serum, the serum passed through the column, naïve serum, anti-OVA mouse IgG1 monoclonal antibody, anti-OVA mouse IgG2a monoclonal antibody, and a mixture of IgG1 antibody and IgG2a antibody were administered the day before each OVA/alum injection and a week before the first OVA/alum injection.

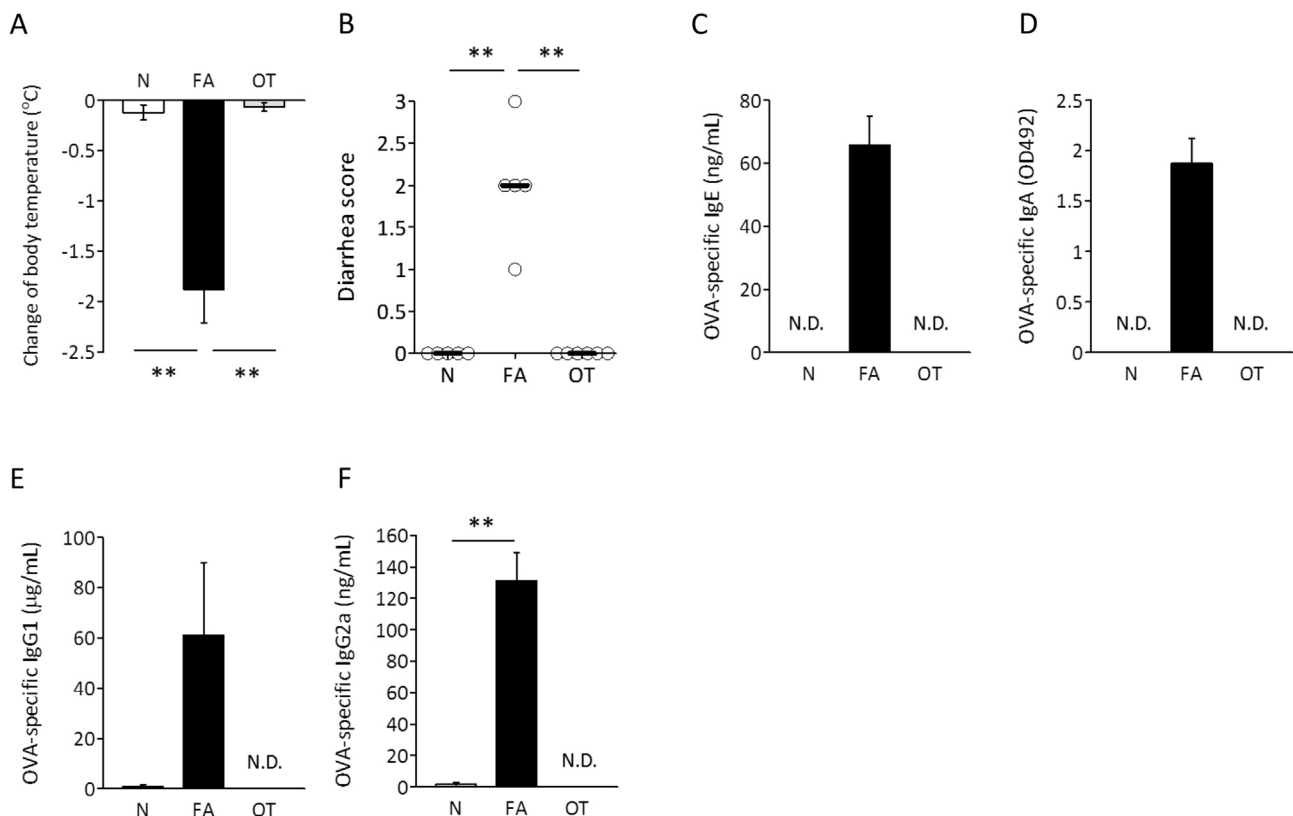


Fig. 2. Induction of food allergy and immunoglobulin levels in female mice. Food allergy in female mice was estimated by decrease in (A) the rectal temperature and (B) diarrheal score. Levels of OVA-specific (C) IgE, (D) IgA, (E) IgG1, and (F) IgG2a in the blood collected on day 38 were measured by ELISA. Each value represents a mean $\pm$ SEM of 5 mice, or each dot means an individual datum (** $P < 0.01$). N, naïve; FA, food allergy; OT, oral tolerance; N.D., not detected.

Western blotting for immune complex

OVA-IgG immune complex was detected using peroxidase-rabbit anti-OVA antibody (Rockland Immunochemicals, Limerick, PA) and peroxidase-goat anti-mouse IgG (Thermo Scientific). Samples of serum passed through G-protein column, and 5 and 10 $\mu\text{g/mL}$ standard OVA were applied to 10% acrylamide gel. After electrophoresis, the separated proteins in the samples were transferred onto a PVDF membrane using a semi-dry blotting system (Trans-blot SD, Bio-Rad Laboratories Inc., Hercules, CA). The transferred proteins were detected by anti-OVA antibody. After imaging using LAS-3000 mini imager (Fuji Photo Film, Tokyo, Japan) or ChemiDoc Touch (Bio-Rad Laboratories), the membrane was dipped into stripping buffer (Aproscience, Naruto, Japan), and re-stained by anti-mouse IgG antibody.

Statistical analyses

Data were expressed as mean $\pm$ SEM. The statistical significance of the difference values was evaluated by Student's or Welch's *t*-tests, or Dunnett's test and Tukey–Kramer's method. Each diarrheal score for a mouse was indicated as a dot, and an average of the score shown as a bar. The statistical significance in diarrheal scores was evaluated by Mann–Whitney *U* test, or Steel's and Steel–Dwass's test. $P < 0.05$ was considered statistically significant.

