Ingested allergens must be absorbed systemically to induce systemic anaphylaxis

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Background: IgE-mediated food allergy is a common cause of enteric disease and is responsible for approximately 100 systemic anaphylaxis deaths in the United States each year. IgG antibodies can protect against IgE-mediated systemic anaphylaxis induced by injected antigens by neutralizing antigens before they can bind to mast cell–associated IgE.
Objective: We have investigated whether IgA and IgG antibodies can similarly protect against systemic, IgE-mediated anaphylaxis induced by ingested antigens and, if so, whether IgA and IgG antibodies protect by neutralizing antigens before or after their systemic absorption.
Methods: Murine passive and active anaphylaxis models were used to study the abilities of serum versus gut lumenal IgA antibodies and serum IgG antibodies to inhibit systemic anaphylaxis induced by ingested allergens in normal mice, mice deficient in the ability to secrete IgA into the intestines, and mice in which intestinal IL-9 overexpression has induced intestinal mastocytosis and increased intestinal permeability.
Results: IgE-mediated systemic anaphylaxis and mast cell degranulation induced by antigen ingestion are suppressed by both serum antigen-specific IgA and IgG, but not by IgA within the gut lumen.
Conclusion: Systemic rather than enteric antibodies protect against systemic anaphylaxis induced by ingested antigen. This implies that ingested antigens must be absorbed systemically to induce anaphylaxis and suggests that immunization protocols that increase serum levels of antigen-specific, non-IgE antibodies should protect against severe food allergy. (J Allergy Clin Immunol 2011;127:982-9.)

Key words: *Mouse, food allergy, IgE, IgA, IgG, allergic diarrhea, blocking antibodies*

Systemic anaphylaxis, which can be characterized by urticaria, angioedema, bronchospasm, diarrhea, dysrhythmias, and cardiovascular collapse, is responsible for approximately 150,000 emergency department visits,[1] 15,000 hospital admissions,[2,3] and 1,500 deaths[4-6] each year in the United States. Although parenteral allergen administration is more likely to trigger systemic anaphylaxis than ingestion of the same allergen, the high prevalence of food allergy, coupled with the much greater likelihood of eating an allergenic protein than being injected with an allergenic protein, makes food allergy responsible for approximately one third to one fifth of the emergency department visits for anaphylaxis[1,7] and 100 to 200 deaths annually[4,7,8] in the United States. The immune mechanisms that cause food-induced systemic anaphylaxis and the immune mechanisms that may protect against food-induced systemic anaphylaxis are not as well understood as those that promote and protect against parenteral allergen-induced anaphylaxis and are likely different. Systemic anaphylaxis elicited by allergen injection, for example, can be induced in mice by either an IgE/FcεRI/mast cell–dependent mechanism or an IgG/low affinity stimulatory IgG receptor (FcγRIII)/basophil-dependent or macrophage-dependent mechanism,[9,10] whereas triggering of systemic anaphylaxis by allergen ingestion appears to be always or nearly always IgE, FcεRI, and mast cell–dependent.[11,12] In addition, although histamine is the predominant mediator responsible for IgE-dependent anaphylaxis induced by injected allergen, platelet activating factor and serotonin appear to have a more important role in IgE-dependent anaphylaxis induced by ingested allergen.[9,11]

The evidence that immunoglobulin isotypes other than IgE have little or no role in food allergen–induced systemic anaphylaxis, when coupled with evidence that non-IgE antibodies can protect against IgE-mediated anaphylaxis caused by injected antigens by binding allergen epitopes before they can react with mast cell–associated, allergen-specific IgE,[13] raises questions about whether these other isotypes can also protect against food allergen–induced anaphylaxis. Furthermore, if allergen-specific non-IgE antibodies are protective, do they protect by binding ingested allergen in the gut lumen, before it has been absorbed, in the same way that IgA neutralizes intestinal toxins and blocks bacterial binding to epithelial receptors,[14-18] or by binding to allergen after it has been adsorbed systemically? This question is related to an additional issue: does ingested allergen induce systemic anaphylaxis predominantly by activating mucosal mast cells that are interspersed with mucosal epithelial cells at the interface of intestinal villi with the gut lumen, in which case systemic allergen absorption may not be necessary, or by interacting with mast cells that are associated with lymphatics and blood

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Abbreviations used
FcγRIIb: Low-affinity inhibitory IgG receptor
Fcα/μR: Receptor for IgM and IgA
IL-4C: A complex of 2 molecules of IL-4 bound by 1 molecule of a monoclonal neutralizing anti-IL-4 mAb
IL-9 tgn: IL-9 transgenic mice in which transgene expression is controlled by the intestinal fatty acid binding protein promoter
MMCP1: Mouse mast cell protease 1
OVA: Ovalbumin
PIgR: Polymeric immunoglobulin receptor
TNP: Trinitrophenyl
TNP-OVA: Trinitrophenylated ovalbumin

vessels, in which case systemic absorption is likely to be important?

