

Induction and suppression of allergic diarrhea and systemic anaphylaxis in a murine model of food allergy

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Background: The clinical manifestations of food allergy include diarrhea and systemic anaphylaxis (shock), which can occur together or by themselves in different subjects. Although ingested food antigens need to be absorbed to induce shock, it is not known whether they need to be absorbed to induce diarrhea.

Objective: We sought to identify mechanisms that determine whether food allergy induces diarrhea versus shock and determine whether diarrhea requires absorption of ingested antigens.

Methods: These issues were studied in mice in active, passive, and hybrid immunization models. The active model was used to determine the allergic diarrhea susceptibility of J chain- and polymeric immunoglobulin receptor-deficient mice, which are unable to secrete IgA. The hybrid model was used to determine whether intravenously administered antigen-specific IgG antibody, which is not secreted into the gut, can protect against allergic diarrhea, as well as shock.

Results: Shock, but not diarrhea, was induced in naive mice by using intravenous IgE anti-trinitrophenyl (TNP) antibody, followed by oral TNP-BSA, whereas both were induced in mice presensitized with intraperitoneal ovalbumin/alum plus oral ovalbumin. More TNP-BSA was required to induce shock than diarrhea in presensitized mice, and intravenous IgG anti-TNP antibody, which is not secreted into the gut, protected these mice against both diarrhea and shock. Consistent with this, chicken ovalbumin-immunized J chain- and polymeric immunoglobulin receptor-deficient mice, which have high serum IgA levels but little intestinal IgA, resisted diarrhea induction.

Conclusion: Intestinal immunity and oral antigen dose determine whether diarrhea, systemic anaphylaxis, or both are

induced, and ingested antigen must be absorbed to induce either response. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: IgA, IgE, IgG, J chain, polymeric immunoglobulin receptor

Food allergy is a general term that includes several different food-induced clinical syndromes. These range from mild abdominal discomfort to severe swelling of the mouth, tongue, and pharynx (angioedema); vomiting and diarrhea; hives; asthma; and shock (systemic anaphylaxis).¹⁻⁴ These syndromes can occur independently or in combination and are not hierarchical: self-limited symptoms, such as diarrhea or vomiting, most frequently occur in the absence of life-threatening shock, whereas shock can occur in the absence of gastrointestinal symptoms.¹⁻⁴ Although mild food allergy can be induced through both antibody-dependent and antibody-independent mechanisms, gastrointestinal symptoms, hives, and shock that develop rapidly after food ingestion are IgE- and mast cell-dependent immediate hypersensitivity reactions.¹⁻⁵ Even though the milder forms of IgE-mediated food allergy far outnumber the life-threatening forms, food ingestion is the most common cause of systemic anaphylaxis, with approximately 50,000 emergency department visits and approximately 150 deaths annually in the United States.^{2,6,7} Additionally, food allergy, in common with most allergic disorders, appears to be dramatically increasing in frequency in developed countries and has no satisfactory treatment.⁶⁻⁹

Several rodent models have been developed to study food allergy.^{5,10,11} All involve the initial administration of an antigen in the context of inflammation, followed by antigen ingestion. In one such model, in which mice were primed by means of intraperitoneal inoculation with chicken ovalbumin (OVA) plus alum and then inoculated several times with OVA by means of oral gavage (OG),^{5,12} shock can develop (detected as hypothermia), which usually becomes evident within 5 minutes and reaches its nadir at 15 to 25 minutes, as can watery diarrhea, which is apparent by 30 to 60 minutes. Although both of these responses depend on IgE, FcεRIα, and mast cells, they differ in the mast cell-secreted mediators that are most important (histamine for shock and platelet-activating factor and possibly serotonin for diarrhea).^{5,13} Furthermore, like human food allergy, murine models can be manipulated so that diarrhea occurs without shock and *vice versa*.