Results

Food allergy and oral tolerance in male and female mice as parents

Food allergy and oral tolerance were induced in male and female BALB/c mice using protocols shown in Figure 1B, C. Decrease in the body temperature and allergic diarrhea was evoked by oral administration of 50 mg OVA in the female mice with food allergy compared with that in naïve and mice with oral tolerance (Fig. 2A, B). In the female mice with food allergy, OVA-specific IgE, IgG₁, IgG_{2a}, and IgA were detected in the blood collected the day before induction; however, OVA-specific immunoglobulins were not detected in female mice with oral tolerance (Fig. 2C–F). Decrease in the body temperature and diarrheal score in the two food allergy-induced male mice were 1.7 and 1.2 $^{\circ}\text{C}$, and 2 and 1, respectively. OVA-specific IgE levels in the two male mice were 134.9 and 102.1 ng/mL, respectively, and IgG₁ levels were 101.1 and 126.1 $\mu\text{g/mL}$, respectively. OVA-specific immunoglobulins were not detected in the male mice with oral tolerance using ELISA.

Induction of food allergy in offspring of mice with maternal or paternal food allergy and oral tolerance

OVA-specific IgG₁ and IgG_{2a}, but not OVA-specific IgE and IgA, were detected in the blood collected at weaning of the offspring (4-week-old) from parents allergic to food (Fig. 3A, B, data not shown). Female offspring from parents with food allergy or oral

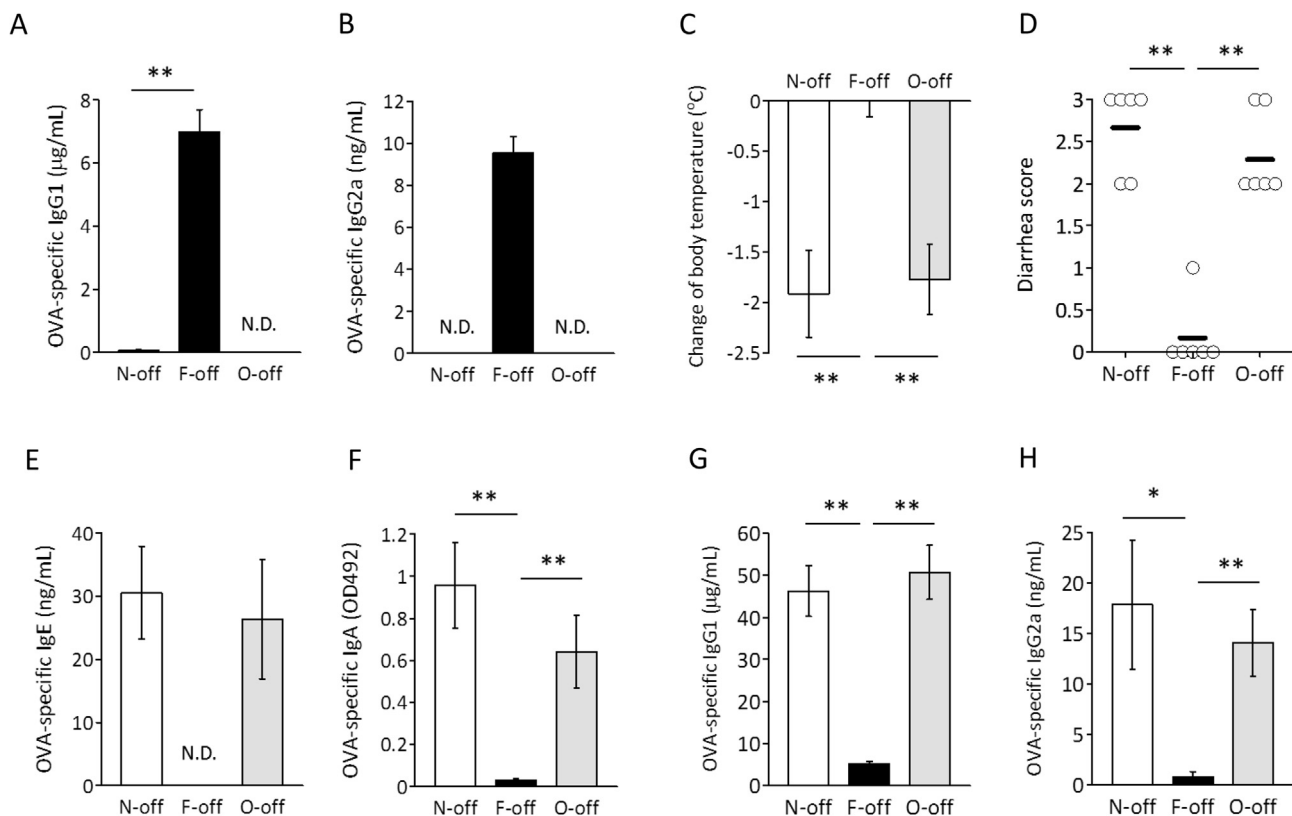


Fig. 3. Induction of food allergy and measurement of immunoglobulin levels in offspring at weaning and on the day before the challenge. Panels (A) and (B) show levels of OVA-specific IgG₁ and IgG_{2a} in the blood of female offspring from parents with food allergy or oral tolerance, or naïve mice after weaning. Food allergy was induced in infant mice. Anaphylaxis for OVA was estimated by measuring the decrease in (C) the rectal temperature and (D) diarrheal score. Levels of OVA-specific (E) IgE, (F) IgA, (G) IgG₁, and (H) IgG_{2a} in the blood collected the day before the induction were measured by ELISA. Each value represents mean $\pm$ SEM of 6 mice, or each dot means an individual datum (* $P < 0.05$, ** $P < 0.01$). N-off, offspring from naïve parents; F-off, offspring from food allergy parents; O-off, offspring from parents with oral tolerance; N.D., not detected.