These questions have clinical implications: if ingested allergens do not have to be absorbed systemically to induce systemic anaphylaxis, allergen-specific antibody, presumably of the IgA isotype, would have to be secreted into the gut lumen to intercept allergen before it could activate mast cells and induce anaphylaxis. In contrast, if induction of systemic anaphylaxis by ingested allergens requires their systemic absorption, then IgG and nonsecretory IgA antibodies should be able to inhibit systemic anaphylaxis induced by ingested allergens. These alternative possibilities should influence strategies for the optimal induction of antibodies able to inhibit food allergy–related systemic anaphylaxis.

To address these issues, we have used both passive and active anaphylaxis models to study the ability of secreted versus nonsecreted IgA antibodies and IgG antibodies to inhibit systemic anaphylaxis induced by ingested allergens in 3 models: (1) normal mice that have been sensitized passively by injection of a trinitrophenyl (TNP)-specific IgE antibody, (2) IgE anti-TNP mAb passively sensitized mice in which intestinal IL-9 overexpression has induced intestinal mastocytosis, and (3) normal mice in which intraperitoneal followed by oral immunization with ovalbumin (OVA) has induced both intestinal mastocytosis and an IgE anti-OVA antibody response. Our observations provide evidence for suppression of systemic anaphylaxis by both IgG and IgA antibodies and for better suppression of systemic anaphylaxis by systemic rather than by enteric IgA. These observations support a requirement for systemic absorption of ingested allergens to induce systemic anaphylaxis and favor the adoption of immunization strategies capable of inducing high titers of IgG antibodies to anaphylaxis-inducing food allergens.

METHODS

Mice

BALB/c mice, BALB/c background IL-9 transgenic mice in which transgene expression is controlled by the intestinal fatty acid binding protein promoter (IL-9 tgn),[12] polymeric immunoglobulin receptor (PIgR)–deficient mice (Jackson Lab, Bar Harbor, Me),[19] Receptor for IgM and IgA (Fcα/μR)–deficient mice,[20] J-chain–deficient mice (a gift from Dennis Metzger, Albany Medical College),[21] and low-affinity inhibitory IgG receptor (FcγRIIb)–deficient mice[22] were 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

TNP-labeled OVA was prepared as previously described.[13] OVA, Pristane and propranolol were purchased from Sigma (St Louis, Mo). Serum levels of mouse mast cell protease 1 (MMCP-1) were measured by ELISA according to the manufacturer's instructions (Moredun Scientific, Midlothian, United Kingdom).

Hybridomas

The following hybridomas were grown as ascites in Pristane-primed athymic nude mice and purified by ammonium sulfate precipitation, followed by DE-52 cation exchange chromatography when appropriate: mouse IgE anti-TNP (IGEL 2a), IgA anti-TNP (2F.11.15), IgA isotype control mAb (J558), and IgG_1 anti-TNP (1B7.11) were from the American Type Culture Collection (Rockville, Md), and rat IgG_1 antimouse IL-4 mAb (11B11) was purchased from Verax (Lebanon, NH). Recombinant mouse IL-4 was purchased from PeproTech (Rocky Hill, NJ).

Polyclonal IgA anti-OVA antibody

Serum rich in IgA anti-OVA antibody was collected from J-chain–deficient mice that were immunized intraperitoneally with 50 μg OVA adsorbed to 1 mg alum and then, starting 2 weeks later, boosted multiple times by oral gavage with 50 mg OVA. Serum pooled from several OVA-immunized mice was heated to 56°C for 30 minutes to inactivate all IgE.

Cytokine administration

IL-4 was administered as a complex (IL-4C) of 2 molecules of IL-4 bound by 1 molecule of a monoclonal neutralizing anti–IL-4 mAb, 11B11, which were mixed 5 minutes or more before injection. This complex dissociates *in vivo* over 3 days, releasing IL-4. IL-4C itself is inactive because 11B11 blocks binding to IL-4Rs. IL-4C does not activate complement or bind more avidly than uncomplexed IgG to Fcγ receptors because it contains a single IgG molecule.[23,24]

Fecal pellet IgA extraction

Five freshly excreted fecal pellets per mouse were collected on ice in a preweighed 1.5-mL tube. The pellets were weighed, and 25 μL protease inhibitor cocktail (P2714; Sigma) in 225 μL PBS was added per 50 mg feces and vigorously mixed to inhibit further protein digestion. Samples were then diluted 10-fold with PBS. Tubes were vigorously mixed until the pellet was completely suspended in solution. Samples were then centrifuged at 3000*g* for 10 minutes and supernatants transferred into fresh 1.5-mL tubes containing 10 μL protease inhibitor cocktail. Samples were then re-centrifuged at 12,000*g* for 5 minutes, and supernatant was collected and stored at –80°C until analyzed.

Passive anaphylaxis model

Mice (5-6/group unless noted otherwise) were injected intravenously with 10 μg IgE anti-TNP mAb followed 24 hours later by oral gavage challenge with trinitrophenylated ovalbumin (TNP-OVA) in 300 μL saline. Some mice were also injected intravenously with 1 μg IL-4C (complex of 1 μg IL-4 with 5 μg 11B11) 24 hours before and with 0.03 mg propranolol 30 minutes before oral gavage challenge. Anaphylaxis severity was determined by change in activity scores and rectal thermometry[9,23] with a Digital Thermocouple Thermometer (Model BAT-12; Physitery Instruments, Clifton, NJ). All studies were repeated at least once to assure reproducibility.