This suggests that further examination of these models might shed light on the factors that alternatively promote shock versus diarrhea. We have taken this approach with studies that evaluated 2 possibilities. Because mice in our model must be inoculated by means of OG with OVA several times before they have allergic diarrhea and this clinical feature is associated with large increases in intestinal mast cell numbers,^{5,12} we hypothesized that local

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Abbreviations used

OVA: Chicken ovalbumin
pIgR: Polymeric immunoglobulin receptor
TNP: Trinitrophenyl
WT: Wild-type

allergic inflammation might be needed to induce allergic diarrhea. We were also influenced by the much greater effectiveness of serum IgA and IgG antibody than gut secretory IgA at blocking IgE-dependent shock that is induced by antigen ingestion.¹² Because serum immunoglobulin could only inhibit an antigen-mediated process if that antigen must be absorbed systemically and a requirement for systemic antigen absorption had not been demonstrated for the induction of allergic diarrhea, we hypothesized that antigen still present in the gut lumen might induce allergic diarrhea, but not shock, by activating mucosal mast cells that are located immediately adjacent to the lumen.¹⁴ By supporting the first hypothesis but disproving the second, the studies reported here suggest that the purposeful induction of a strong IgG antibody response to a food allergen should be useful for treating both the intestinal and systemic features of food allergy.

METHODS**Mice**

BALB/c-background wild-type (WT) and transgenic mice in which an IL-9 transgene is regulated by the intestinal fatty acid-binding protein promoter (iIL-9 Tgn mice),¹⁵ BALB/c-background polymeric immunoglobulin receptor (pIgR)-deficient mice (Jackson Laboratories, Bar Harbor, Me),¹⁶ BALB/c-background J-chain-deficient mice (a gift from Dennis Metzger, Albany Medical College, Albany, NY),¹⁷ and BALB/c-background FcγRIIb-deficient mice¹⁸ were all bred in house. All experimental procedures were performed with approval from the Institutional Animal Care and Use Committees of the Cincinnati Children's Hospital Research Foundation, which follows the "Guide for the care and use of laboratory animals" prepared by the Institute of Laboratory Animal Resources, National Research Council and published by the National Academy Press.

Reagents

OVA, BSA, and Pristane were purchased from Sigma (St Louis, Mo). OVA and BSA were labeled with trinitrophenyl (TNP).¹⁹

Hybridomas

Hybridomas that secrete murine IgE anti-TNP (IGEL 2a) and murine IgG₁ anti-TNP (1B7.11) were purchased from the American Type Culture Collection (Rockville, Md) and grown in Pristane-primed athymic nude mice. A hybridoma that secretes murine IgG₁ anti-fluorescein isothiocyanate (CG7) was produced in house. IgG₁ mAbs were purified by means of ammonium sulfate precipitation, followed by diethylaminoethyl cellulose (DE-52) cation exchange chromatography, whereas IgE anti-TNP ascites were used without purification.

Fecal pellet IgA extraction

IgA was extracted from fecal pellets, as previously described.¹²

Passive anaphylaxis

Mice (5-6 per group unless otherwise noted) were injected intravenously with 10 μg of IgE anti-TNP mAb followed 24 hours later by OG

with TNP-OVA or TNP-BSA plus Evans blue in 300 μL of 0.15 mol/L NaCl. Systemic anaphylaxis severity, as reflected by hypothermia, was determined by means of rectal thermometry.¹³ All studies were repeated at least twice.

Active anaphylaxis

Mice (5-6 per group) were injected intraperitoneally with 50 μg of OVA adsorbed to 1 mg of alum. Starting 14 days later, these mice were inoculated by means of OG 3 times per week with 50 mg of OVA plus Evans blue in 300 μL of 0.15 mol/L NaCl. Mice were observed for up to 60 minutes after OVA inoculation for the development of diarrhea (intestinal anaphylaxis) and hypothermia.

Mixed (hybrid) anaphylaxis

Mice (5-6 per group) were inoculated intraperitoneally with OVA/alum. Fourteen days later, they were inoculated by means of OG every 2 to 3 days with OVA until they had both hypothermia and diarrhea. Mice were then injected intravenously with 10 μg of IgE anti-TNP mAb or saline. The next day, mice were injected intravenously with 2 mg of IgG₁ anti-TNP mAb or IgG₁ isotype control mAb and challenged 1 hour later by means of OG with 5 to 50 mg of TNP-BSA plus Evans blue and observed for the next hour for development of hypothermia and diarrhea.

Evaluation of diarrhea

Mice were evaluated by a blinded observer for the development of diarrhea, which was defined as the development of watery green stools by 60 minutes after OG inoculation with antigen plus Evans blue. Attempts to quantitate the severity of diarrhea by measuring stool volume between the time of OG inoculation and the development of diarrheal stool were not productive.