tolerance, and naïve mice were sensitized with OVA/alum and challenged by oral administration with OVA. Food allergy was suppressed in the offspring from parents with food allergy (Fig. 3C, D). The production of OVA-specific IgE was inhibited completely (Fig. 3E), and that of IgA, IgG₁, and IgG_{2a} was strongly suppressed in the offspring of parents allergic to food (Fig. 3F–H).

Next, we analyzed whether the immune suppression for food allergy was dependent on maternal or paternal factors. Food allergy was induced in progenies of all the mating combinations of food allergy, oral tolerance, and naïve mice. Hypothermia and allergic diarrhea were suppressed or tended to suppress in the offspring of female mice allergic to food than naïve and oral tolerance in spite of fathers' immune condition (Fig. 4A, B). In a case of food allergy father mated with food allergy mother, diarrhea was suppressed to compare with in a case of father of oral tolerance mated with food allergy mother (Fig. 4B). Additionally, the production of OVA-specific IgE was completely suppressed in offspring of female mice allergic to food (Fig. 4C). Taken together, the immune suppression

for food allergy in the offspring might depend on the IgG from the mother.

Suppression of food allergy by IgG administration

To confirm the suppression of food allergy by IgG administration, food allergy was induced in mice supplied with OVA-specific IgG. IgG was obtained from the serum of mice allergic to food containing a large amount of OVA-specific IgG and IgE, and from food allergy serum passed through the protein G column to remove IgE and IgA. Additionally, OVA-specific monoclonal IgG₁, IgG_{2a}, and mixture of IgG₁ and IgG_{2a} were assessed. Each IgG sample was administered 8 days before the first sensitization for OVA, and the day before each sensitization (Fig. 5A). The results suggested that the decrease in the body temperature by anaphylaxis showed no significant differences among the groups (Fig. 5B). However, allergic diarrhea was inhibited by administration of purified IgG from food allergy serum (Fig. 5C). OVA-specific IgE and IgG₁ levels