Active anaphylaxis model

Mice (10/group) were injected intraperitoneally with 50 μg OVA adsorbed to 1 mg alum. Starting 14 days later, these mice were inoculated by oral gavage

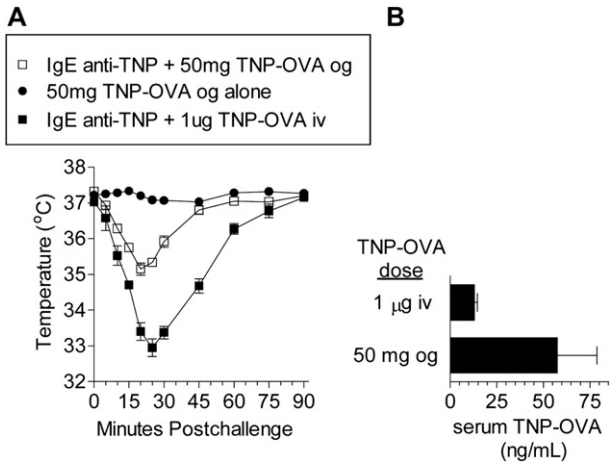

FIG 1. Ingested antigen is rapidly absorbed and can rapidly induce systemic anaphylaxis. **A,** BALB/c mice were injected with 10 μg IgE anti-TNP mAb intravenously *(iv)* and then challenged 24 hours later by oral gavage *(og)* with 50 mg TNP-OVA in 300 μL saline or intravenously with 1 μg TNP-OVA. Another group of mice, not given IgE anti-TNP mAb, was challenged with 50 mg TNP-OVA by oral gavage. Rectal temperatures were followed for 90 minutes (means ± SEMs shown in this and subsequent figures). **B,** BALB/c mice (9-10/group) were inoculated intravenously with 1 μg or by oral gavage with 50 mg of TNP-OVA. Blood was drawn 5 minutes later, and serum TNP-OVA levels were measured by ELISA.

3 times a week with 50 mg OVA in 300 μL saline. Mice were followed for up to 120 minutes after OVA challenges for the development of diarrhea (intestinal anaphylaxis) and hypothermia (an indicator of systemic shock).

Measurement of serum TNP-OVA

A standard sandwich ELISA technique was used with 96-well microtiter plates coated with anti–chicken egg albumin mAb (Sigma) followed by anti—chicken–OVA–horseradish peroxidase as detection antibody (Sigma) and SuperSignal ELISA substrate (Pierce Biotechnology, Cheshire, United Kingdom). Known quantities of TNP-OVA were used as standard.

Measurement of IgA

IgA levels were determined by standard sandwich ELISA, with microtiter plate wells coated with antimouse IgA mAb (BD Bioscience, Franklin Lakes, NJ) followed by sample and standard. After incubation for 60 minutes, antimouse IgA-biotin (BD Bioscience), streptavidin–horseradish peroxidase, and SuperSignal ELISA substrate (Pierce Biotechnology) were added sequentially. Purified mouse IgA (BD Bioscience) was used as standard.

Statistics

The significance of differences in temperature, TNP-OVA, MMCP-1, and IgA concentrations between groups of mice was compared by using the Mann-Whitney *t* test (GraphPad Prism 5.0; GraphPad software, La Jolla, Calif). A P value <.05 was considered significant.

RESULTS

Orally administered TNP-OVA is absorbed systemically and can induce systemic anaphylaxis in mice primed with IgE anti-TNP mAb

In human beings, systemic anaphylaxis can occur within minutes of ingestion of an allergen,[25] suggesting that triggering of anaphylaxis occurs at or near the surface of the gut lumen or that the offending allergen is rapidly absorbed into the systemic circulation in an amount sufficient to induce anaphylaxis. To test the hypothesis that ingested antigens must be rapidly absorbed systemically to induce systemic anaphylaxis, we first evaluated whether ingested antigen can rapidly induce systemic anaphylaxis and be systemically absorbed in sufficient quantity and with sufficient speed to account for systemic shock. BALB/c mice sensitized by intravenous injection of 10 μg of an IgE anti-TNP mAb all developed mild clinical anaphylaxis, manifested as reduced movement, 5 to 10 minutes after intravenous injection of 1 μg TNP-OVA or oral gavage of 50 mg TNP-OVA, although the hypothermia induced by the oral TNP-OVA was considerably less severe than that induced by the intravenous TNP-OVA (Fig 1, *A*). This difference in severity probably resulted from the much higher concentration of TNP-OVA immediately after intravenous challenge with 1 μg TNP-OVA (calculated to be ~800 ng/mL on the basis of a mouse plasma volume of ~1.25 mL) than that induced by oral gavage with 50 mg TNP-OVA, which reaches ~80 ng/mL 5 minutes after gavage (Fig 1, *B*). At this 5-minute timepoint, plasma TNP levels in the intravenously challenged mice had declined to ~10 μg/mL. These observations demonstrate that (1) ingested antigen can be absorbed systemically with a speed consistent with the kinetics of development of systemic anaphylaxis, and (2) the severity of systemic anaphylaxis induced in this system appears to be related more closely to the initial or the peak plasma concentration of antigen to which mast cells are sensitized, rather than to how long the antigen concentration remains elevated.