ELISA

IgA and IgE concentrations were determined by using sandwich ELISAs in which microtiter plate wells were coated with anti-mouse IgA mAb (BD Biosciences, Franklin Lakes, NJ) or rat IgG2a anti-mouse IgE mAb (EM-95) followed by sample and purified IgA or IgE standard (BD Biosciences). After incubation for 60 minutes, bound IgA or IgE was detected with biotin-anti-mouse IgA mAb (BD Biosciences) or biotin-rat IgG2a anti-mouse IgE mAb (RIE4), respectively, followed sequentially by horseradish peroxidase-streptavidin and SuperSignal ELISA substrate (Pierce Biotechnology, Rockford, Ill).

Statistics

The significance of differences in temperature and immunoglobulin concentrations between groups of mice was compared with the Mann-Whitney *U* test. Differences in the occurrence of diarrhea between groups were compared with the Pearson χ^2 test (GraphPad Prism 5.0; GraphPad Software, Inc, La Jolla, Calif). A *P* value of less than .05 was considered significant. Means $\pm$ SEMs are shown in all figures. All studies were performed at least twice with consistent results.

RESULTS**Passive immunization with IgE antibody is sufficient for antigen ingestion to induce shock, but not diarrhea, in immunologically naive mice**

Initial experiments determined whether increased levels of antigen-specific IgE antibody are sufficient for ingestion of the targeted antigen to induce systemic shock, allergic diarrhea, or both. By itself, intravenous injection of WT mice with IgE anti-TNP mAb did not induce shock (hypothermia) or diarrhea (data