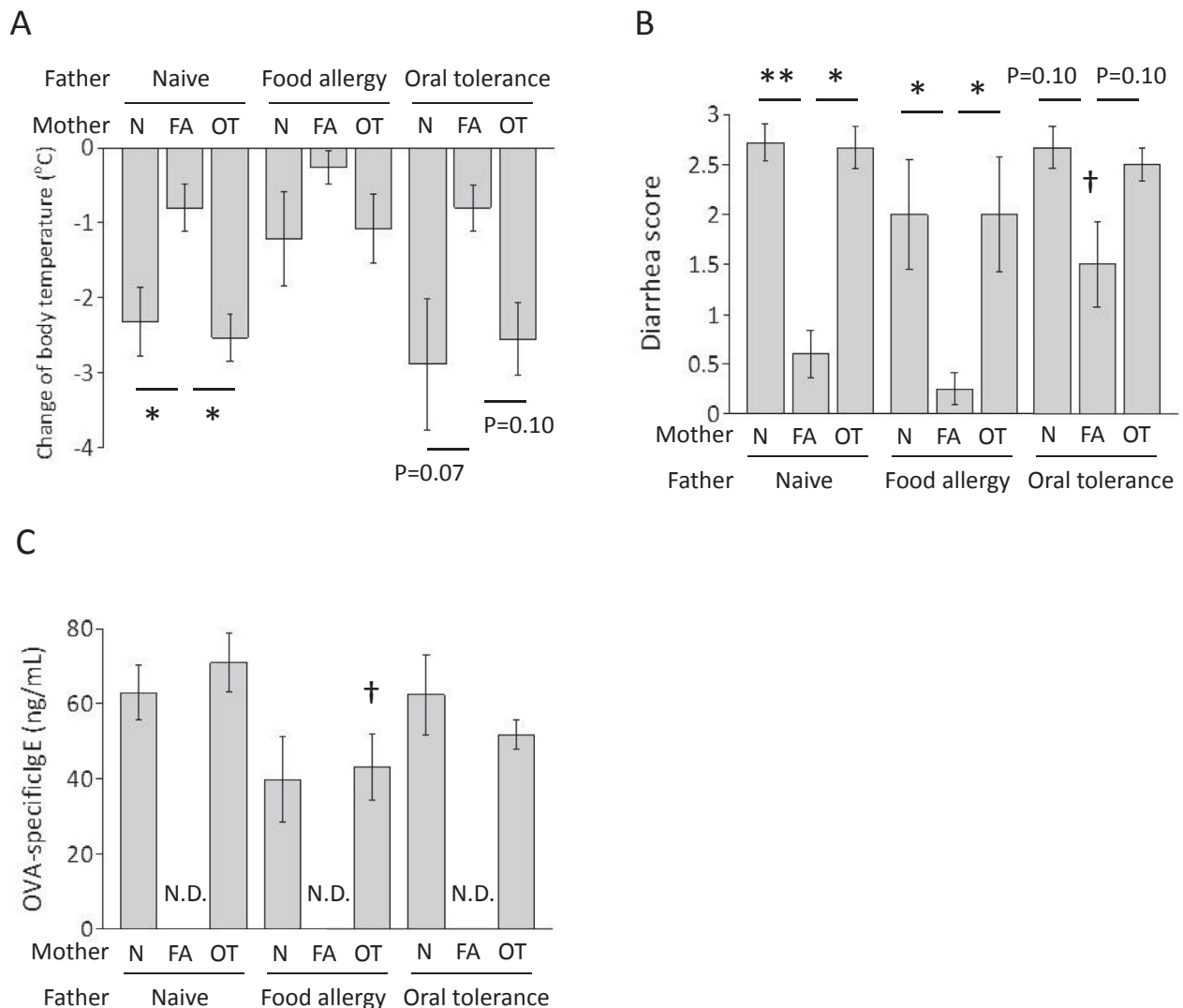


Fig. 4. Shuffled mating of female and male mice with food allergy or oral tolerance, or naïve mice. Food allergy or oral tolerance was induced in female and male mice. Naïve mice were used as controls. Offspring were obtained from crosses between naïve male mice and naïve (N), food allergy-induced (FA), or oral tolerance-induced (OT) female mice. Similarly, offspring were obtained from crosses between food allergy-induced or oral tolerance-induced male mice and female mice with the indicated treatment. Food allergy was induced in female infant mice. Anaphylaxis for OVA was estimated by measuring the decrease in (A) the rectal temperature and (B) diarrheal score. Levels of (C) OVA-specific IgE in the blood collected the day before the induction were measured by ELISA. Statistics were conducted by Tukey–Kramer's or Steel–Dwass's test among the same treated fathers or mothers (* $P < 0.05$ and ** $P < 0.01$ and indicated provability in the same treated fathers, or † $P < 0.05$ in the same treated mothers). N.D., not detected.