Pretreatment of mice with IL-4C and propranolol increases sensitivity to anaphylaxis induced by ingested antigen

The requirement for a high oral dose of TNP-OVA to induce anaphylaxis in our system had 2 disadvantages: (1) after adjusting for differences between mouse and human weight, it was disproportionate to the doses of ingested antigen that are known to induce anaphylaxis in some sensitized human beings; and (2) it was too large for it to be practical for us to try to neutralize it by mixing it with an equimolar amount of anti-TNP mAb. To address both issues, we adopted a previous observation that pretreatment with a long-acting form of IL-4 (IL-4C) decreases the dose of injected antigen required to induce anaphylaxis by making mice more sensitive to mediators released by mast cells.[23] This IL-4–dependent increase in sensitivity is observed in mice that have been induced to generate a strong T_H2 response and may also occur in human beings with food allergy. As expected, IL-4C pretreatment decreased the dose of ingested TNP-OVA required to induce measurable shock by a factor of ~50 (see this article's Fig E1 in the Online Repository at www.jacionline.org). To increase sensitivity to ingested antigen further, we also treated mice with the β-adrenergic antagonist propranolol, which inhibits the ability to compensate for the decreased intravascular volume caused by vascular leak, the predominant pathophysiologic mechanism responsible for murine anaphylaxis. Similar to IL-4C, propranolol pretreatment decreased the dose of ingested TNP-OVA required to induce measurable shock around 50-fold (Fig E1). Together, IL-4C and propranolol increased sensitivity to oral antigen challenge ~250-fold, causing mice sensitized by IgE anti-TNP mAb to develop mild hypothermic shock after oral gavage challenge with 100 μg TNP-OVA and severe hypothermic shock after oral gavage challenge with 1000 μg TNP-OVA (Fig E1). This

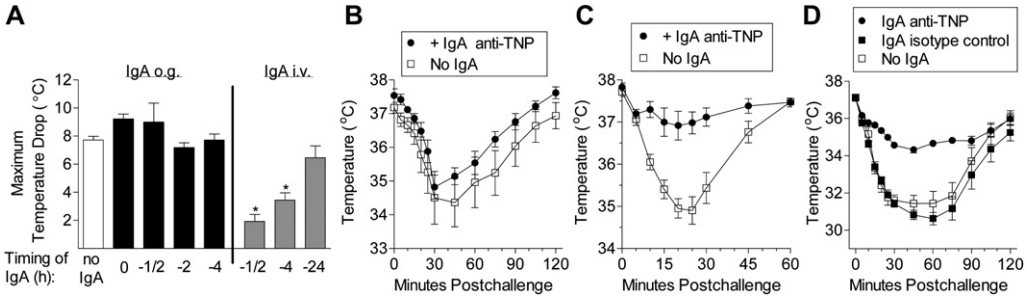

FIG 2. Oral antigen-induced systemic anaphylaxis is inhibited by systemic but not enteric IgA. **A,** BALB/c mice were primed/sensitized with IgE anti-TNP mAb/IL-4C/propranolol (see Methods), then challenged with 1 mg TNP-OVA oral gavage *(o.g.)*. Some mice were also pretreated with IgA anti-TNP mAb (2 mg oral gavage or 1 mg intravenously *[i.v.]*) at the times shown before antigen challenge. Maximum temperature drop ± SEM for each group of mice is shown. *P <.05 compared with no IgA group. **B,** BALB/c mice were primed/sensitized as in *A*, then challenged with 0.3 mg TNP-OVA ± 10 mg IgA anti-TNP mAb oral gavage. Temperatures were followed for 120 minutes. **C,** BALB/c mice were primed with IgE anti-TNP mAb and injected intravenously the next day mice with either saline or 2 mg IgA anti-TNP mAb. Mice were challenged 30 minutes later with 50 mg TNP-OVA oral gavage. Temperatures were followed for 60 minutes. **D,** BALB/c mice were primed/sensitized as in *A*, then injected intravenously with saline, 2 mg IgA anti-TNP, or IgA control mAb (J558). Mice were challenged 30 minutes later with 1 mg TNP-OVA oral gavage, and temperatures were followed for 2 hours.

amount would be closely equivalent on a weight basis to the ingestion of 1 average-size peanut by a typical 8-year-old child.