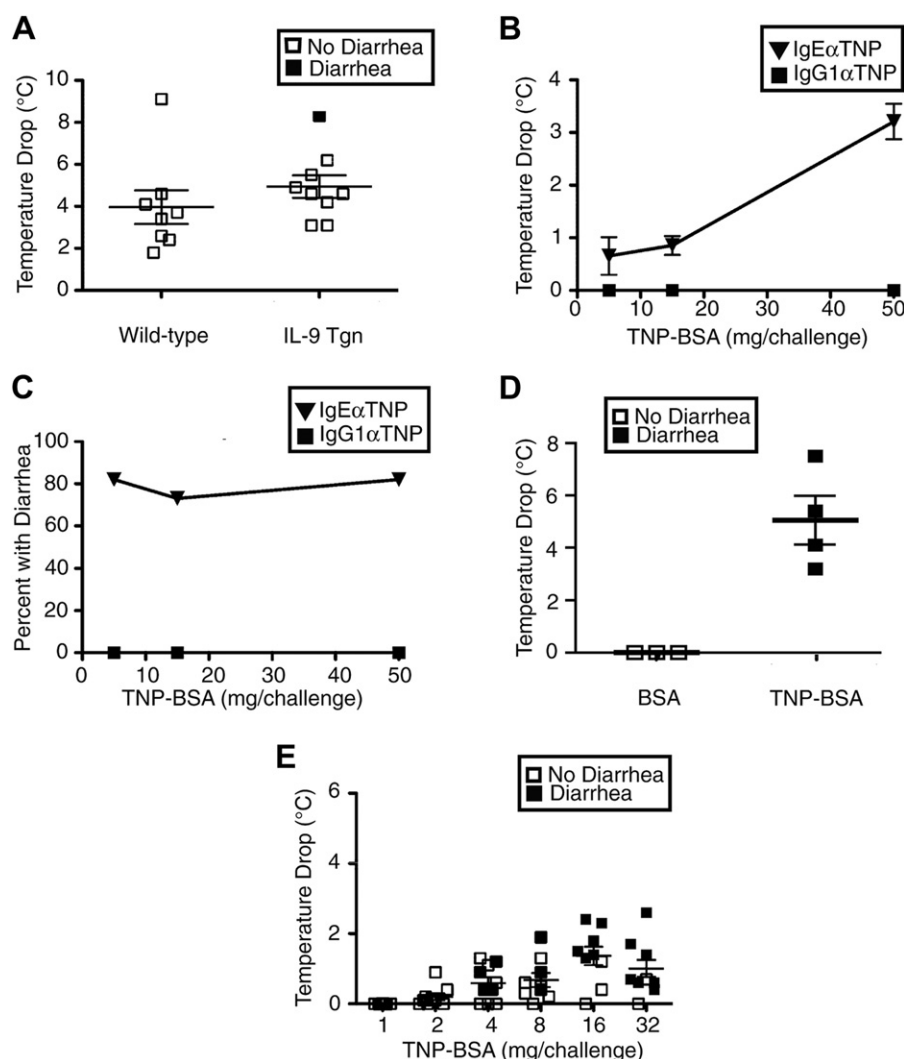


FIG 1. IgE-mediated shock and diarrheal responses to ingested antigens. **A**, Naive BALB/c WT and iIL-9 Tgn mice were injected intravenously with 10 μ g of IgE anti-TNP mAb and then challenged by means of OG 24 hours later with 50 mg of TNP-BSA. Rectal temperatures and development of diarrhea were assessed during the next 60 minutes. **B** and **C**, BALB/c mice (5-6 per group) were inoculated intraperitoneally with OVA/alum and then OG every 2 to 3 days with OVA until they had both hypothermia and diarrhea. Mice were then injected intravenously with either 10 μ g of IgE anti-TNP mAb or 2 mg of IgG anti-TNP mAb; challenged the next day by means of OG with 5, 15, or 50 mg of TNP-BSA; and followed for the next hour for the development of hypothermia and diarrhea. **D**, BALB/c mice sensitized to OVA as in Fig 1, **B**, were challenged by means of OG with 50 mg of BSA or TNP-BSA and followed for the next hour for development of hypothermia and diarrhea. **E**, BALB/c mice sensitized to OVA as in Fig 1, **B**, were injected with 10 μ g of IgE anti-TNP mAb, challenged by means of OG the next day with the doses of TNP-BSA shown, and followed for the next hour for development of hypothermia and diarrhea.

not shown); however, OG of these IgE-primed mice the next day with 50 mg of TNP-BSA induced shock (hypothermia), although it did not induce diarrhea (Fig 1, **A**). To determine whether the presence of small intestinal mastocytosis and increased intestinal permeability would be sufficient for IgE priming and antigen challenge to induce diarrhea and shock, we subjected iIL-9 Tgn mice to the same treatment that had been given to WT mice. Despite the constitutive small intestinal mastocytosis and increased intestinal permeability seen in iIL-9 Tgn mice,¹⁵ only 1 of 9 had diarrhea (Fig 1, **A**). Thus IgE antibody is sufficient to prime immunologically intact naive mice for the development of systemic anaphylaxis in response to antigen ingestion, whereas

induction of allergic diarrhea requires additional stimuli that go beyond increased small intestinal mastocytosis and permeability.

Active intestinal immunization allows passive immunization with IgE mAb, but not IgG₁ mAb, to prime for intestinal anaphylaxis

We hypothesized that pre-existing intestinal allergic inflammation could allow IgE mAb to induce allergic diarrhea in response to ingested antigen. To test this, mice were first immunized intraperitoneally with OVA/alum and then challenged by means of OG every 2 to 3 days with OVA until challenge