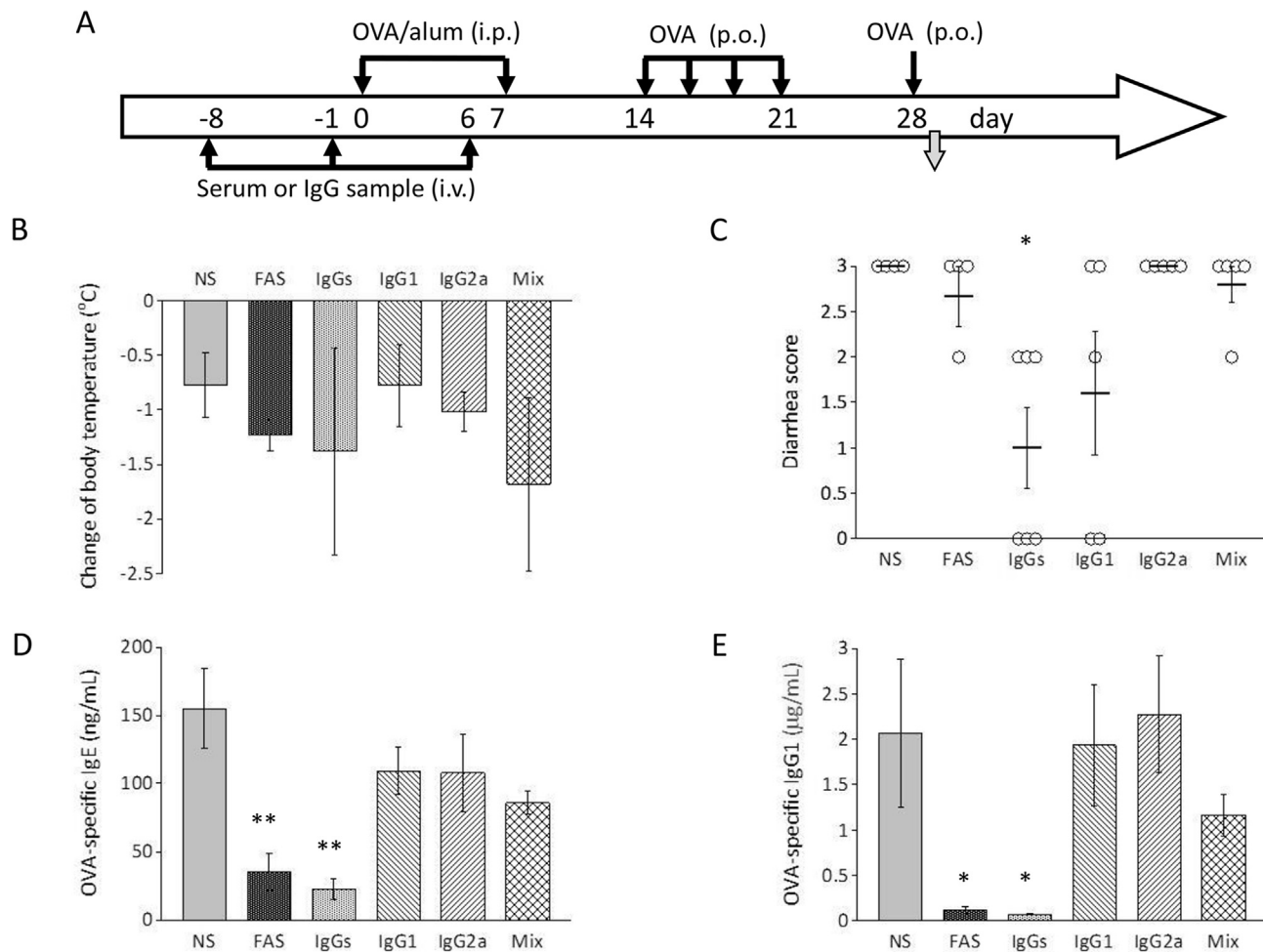


Fig. 5. Prevention of anaphylaxis by administration of OVA-specific IgGs. **(A)** Food allergy serum (FAS), the serum passed through protein G-column (IgGs) or naïve (NS), or OVA-specific IgG₁ or/and IgG_{2a} monoclonal antibodies (IgG₁, IgG_{2a}, and Mix) were administered 8 days before the first sensitization with OVA/alum, and the day before each sensitization. Anaphylaxis for OVA was estimated by measuring the decrease in **(B)** the rectal temperature and **(C)** diarrheal score. Levels of OVA-specific **(D)** IgE and **(E)** IgG₁ in the blood collected the day before the induction were measured by ELISA. Each value represents a mean $\pm$ SEM of 4–6 mice, or each dot means an individual datum (* $P < 0.05$, ** $P < 0.01$).

on the day before the last induction were attenuated in the mice administered with food allergy serum or the purified IgG compared with those in mice administered with monoclonal IgG₁ and IgG_{2a} (Fig. 5D, E).

Detection of IgG-immune complex

Because monoclonal IgG administration failed to suppress the food allergy, inhibition by IgG-immune complex was suspected. Therefore, immune complex of OVA with IgG was confirmed by western blotting in the serum of the mother allergic to food. The protein was detected by anti-OVA antibody in the serum of mice allergic to food, but not in the serum of naïve mice or those administered monoclonal IgG (Fig. 6A). The size of the protein was larger than that of OVA and similar to that of IgG (Fig. 6B). We believe that the protein with the size of about 50 kDa was the OVA-IgG immune complex.

Next, we confirmed OVA-IgG immune complex in sera collected 1, 2, 4, 8 weeks after the last challenge with OVA. As results, we could detect OVA protein even in the serum collected 8 weeks after the challenge (Fig. 6C, D). We tried to detect OVA-IgG complex from sera in offspring at the age of 4 weeks, but we could not detect the bands of OVA in the samples of offspring from naïve, food allergy, and oral tolerance patients by western blotting (data not shown).

Discussion

Food allergy is defined as a phenomenon in which adverse reactions are caused through antigen-specific immunological mechanisms after exposure to given food.³² Generally, immune tolerance for proteins in food is acquired by oral intake of the food in a healthy person; however, anaphylaxis is evoked after intake of the food in patients with food allergy. Because food allergy almost develops early in the life, some of its causes depend on the environment during the fetal period and infancy. In this study, we focused on the relationship between parental conditions for a food allergen and descendent allergy.

Because parental allergy is a risk factor for offspring allergy,¹⁹ we formulated a hypothesis that parental food allergy is a risk factor for developing food allergy in their children. We inspected this hypothesis by producing offspring of parents with food allergy or oral tolerance using murine models. Unexpectedly, development of food allergy was suppressed in the offspring of parents with food allergy. On the contrary, anaphylaxis was observed in the offspring of parents with oral tolerance. Next, we analyzed whether immune suppression could be traced to the male or female mice. The results revealed that antigen-specific IgE production was inhibited in female mice with food allergy; some factors from the mother with food allergy were able to prevent sensitization to food antigens.