Anaphylaxis induced by ingested antigen is inhibited by systemic, but not enteric, neutralizing IgA mAb

Because IgG antibody in blood can inhibit IgE-mediated anaphylaxis induced by intravenous antigen injection,[9,13] we hypothesized that IgA antibody, which can be induced by oral vaccination, might be able to suppress anaphylaxis induced by oral antigen ingestion similarly. Surprisingly, mixing TNP-OVA with IgA anti-TNP mAb before oral gavage had no effect on the severity of the systemic anaphylaxis (Fig 2, *A*). Similar negative results were observed when IgA anti-TNP mAb was administered by oral gavage minutes to hours before oral gavage challenge with TNP-OVA (Fig 2, *A*) and when the dose of IgA anti-TNP mAb was increased to make the molar ratio of anti-TNP mAb to TNP 1.9. (Fig 2, *B*). In contrast, intravenous injection of a considerably lower amount of IgA anti-TNP mAb (molar mAb to antigen ratio of 0.05) in similarly sensitized mice immediately before TNP-OVA oral gavage significantly inhibited anaphylaxis (Fig 2, *A*). Intravenous injection of IgA anti-TNP mAb even inhibited systemic anaphylaxis when mice that had not been pretreated with IL-4C or propranolol were inoculated with 50 mg TNP-OVA by oral gavage (Fig 2, *C*). The greater inhibitory effect of intravenous injected IgA mAb than ingested IgA mAb makes sense—the molar ratio of intravenous injected IgA anti-TNP mAb to the small percentage of ingested TNP-OVA that is absorbed systemically is much higher than the molar ratio of ingested IgA anti-TNP mAb to ingested TNP-OVA in all of these experiments—but only if ingested antigen has to be absorbed systemically to induce systemic anaphylaxis.

These results could also be explained, however, by the trivial possibility that intravenous IgA mAb injection protected against systemic anaphylaxis by increasing plasma volume and oncotic pressure rather than by neutralizing antigen. Although this seemed unlikely, because all IgA mAb injections were controlled by injection of an equal volume of saline and the oncotic contribution of injected IgA mAb would be small relative to that of plasma albumin, an experiment was performed in which injection of an IgA mAb that lacks relevant antigen specificity was used as a control for IgA anti-TNP mAb. Results of this study (Fig 2, *D*) confirmed the antigen-specific protective effect of IgA anti-TNP mAb.

Intravenous IgA does not have to be secreted to protect against systemic anaphylaxis

Although these observations provide strong evidence that IgA mAb protects against systemic anaphylaxis caused by ingested antigen by neutralizing antigen that had been absorbed systemically, it remained possible that neutralization of antigen by IgA that had been actively secreted into the gut (or was in the process of being secreted) was also important. Indeed, some intravenous injected IgA mAb appears 2 to 4 hours later in defecated feces, although the amount is ~100-fold less than the amount of ingested IgA that is recovered in feces within 1 hour (Fig 3, *A and B*). To limit the amount of intravenous injected IgA anti-TNP mAb that could be secreted into the gut lumen, we injected this mAb into mice deficient in PIgR, which is required to secrete IgA.[19,26] Only trivial quantities of IgA mAb injected into these mice could be recovered in feces, even when PIgR mice had first been induced by an active OVA immunization protocol to develop allergic diarrhea, which is accompanied by a considerable increase in intestinal permeability (Fig 3, *C*). Despite the very limited passage of intravenous injected IgA mAb into the gut lumen in PIgR mice, intravenous injected IgA anti-TNP mAb protected PIgR-sufficient and deficient mice equally well against anaphylaxis induced by TNP-OVA ingestion (Fig 3, *D*). Similar results were obtained by using J-chain–deficient mice, which are also defective in their ability to secrete IgA into the intestinal lumen[18,21] (data not shown). Thus, active secretion of IgA antibody does not contribute to its ability to protect against anaphylaxis.

To confirm this conclusion, we compared the abilities of IgA anti-TNP mAb (which can be secreted into the gut of