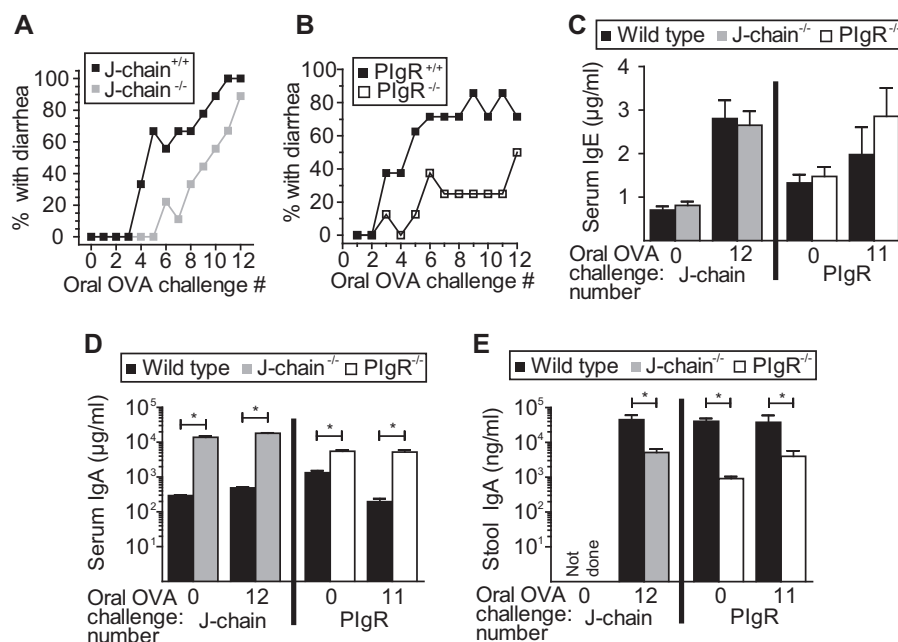


FIG 2. Serum but not secreted IgA delays the development of intestinal anaphylaxis. In separate experiments J chain^{-/-} or pIgR-sufficient or J chain^{-/-} or pIgR-deficient mice (10 per group) were inoculated intraperitoneally with OVA/alum and then challenged multiple times with OVA by means of OG until they had allergic diarrhea. **A** and **B**, The percentages of WT and either J chain^{-/-} (Fig 2, **A**) or pIgR-deficient (Fig 2, **B**) mice with diarrhea within 60 minutes after each oral OVA challenge are shown. Blood was drawn, and fresh feces was collected before the initial OG OVA challenge and after the 11th or 12th challenge. **C-E**, Total serum IgE (Fig 2, **C**), total serum IgA (Fig 2, **D**), and total stool IgA (Fig 2, **E**) levels were determined by means of ELISA. **P* < .05.

induced both diarrhea and shock. These mice were then injected intravenously with IgE anti-TNP mAb and challenged by means of OG the next day with 5 to 50 mg of TNP-BSA. TNP-BSA-challenged mice had both shock (hypothermia; Fig 1, **B**) and diarrhea (Fig 1, **C**), whereas mice challenged with BSA that was not haptenated had neither shock nor diarrhea (Fig 1, **D**). Most mice with shock (temperature decrease >1°C) also had diarrhea, whereas many mice that had diarrhea did not have shock (Fig 1, **E**). Thus once allergic inflammation has been induced in the gut, less antigen is required to induce diarrhea than to induce shock (Fig 1, **B-E**). In contrast to priming with IgE anti-TNP mAb, priming OVA-immune mice with IgG₁ anti-TNP mAb did not allow the development of either shock (Fig 1, **B**) or allergic diarrhea (Fig 1, **C**) in response to TNP-BSA ingestion.

Serum IgA rather than secreted IgA delays development of intestinal anaphylaxis

We have previously demonstrated that antigen-specific IgA antibody is much more effective at blocking the IgE-mediated systemic anaphylactic (shock) response to ingested antigen if it is present in serum than in gut secretions.¹² This indicated that ingested antigen must be absorbed systemically to induce systemic shock. (We define systemic absorption as transit of antigen in the intestinal lumen through the intestinal villus epithelium; by this definition, antigen in the lamina propria of the intestine has been systemically absorbed.) The same requirement was not necessarily true for the induction of IgE-mediated allergic diarrhea by ingested antigen inasmuch as allergic diarrhea is a local rather than a systemic process that requires pre-existing local allergic

inflammation and requires less ingested antigen than induction of shock. In addition, the presence of some intestinal mast cells immediately adjacent to the intestinal lumen¹⁴ suggested that allergic diarrhea might be induced directly by antigen in the gut without a requirement for its systemic absorption. To evaluate this possibility, we first compared the development of allergic diarrhea in actively immunized WT versus J chain^{-/-} mice (Fig 2, **A**) and WT versus pIgR-deficient mice (Fig 2, **B**), with the foreknowledge that defects in immunoglobulin secretion allow normal serum levels of IgE (Fig 2, **C**) in the J chain^{-/-} and pIgR-deficient mice, whereas they cause increased levels of serum IgA (Fig 2, **D**) and decreased levels of intestinal IgA (Fig 2, **E**) in these mice. In contrast to our expectations, allergic diarrhea was slower to develop in the J chain^{-/-} (Fig 2, **A**) and pIgR-deficient (Fig 2, **B**) mice than in WT mice. Because antigen in the gut lumen can only be neutralized by luminal immunoglobulin whereas serum immunoglobulin can neutralize antigen that has been systemically absorbed, the slower development of allergic diarrhea in mice that have high serum IgA but low luminal IgA levels suggests that ingested antigen needs to be absorbed systemically to induce allergic diarrhea.