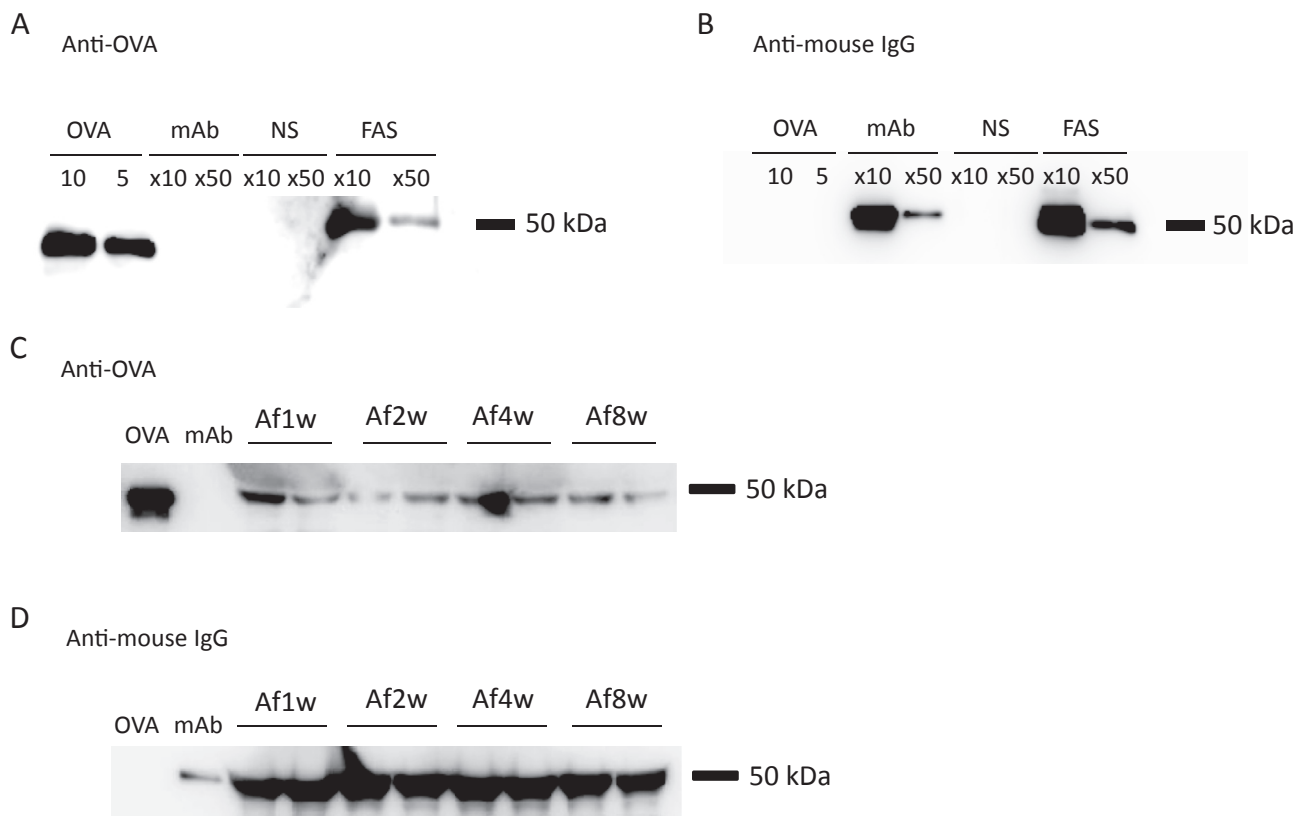


Fig. 6. Western blotting for OVA-IgG immunocomplex. **(A)** OVA proteins in food allergy serum (FAS), naïve serum (NS), monoclonal IgG₁ (mAb), or 5 and 10 µg/mL OVA solutions were detected by western blotting. **(B)** Proteins of IgG in the same samples were detected by anti-murine IgG antibody. Applied samples were diluted naïve (NS) or food allergy (FAS) serum, or monoclonal IgG₁ used in the mouse model 10 times ($\times 10$) or 50 times ($\times 50$). In panels **(C)** and **(D)**, OVA and IgG were detected from the serum of two mice collected 1, 2, 4, 8 after the last challenge with OVA in food allergy model (shown as Af1w, Af2w, Af4w, and Af8w). Applied serum sample was diluted 10 times with PBS, and monoclonal IgG (mAb) was diluted 50 times. Control OVA was used at a concentration of 10 µg/mL.

In the offspring of mice allergic to food, OVA-specific IgE production was completely suppressed and OVA-specific IgG₁ level was maintained from the weaning period. Because the mother was not treated with OVA during mating and pregnancy, “some factors” were probably not directly referring to OVA proteins, which could induce oral tolerance, from mother to fetuses or babies. We detected OVA-specific IgG in the offspring of parents allergic to food, and maternal IgG with antigens could across the placenta through FcRn and be transferred into the bloodstream in neonates.³³ Therefore, we suspected that the factor causing prevention of food allergy by OVA-specific IgG came from the mother. This supposition was consistent with the data of cross-mating among mice with food allergy or oral tolerance, and naïve mice, in which OVA-specific IgE production was not detected in the offspring of female mice allergic to food administered OVA/alum.

To confirm the suppression of food allergy by IgG, food allergy serum containing a large amount of IgG, food allergy serum purified by passing through protein G column to remove immunoglobulins (IgE and IgA) other than IgG, and OVA-specific monoclonal IgG₁ or IgG_{2a} were administered to naïve mice followed by induction of food allergy. The results indicated that IgG from the serum (mixture of polyclonal IgG subclasses) inhibited the elevation of IgE significantly, but partially. Decrease in the body temperature due to anaphylaxis was not suppressed by administration of IgG from both food allergy serum and column-through serum. Allergic diarrhea was suppressed by administration of the column-through serum. The reason that food allergy serum, but not column-through serum, could not suppress diarrhea might be because of the inclusion of IgE. However, in mice administered the food allergy serum, the

reason for partial suppression of IgE production was unclear. We believe that complicated immune mechanisms are involved in immune tolerance to food antigens.

We believe that there may be two possibilities for the inhibition by IgG, with one being blocking sensitization. OVA-specific IgG would bind to OVA from alum after the sensitization injection followed by eliminating OVA in the body. The elimination could spoil sensitization for OVA and inhibit the elevation of OVA-specific IgE. However, the results of monoclonal IgG antibody administration rejected the hypothesis. Administration of a large amount of OVA-specific monoclonal IgG₁ failed to suppress the production of OVA-specific IgE. The other possibility was inhibitory signaling via FcγRIIb on immune cells. In rodents, FcγR is classified into 4 types—FcγRI, FcγRII, FcγRIII, and FcγRIV. Murine FcγRIa, FcγRIIIa, and FcγRIV transmit activation signal via immunoreceptor tyrosine-based activation motifs (ITAM) in cytoplasmic domain, and FcγRIIb transmits inhibitory signal via immunoreceptor tyrosine-based inhibitory motifs (ITIM).³⁴ FcγRIIb negatively regulates cell activation and proliferation by co-engaging with surface activating receptors containing ITAM on the same cell.³⁵ Also, blocking antibody to FcγRIIb enhanced IgE-mediated anaphylaxis in mice.³⁶

It was reported that the immune complex of IgG₁ linked FcγRIIb to block the migration of neutrophils and complement-mediated inflammation.³⁷ Synergistic effect of plural anti-virus specific IgG inhibits activation of B cell receptor responses by binding to FcγRIIb.³⁸ Additionally, IgG immune complexes suppress IL-1α and IL-1β secretion through inhibition of inflammasome activation via ligation of immune complex to activating FcγRs. The IgG immune complexes prevent sensitization by OVA/alum and attenuate

adaptive immune responses inducing IgE production in murine models.³⁹ The serum collected from mice allergic to food contains a large amount of polyclonal IgG, and polyclonal IgG would constitute the immune complex with antigen. In our study, the immunosuppression might be associated with the IgG-immune complex.