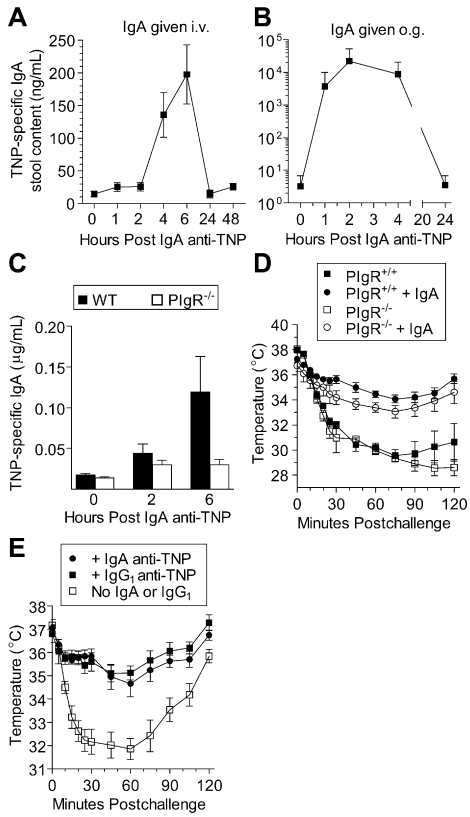

FIG 3. IgA does not need to be secreted into the gut to inhibit systemic oral antigen-induced anaphylaxis. **A,** BALB/c mice (n = 5) were injected intravenously *(i.v.)* with 2 mg IgA anti-TNP mAb; 5 fresh stool pellets were collected from each mouse 0 to 48 hours postinjection. Stool IgA anti-TNP mAb content was determined by ELISA. **B,** BALB/c mice (n = 5) were inoculated by oral gavage *(o.g.)* with 2 mg IgA anti-TNP mAb. Stool IgA was analyzed as in *A*. **C,** PIgR-sufficient and deficient mice (n = 8-9/group) were sensitized by using our active anaphylaxis model. After 6 OVA oral gavage challenges, the mice were rested 3 days, then injected intravenously with 2 mg IgA anti-TNP mAb; 5 fresh stool pellets were collected from each mouse 0 to 6 hours postinjection. Stool IgA was analyzed as in *A*. **D,** PIgR-deficient and sufficient mice were primed/sensitized by using our passive anaphylaxis model; half also received 2 mg IgA anti-TNP mAb intravenously. All mice were challenged 30 minutes later by oral gavage with 1 mg TNP-OVA. Temperatures were followed for 2 hours. **E,** BALB/c mice were primed/sensitized by using our passive anaphylaxis model. The next day, mice were injected intravenously with 2 mg IgA anti-TNP mAb, 2 mg IgG_1 anti-TNP mAb, or saline, and 30 minutes later challenged by oral gavage with 1 mg TNP-OVA. Temperatures were followed for 2 hours.

PIgR-sufficient and J-chain–sufficient mice) and IgG_1 anti-TNP mAb (which cannot be secreted) to protect wild-type, IgE anti-TNP mAb-sensitized BALB/c mice against systemic anaphylaxis induced by TNP-OVA ingestion. Both IgA and IgG_1 mAbs bound TNP-OVA with high affinity (not shown). The 2 immunoglobulin isotypes protected equally well against anaphylaxis induced by ingested TNP-OVA (Fig 3, *E*), confirming the lack of importance of antibody secretion in protection against oral antigen-induced systemic anaphylaxis.

Systemic IgA mAb protects against mast cell degranulation induced by ingested antigen

Because systemic anaphylaxis in our system is mast cell–dependent,[9,13] IgA mAb would be expected to protect against anaphylaxis by inhibiting mast cell degranulation. To evaluate this expectation more directly, we measured serum levels of MMCP-1, an enzyme released by degranulating mast cells,[9,11,12,23] in IgE–anti-TNP mAb-sensitized mice that had been challenged orally with TNP-OVA after injection of IgA anti-TNP mAb or saline. As expected, IgA anti-TNP pretreatment considerably reduced the MMCP-1 response to oral TNP-OVA challenge (Fig 4).

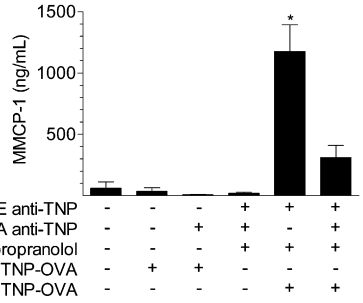

FIG 4. Systemic IgA inhibits mast degranulation induced by ingested antigen. BALB/c mice (n = 5/group) were left untreated or injected intravenously with the reagents shown. Some mice were challenged by oral gavage with 1 or 50 mg TNP-OVA. Blood was obtained 2 hours post-challenge or, for unchallenged mice, 2.5 hours after propranolol or IgA injection. Serum MMCP-1 levels (means ± SEMs) were measured by ELISA. **P* <.05 compared with untreated/unchallenged mice.

IgA anti-TNP suppresses passive IgE anaphylaxis in mice with increased intestinal mast cell load

All studies up to this point had been performed with wild-type mice that had been passively sensitized with IgE and therefore had baseline numbers of intestinal mast cells. Because human beings with food allergies have increased numbers of intestinal mast cells[27,28] and increased numbers of intestinal mast cells are associated in mice with increased intestinal permeability,[12,29] it was possible that mastocytosis and increased intestinal permeability might eliminate the systemic absorption requirement for ingested antigen to induce systemic anaphylaxis. If so, injected IgA mAb would lose its ability to protect against systemic anaphylaxis. To test this possibility, we studied mice that express an IL-9 transgene regulated by the intestinal fatty acid binding promoter (which induces gene expression only in small intestinal enterocytes).[12] IL-9 tgn mice have increased intestinal permeability as well as a large increase in the number of intestinal mucosal mast cells; these abnormalities make it possible to induce systemic anaphylaxis in these mice by challenge with a relatively low concentration of TNP-OVA after sensitization with IgE anti-TNP mAb in the absence of pretreatment with IL-4C or propranolol. Even in this system, intravenous injection of IgA anti-TNP mAb still protected against anaphylaxis induced by oral ingestion of TNP-OVA (Fig 5). Thus, systemic IgA protects against induction of anaphylaxis by ingested antigen even in mice that have the intestinal mastocytosis characteristic of chronic allergic inflammation. Antigen must be absorbed systemically, even under these circumstances, to induce systemic anaphylaxis.