Antigen-specific IgG₁ suppresses IgE-mediated shock and diarrheal responses to oral antigen challenge through an FcγRIIb-independent mechanism

To confirm this interpretation, we used our hybrid active/passive immunization model to determine whether antigen-specific IgG mAb, which is not secreted into the gut,²⁰ can suppress

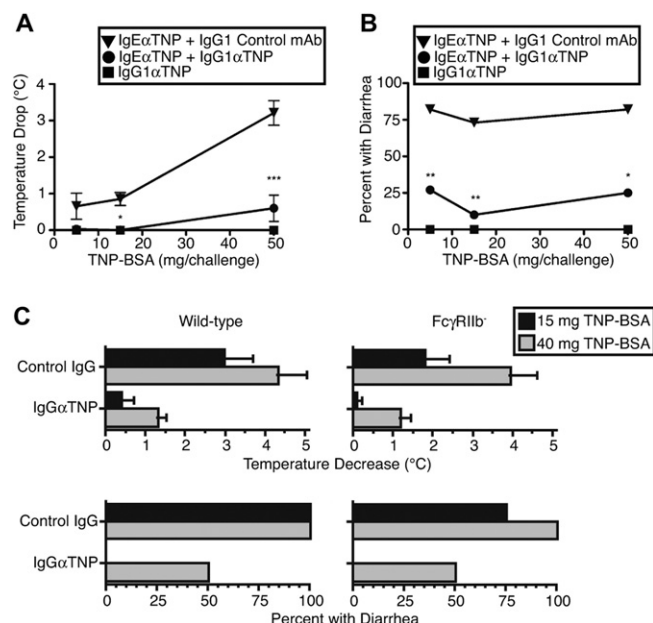


FIG 3. Antigen-specific IgG₁ suppresses IgE-mediated shock and diarrheal responses to oral antigen challenge through an FcγRIIb-independent mechanism. BALB/c mice (5-6 per group) that had been sensitized to OVA as in Fig 1, B, were injected intravenously with 10 μg of IgE anti-TNP mAb. Twenty-four hours later, these mice were injected intravenously with 2 mg of IgG₁ anti-TNP mAb or control IgG₁ mAb and challenged by means of OG 30 minutes later with 5, 15, or 50 mg of TNP-BSA. **A** and **B**, Rectal temperature (Fig 3, A) and diarrhea (Fig 3, B) were assessed for 1 hour. In a separate experiment BALB/c-background WT and FcγRIIb-deficient mice (5-6 per group) were sensitized with OVA as in Fig 1, B, and injected intravenously with 10 μg of IgE anti-TNP mAb. Twenty-four hours later, these mice were inoculated intravenously with 2 mg of IgG₁ anti-TNP mAb or control IgG₁ mAb. Mice were challenged by means of OG 1 hour later with 15 or 40 mg of TNP-BSA. **C** and **D**, Rectal temperature (Fig 3, C) and diarrhea (Fig 3, D) were assessed for the following hour. **P* < .05, ***P* ≤ .01, ****P* < .001.

IgE-mediated allergic diarrheal, as well as shock responses to ingested antigen. Indeed, IgG₁ anti-TNP blocked both shock and diarrhea in OVA-immune mice that were primed with IgE anti-TNP mAb and challenged orally with 5 to 50 mg of TNP-BSA (Fig 3, A and B). IgG₁ might inhibit IgE-mediated shock and allergic diarrhea by intercepting antigen before it could be bound by mast cell-associated IgE, by forming an IgG₁/antigen complex that inhibits FcεRI-mediated mast cell degranulation by cross-linking FcεRI with the inhibitory receptor FcγRIIb, or both.