Maternal multiple antibodies are useful in the neonatal immune system against infectious diseases.⁴⁰ In a study on allergy in rats, maternal exposure to OVA during pregnancy and lactation attenuated OVA-specific IgG production in the next generation of rats.⁴¹ In a murine asthma model, exposure of OVA-aerosol to the mother during lactation prevented induction of airway hyper-reactivity in the offspring according to reduction in OVA-specific IgE and IgG₁ in the blood.^{42,43} The reports showed that OVA-IgG₁ immune complexes in the breast milk prevent asthma. We attempted to detect OVA and IgG in the breast milk of allergic mother; however, we could not detect them because the amount of sample was too less (data not shown). Therefore, we detected the OVA-IgG immune complex in the serum of mother mice collected a week after the final oral challenge of OVA. We could detect a band of OVA and IgG of similar size. We believe that the band detected by anti-OVA antibody is the OVA-IgG immune complex because the size of the protein detected by anti-mouse IgG was also the same. We attempted to detect the band of OVA from the sera collected 1, 2, 4, and 8 weeks after the last challenge with OVA. We presume that each time is in mating, during pregnancy, in childbirth, and with lactation. Interestingly, we detected the bands of OVA even in 8 weeks after the challenge. However, we could not detect the bands of OVA in samples of 4 week-old pups from food allergy- and oral tolerance-parents. Namely, OVA-IgG immune complex was comparatively stable in blood; however, we could not confirm transferring the immune complex from mother to pup clearly. We speculated that the complex was too small amount to detect in sample of offspring, or that the complex became unstable in the transferring process.

Taken together, we investigated that parental food allergy affected the development of food allergy in the offspring. The results indicated that maternal food allergy prevented the development of food allergy in the offspring by administration of OVA-specific polyclonal IgG and OVA-IgG immune complex via placenta or breast milk. We think that the prevention by immune complex is a system of the natural maternal immunity because the condition of absent of antigen-specific immunoglobulin in naïve and experimental oral tolerance is unnatural immunity. We think maternal antigen-IgG immune complex is one of tolerogenic systems.

We have no idea why antigen-IgG complex from mother provided a tolerogenic memory for the antigen to pups. More analyses need to be conducted for elucidating the detailed mechanisms underlying the suppression. However, the data suggested a possibility of prevention of food allergy by administration of food antigen specific IgG.

Acknowledgments

We thank Dr. Mamoru Kiniwa (Kyushu University) for providing valuable hybridomas. This work was supported in part by Takeda Science Foundation.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

TH, KS, and HM performed the experiments. TM, HT, and NI participated in the design of the study and helped to draft the manuscript. HY conceived the study, participated in its design, performed the experiments, and drafted the manuscript. All authors concurred with the submission.