Fcα/μR is not involved in IgA-mediated protection

Mice express a functional receptor for both IgA and IgM (Fcα/μR).[30,31] Because it is not known whether this receptor has stimulatory or inhibitory effects on cellular activation or whether

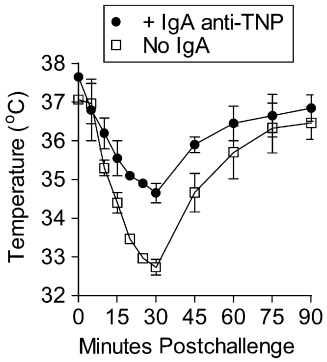

FIG 5. IgA suppresses oral antigen–induced IgE-mediated anaphylaxis in IL-9 tgn mice. Intestinal IL-9 tgn mice (7-8/group) were injected intravenously with 10 μg IgE anti-TNP mAb, and then 24 hours later injected intravenously with 2 mg IgA anti-TNP mAb or control mouse immunoglobulin. Thirty minutes later, all mice were challenged by oral gavage with 10 mg TNP-OVA, and temperatures were followed for 2 hours.

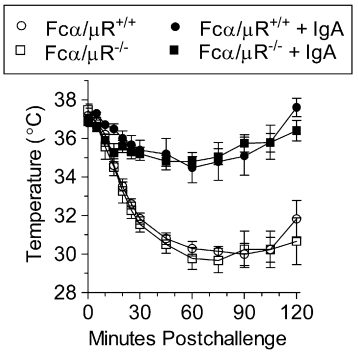

FIG 6. Fcα/μR is not involved in IgA-mediated protection against oral antigen-induced anaphylaxis. Fcα/μR-sufficient and deficient mice were primed/sensitized by using our passive anaphylaxis model. The next day, some mice were injected intravenously with 2 mg IgA anti-TNP mAb. Mice were challenged by oral gavage 30 minutes later with 1 mg TNP-OVA, and temperatures were followed for 2 hours.

it is expressed on mast cells, we could not rule out the possibility that signaling through this receptor, rather than IgA interception of antigen, was involved in IgA protection against systemic anaphylaxis. To address this possibility, we evaluated the ability of IgA anti-TNP mAb to protect Fcα/μR-sufficient and deficient mice[20] against the induction of IgE-mediated anaphylaxis by ingested TNP-OVA. Results of this experiment demonstrate equal protection by IgA anti-TNP mAb in Fcα/μR-sufficient and deficient mice (Fig 6); thus, signaling through this receptor does not contribute to IgA-mediated protection against IgE-dependent anaphylaxis. Similar studies with FcγRIIb-deficient mice demonstrated that this inhibitory receptor, which has been described to interact indirectly with IgA through galectin-3,[32-34] is also not involved in IgA-mediated protection against IgE-dependent anaphylaxis (data not shown).

Antigen-specific IgA-rich serum can prevent oral antigen–induced systemic anaphylaxis in an active immunization food anaphylaxis model

Although the use of a passive anaphylaxis model facilitates interpretation of experiments by allowing tight definition of the isotypes and quantities of antibodies present, it was possible that the requirement for systemic absorption of ingested antigen might differ in a more complex active anaphylaxis model, in which immunized mice produce multiple isotypes of antigen-specific antibody. To evaluate the possible role of IgA as an inhibitor in an active anaphylaxis model, we sensitized wild-type mice to OVA by injecting them with OVA/alum intraperitoneally followed by several oral OVA challenges until the mice developed systemic anaphylaxis to the oral OVA challenge. After 3 days of rest, mice were injected intravenously with two 0.5-mL aliquots of pooled heat-inactivated serum from OVA-immunized J-chain–deficient mice, which has several fold higher titers of OVA-specific IgA than serum from similarly immunized WT mice (data not shown), or with heat-inactivated serum from unsensitized mice. Fig 7 demonstrates that the IgA-rich OVA-immune serum from J chain–deficient mice effectively inhibited systemic anaphylaxis. Although we cannot exclude the possibility that protection is mediated by transferred serum IgG, instead of IgA, this would still be consistent with our central hypothesis that ingested antigen must

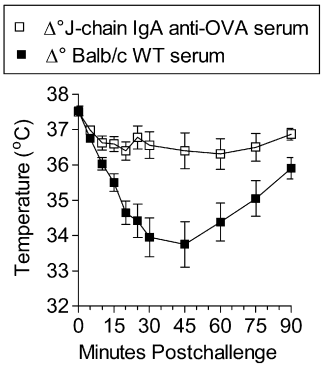

FIG 7. Intravenous antigen-specific IgA inhibits oral antigen–induced systemic anaphylaxis in an active immunization food anaphylaxis model. BALB/c mice (n = 10/group) were sensitized to OVA using our active anaphylaxis model. After the sixth OVA challenge and 3 days of rest, mice were injected intravenously with a total of 1 mL heat-inactivated (Δ°) serum from OVA-immunized J-chain–deficient mice or heat-inactivated serum from naive WT mice. Thirty minutes later, the mice were challenged by oral gavage with 50 mg OVA. Temperatures were followed for 90 minutes.

be absorbed systemically to induce systemic anaphylaxis, even when mice are sensitized by active immunization.