To determine whether IgG inhibition of IgE-mediated anaphylactic shock and allergic diarrhea involves an interaction between IgG-containing immune complexes and FcγRIIb, we compared the ability of IgG₁ anti-TNP to inhibit these IgE-mediated responses in WT versus FcγRIIb-deficient mice on the same BALB/c background. Results show that IgG-mediated inhibition of IgE-mediated allergic diarrhea is at least as effective in FcγRIIb-deficient mice as in WT mice (Fig 3, C). Thus IgG-mediated inhibition of IgE-mediated allergic diarrhea most likely results from interception of absorbed antigen by IgG antibody before it can bind to mast cell-associated IgE mAb rather than from inhibition of signaling by IgG immune complex ligation of FcγRIIb.

DISCUSSION

The studies described here (1) explain how allergic diarrhea and systemic anaphylaxis can each occur by itself in patients with

food allergy and (2) demonstrate that even allergic diarrhea requires systemic absorption of ingested allergen. Our observation that passive immunization with IgE anti-TNP, followed by ingestion of TNP-BSA, causes shock but not diarrhea indicates that basal mast cell numbers and quantities of antigen absorption are sufficient to induce shock but not diarrhea. This occurs even when iIL-9 Tgn mice, which have increased permeability and mast cell numbers in the small intestine, are passively immunized and challenged. Because most fluid resorption occurs in the large intestine, it seems likely that allergic diarrhea requires increased permeability (and perhaps mastocytosis) of this organ in addition to the small intestine. This is consistent with the requirement for active enteral immunization, which induces mastocytosis and increased permeability in both the small and large intestines,^{15,20,21} to allow allergic diarrhea to develop in mice in response to passive IgE sensitization and oral antigen challenge.

Once actively immunized mice become able to have allergic diarrhea, however, we find that diarrhea can be triggered by less antigen than is required to trigger shock. Because shock is caused by vascular leak (which decreases intravascular volume),²² this most likely reflects a requirement for antigen activation of vascular mast cells rather than gut mast cells to induce systemic anaphylaxis. If so, the induction of diarrhea without shock could be explained by the higher concentration of absorbed antigen in intestinal tissue than in the vasculature after ingestion of a given antigen dose. In contrast, a requirement for induction of gut mastocytosis to induce diarrhea while basal numbers of vascular mast cells are sufficient to induce systemic anaphylaxis could explain why some subjects experience systemic disease without intestinal symptoms. However, we cannot rule out other possible mechanisms that could differentially induce allergic diarrhea versus shock.

One possibility is the production of different sets of vasoactive mediators (eg, predominant production of platelet-activating factor and serotonin, which are associated with allergic diarrhea,⁵ vs predominant production of histamine, which is associated with mast cell-dependent shock¹³) by different mast cell populations. However, although different mast cell populations produce distinct proteases,²³ we are not aware of evidence that different mast cell populations in the mouse secrete distinct subsets or different quantities of vasoactive mediators.

A second possibility is that intestinal inflammation induced by repeated OVA inoculation might permit the selective development of allergic diarrhea by increasing the sensitivity of gut epithelial, smooth muscle, and/or vascular endothelial cells to mast cell-produced mediators. In this regard we have demonstrated that IL-4 and IL-13 have this effect, even at relatively low concentrations.²² Increased concentrations of IL-4 or IL-13 produced by T cells, basophils, and/or nuocytes in the lamina propria might promote diarrhea without systemic shock by predominantly acting locally to increase the sensitivity of intestinal cells but not systemic vascular endothelial cells to mast cell-produced mediators.

In contrast, we find that the induction of both localized and systemic anaphylaxis requires the absorption of ingested antigen, as shown by (1) the delayed development of allergic diarrhea in J chain-deficient and pIgR-deficient mice, both of which have increased plasma IgA levels but very little secreted IgA, and (2) the ability of plasma IgG antibody to block both. Although cross-linking FcεRI with FcγRIIb inhibits IgE-dependent mast cell activation, IgG strongly inhibited both allergic diarrhea and shock in both FcγRIIb-sufficient and FcγRIIb-deficient mice, suggesting

that IgG inhibits IgE-mediated anaphylaxis primarily by intercepting antigen before it can cross-link FcεRI-associated IgE.

Our results have the important practical consequence that induction of high levels of allergen-specific IgG, which can be induced by systemic immunization, should block both allergic diarrhea and systemic anaphylaxis. This is consistent with clinical observations that systemic desensitization therapy that increases IgG antibody levels without decreasing IgE antibody levels can reduce food allergy symptoms²⁴ and provides a mechanistic explanation of why specific antigen desensitization should be an efficacious and, provided that it can be done safely, useful therapy for food allergy.