References

- Platts-Mills TA. The allergy epidemics: 1870–2010. *J Allergy Clin Immunol* 2015;**136**:3–13.
- Woodfolk JA, Commins SP, Schuyler AJ, Erwin EA, Platts-Mills TA. Allergens, sources, particles, and molecules: why do we make IgE responses? *Allergol Int* 2015;**64**:295–303.
- Bunyavanich S, Rifas-Shiman SL, Platts-Mills TA, Workman L, Sordillo JE, Gillman MW, et al. Peanut allergy prevalence among school-age children in a US cohort not selected for any disease. *J Allergy Clin Immunol* 2014;**134**:753–5.
- Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. *J Allergy Clin Immunol* 2007;**120**:638–46.
- Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy* 2014;**69**:992–1007.
- Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. *Allergy* 2014;**69**:62–75.
- Okada Y, Kumagai H, Morikawa Y, Akasawa A. Epidemiology of pediatric allergic diseases in the Ogasawara Islands. *Allergol Int* 2016;**65**:37–43.
- Hong X, Hao K, Ladd-Acosta C, Hansen KD, Tsai H-J, Liu X, et al. Genome-wide association study identifies peanut allergy-specific loci and evidence of epigenetic mediation in US children. *Nat Commun* 2015;**6**:6304.
- Rubicz R, Yolken R, Alaedini A, Drigalenko E, Charlesworth JC, Carless MA, et al. Genome-wide genetic and transcriptomic investigation of variation in antibody response to dietary antigens. *Genet Epidemiol* 2014;**38**:439–46.
- Brown SJ, Asai Y, Cordell HJ, Campbell LE, Zhao Y, Liao H, et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol* 2011;**127**:661–7.
- Bunyavanich S, Shen N, Grishin A, Wood R, Burks W, Dawson P, et al. Early-life gut microbiome composition and milk allergy resolution. *J Allergy Clin Immunol* 2016;**138**:1122–30.
- Matsui T, Tanaka K, Nakagawa T, Sasaki K, Nakata J, Sugiura S, et al. Sun exposure inversely related to food sensitization during infancy. *Pediatr Allergy Immunol* 2015;**26**:628–33.
- Matsui T, Yamashita H, Saneyasu K, Tanaka H, Ito K, Inagaki N. Vitamin D deficiency exacerbates sensitization and allergic diarrhea in a murine food allergy model. *Allergol Int* 2018;**67**:289–91.
- Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M, et al. Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. *Allergy* 2013;**68**:220–8.
- Allen KJ, Koplin JJ, Ponsonby AL, Gurrin LC, Wake M, Vuillermier P, et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol* 2013;**131**:1109–16.
- Yamashita H, Matsuhara H, Miotani S, Sako Y, Matsui T, Tanaka H, et al. Artificial sweeteners and mixture of food additives cause to break oral tolerance and induce food allergy in murine oral tolerance model for food allergy. *Clin Exp Allergy* 2017;**47**:1204–13.
- du Toit G, Tsakok T, Lack S, Lack G. Prevention of food allergy. *J Allergy Clin Immunol* 2016;**137**:998–1010.
- Paaso EM, Jaakkola MS, Rantala AK, Hugg TT, Jaakkola JJ. Allergic diseases and asthma in the family predict the persistence and onset-age of asthma: a prospective cohort study. *Respir Res* 2014;**15**:152.
- Westman M, Kull I, Lind T, Melen E, Stjärne P, Toskala E, et al. The link between parental allergy and offspring allergic and nonallergic rhinitis. *Allergy* 2013;**68**:1571–8.
- Tsai HJ, Kumar R, Pongracic J, Liu X, Story R, Yu Y, et al. Familial aggregation of food allergy and sensitization to food allergens: a family-based study. *Clin Exp Allergy* 2009;**39**:101–9.
- Sicherer SH, Furlong TJ, Maes HH, Desnick RJ, Sampson HA, Gelb BD, et al. Genetics of peanut allergy: a twin study. *J Allergy Clin Immunol* 2000;**106**:53–6.
- Herberth G, Hinz D, Roder S, Schlink U, Sack U, Diez U, et al. Maternal immune status in pregnancy is related to offspring's immune responses and atopy risk. *Allergy* 2011;**66**:1065–74.
- Hinz D, Bauer M, Roder S, Olek S, Huehn J, Sack U, et al. Cord blood Tregs with stable FOXP3 expression are influenced by prenatal environment and associated with atopic dermatitis at the age of one year. *Allergy* 2012;**67**:380–9.
- Sicherer SH, Wood RA, Stablein D, Lindblad R, Burks AW, Liu AH, et al. Maternal consumption of peanut during pregnancy is associated with peanut sensitization in atopic infants. *J Allergy Clin Immunol* 2010;**126**:1191–7.
- Bunyavanich S, Rifas-Shiman SL, Platts-Mills TA, Workman L, Sordillo JE, Camargo Jr CA, et al. Peanut, milk, and wheat intake during pregnancy is associated with reduced allergy and asthma in children. *J Allergy Clin Immunol* 2014;**133**:1373–82.
- Yamashita H, Takahashi K, Tanaka H, Nagai H, Inagaki N. Overcoming food allergy through acquired tolerance conferred by transfer of Tregs in a murine model. *Allergy* 2012;**67**:201–9.
- Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH, et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *J Allergy Clin Immunol* 2000;**106**:150–8.
- Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. *J Allergy Clin Immunol* 2014;**133**:1390–9.

29. Mathias CB, Hobson SA, Garcia-Lloret M, Lawson G, Poddighe D, Freyschmidt EJ, et al. IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *J Allergy Clin Immunol* 2011;**127**:795–805.
30. Nagai H, Maeda Y, Tanaka H. The effect of anti-IL-4 monoclonal antibody, rapamycin and interferon-gamma on airway hyperreactivity to acetylcholine in mice. *Clin Exp Allergy* 1997;**27**:218–24.
31. Tanaka H, Masuda T, Tokuoka S, Komai M, Nagao K, Takahashi Y, et al. The effect of allergen-induced airway inflammation on airway remodeling in a murine model of allergic asthma. *Inflamm Res* 2001;**50**:616–24.
32. Urisu A, Ebisawa M, Ito K, Aihara Y, Ito S, Mayumi M, et al. Japanese guideline for food allergy 2014. *Allergol Int* 2014;**63**:399–419.
33. Rath T, Kuo TT, Baker K, Qiao SW, Kobayashi K, Yoshida M, et al. The immunologic functions of the neonatal Fc receptor for IgG. *J Clin Immunol* 2013;**33**:S9–17.
34. Nimmerjahn F, Ravetch JV. Fcγ Receptors: old friends and new family members. *Immunity* 2006;**24**:19–28.
35. Daeron M, Lesourne R. Negative signaling in Fc receptor complexes. *Adv Immunol* 2006;**89**:39–86.
36. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIb cross-linking. *J Clin Invest* 2006;**116**:833–41.
37. Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, et al. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIb and dectin-1. *Nat Med* 2012;**18**:1401–6.
38. Kim D, Huey D, Oglesbee M, Niewiesk S. Insights into the regulatory mechanism controlling the inhibition of vaccine-induced seroconversion by maternal antibodies. *Blood* 2011;**117**:6143–51.
39. Janczy JR, Ciraci C, Haasken S, Iwakura Y, Olivier AK, Cassel SL, et al. Immune complexes inhibit IL-1 secretion and inflammasome activation. *J Immunol* 2014;**193**:5190–8.
40. Niewiesk S. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Front Immunol* 2014;**5**:446.
41. Kato T, Chen R, Tang X, Fan B, Liu J, Jia X, et al. IgG expression upon oral sensitization in association with maternal exposure to ovalbumin. *PLoS One* 2016;**11**. e0148251.
42. Verhasselt V, Milcent V, Cazareth J, Kanda A, Fleury S, Dombrowicz D, et al. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat Med* 2008;**14**:170–5.
43. Mosconi E, Rekima A, Seitz-Polski B, Kanda A, Fleury S, Tissandie E, et al. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol* 2010;**3**:461–74.