Key messages

- Intestinal immunity and oral antigen dose determine whether food allergy elicits local symptoms, such as diarrhea, systemic anaphylaxis, or both.
- Ingested antigen must be absorbed to induce either response.
- Desensitization procedures that induce systemic IgG secretion should suppress both local and systemic food allergy.

REFERENCES

1. Wang J, Sampson HA. Food allergy. *J Clin Invest* 2011;121:827-35.
2. Sicherer SH, Sampson HA. Food allergy. *J Allergy Clin Immunol* 2010;125(suppl):S116-25.
3. Berin MC, Mayer L. Immunophysiology of experimental food allergy. *Mucosal Immunol* 2009;2:24-32.
4. Sampson HA. 9. Food allergy. *J Allergy Clin Immunol* 2003;111(suppl):S540-7.
5. Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. *J Clin Invest* 2003;112:1666-77.
6. Gupta R, Sheikh A, Strachan DP, Anderson HR. Time trends in allergic disorders in the UK. *Thorax* 2007;62:91-6.
7. Sicherer SH. Epidemiology of food allergy. *J Allergy Clin Immunol* 2010;127:594-602.
8. Branum AM, Lukacs SL. Food allergy among children in the United States. *Pediatrics* 2009;124:1549-55.
9. 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.
10. Hsieh KY, Tsai CC, Wu CH, Lin RH. Epicutaneous exposure to protein antigen and food allergy. *Clin Exp Allergy* 2003;33:1067-75.
11. Li XM, Schofield BH, Huang CK, Kleiner GI, Sampson HA. A murine model of IgE-mediated cow's milk hypersensitivity. *J Allergy Clin Immunol* 1999;103:206-14.
12. Strait RT, Mahler A, Hogan S, Khodoun M, Shibuya A, Finkelman FD. Ingested allergens must be absorbed systemically to induce systemic anaphylaxis. *J Allergy Clin Immunol* 2011;127:982-9.e1.
13. Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. *J Allergy Clin Immunol* 2002;109:658-68.
14. Alizadeh H, Urban JF Jr, Katona IM, Finkelman FD. Cells containing IgE in the intestinal mucosa of mice infected with the nematode parasite *Trichinella spiralis* are predominantly of a mast cell lineage. *J Immunol* 1986;137:2555-60.
15. Forbes EE, Groschwitz K, Abonia JP, Brandt EB, Cohen E, Blanchard C, et al. IL-9- and mast cell-mediated intestinal permeability predisposes to oral antigen hypersensitivity. *J Exp Med* 2008;205:897-913.
16. Johansen FE, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, et al. Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. *J Exp Med* 1999;190:915-22.
17. Hendrickson BA, Conner DA, Ladd DJ, Kendall D, Casanova JE, Cortesby B, et al. Altered hepatic transport of immunoglobulin A in mice lacking the J chain. *J Exp Med* 1995;182:1905-11.
18. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV. Augmented humoral and anaphylactic responses in FcγRII-deficient mice. *Nature* 1996;379:346-9.
19. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and FcγRIIb cross-linking. *J Clin Invest* 2006;116:833-41.
20. Brandtzaeg P, Bjerke K, Kett K, Kvale D, Rognum TO, Scott H, et al. Production and secretion of immunoglobulins in the gastrointestinal tract. *Ann Allergy* 1987;59:21-39.
21. Forbes D, Patrick M, Perdue M, Buret A, Gall DG. Intestinal anaphylaxis: in vivo and in vitro studies of the rat proximal colon. *Am J Physiol Gastrointest Liver Physiol* 1988;255:G201-5.
22. Strait RT, Morris SC, Smiley K, Urban JF Jr, Finkelman FD. IL-4 exacerbates anaphylaxis. *J Immunol* 2003;170:3835-42.
23. Reynolds DS, Stevens RL, Lane WS, Carr MH, Austen KF, Serafin WE. Different mouse mast cell populations express various combinations of at least six distinct mast cell serine proteases. *Proc Natl Acad Sci U S A* 1990;87:3230-4.
24. Nowak-Węgrzyn A, Sampson HA. Future therapies for food allergies. *J Allergy Clin Immunol* 2011;127:558-75.