DISCUSSION

Inspired by the demonstration that IgG antibody can suppress IgE-mediated anaphylaxis induced by intravenously injected antigen,[13] we evaluated whether a non-IgE antibody isotype can also protect against IgE-mediated systemic anaphylaxis induced by an ingested antigen. These studies, which are justified by the high frequency and potential severity of IgE-mediated food allergy,[3,5-7] focused on IgA antibody. This focus was warranted by the greater production of IgA than any other isotype, with most IgA produced and secreted at mucosal surfaces[35]; by demonstration in murine IgE-mediated food allergy models of an inverse correlation between food allergy severity and IgA titers[36,37]; and by the association of human IgA deficiency with increased atopic disease and asthma.[38] Furthermore, studies of the protective effects of IgA against enteric pathogens[14-16] and toxins[17,18] seemed to provide a model of how IgA might prevent anaphylaxis caused by ingested allergens. IgA can inhibit systemic disease

caused by pathogens that gain entry to mucosal surfaces by blocking pathogen molecules required for binding to mucosal surfaces and can similarly inhibit the effects of toxins produced by intestinal pathogens by neutralizing those toxins before they can bind to host receptors. Consequently, it seemed reasonable to hypothesize that IgA antibodies to allergens could inhibit food allergen–induced anaphylaxis by binding to allergens before they could be absorbed systemically.

However, our experimental results do not support this hypothesis and instead point to the surprising conclusion that ingested antigen must be absorbed systemically to induce systemic anaphylaxis. Mixing 10 mg IgA anti-TNP mAb with 300 μg TNP-OVA before oral gavage inoculation failed to inhibit systemic anaphylaxis in mice primed with IgE anti-TNP (Fig 2, *B*), whereas intravenous injection of a considerably lower dose of IgA anti-TNP mAb inhibited systemic anaphylaxis induced by ingestion of an even higher dose of TNP-OVA (Fig 2, *A*). Intravenously injected IgA anti-TNP mAb was just as effective at inhibiting TNP-OVA–induced IgE-mediated anaphylaxis in PIgR-deficient and J chain–deficient mice (Fig 3, *D*), which secrete only trivial quantities of IgA even in the presence of allergic intestinal inflammation, as in nonimmune wild-type mice (Fig 3, *C*). Similarly, IgG anti-TNP mAb, which cannot be secreted into the gut lumen even in WT mice, was as effective as IgA anti-TNP mAb at suppressing IgE-mediated systemic anaphylaxis induced by ingested TNP-OVA (Fig 3, *E*). The trivial quantities of IgA (or IgG) that leak into the intestinal lumen in these mice (Fig 3, *C*) could not possibly have neutralized the relatively much larger quantities of ingested antigen, inasmuch as the much higher quantities of luminal IgA in mice that had ingested this mAb had no inhibitory effect (Fig 2, *B*). TNP-specific IgA mAb most likely suppressed anaphylaxis induced by TNP-OVA in mice primed with IgE anti-TNP mAb by blocking TNP epitopes before TNP-OVA could bind to and crosslink mast cell–bound IgE anti-TNP rather than by activating inhibitory receptors, as demonstrated by suppression of the MMCP-1 response in our model (Fig 4) and by equivalent suppression in wild-type, Fcα/μR-deficient and FcγRIIb-deficient mice (Fig 6; data not shown). We and others have reported on similar epitope masking by noninflammatory IgG isotypes in other systems.[13,39]

If IgA antibody indeed protects against anaphylaxis by blocking antigen binding to mast cell–associated IgE, it might seem more effective for antibody to bind ingested antigen before it is absorbed systemically, when the distance between antigen and mast cell is relatively great, rather than neutralize antigen only after it has been absorbed, when it is much closer to mast cells. This perspective, however, ignores the difference between the relatively high concentration of ingested antigen in the gut lumen and the much lower systemic concentration of intact ingested antigen, a result of proteolytic digestion of most ingested antigen. Thus, although a small percentage of ingested antigen is absorbed intact with sufficient rapidity to account for the kinetics of the systemic anaphylaxis (Fig 1), the ratio of specific antibody to antigen is much higher systemically than it is in the gut lumen. This should greatly increase the effectiveness of systemic, as opposed to enteric, blocking of critical antigen epitopes by specific antibodies. Although it remains possible that intestinal antibody could sufficiently neutralize antigen in the gut lumen before systemic antigen absorption and block systemic anaphylaxis by this mechanism, the concentrations of specific antibody in the gut would have to be extraordinarily high, because we find that an ~2:1 molar ratio of IgA mAb to antigen has no detectable effect (Fig 2, *B*).

The observation that IgA blocks systemic anaphylaxis predominantly by binding to allergen that has been systemically absorbed indicates that allergen must be absorbed systemically to induce systemic anaphylaxis, as opposed to inducing systemic disease by triggering mast cells at the interface between the villus and the gut lumen. If antigen could induce systemic anaphylaxis by interacting solely with mast cells at the luminal interface, without being absorbed systemically, anaphylaxis would not have been inhibited by mAb restricted to systemic tissues. Our demonstration that this is true even in IL-9 transgenic mice (Fig 5) and in mice actively immunized with OVA (Fig 7), which both have high numbers of intestinal mast cells and increased intestinal permeability, suggests that this will also be true for individuals who have intestinal atopic disease, in whom similar changes develop.[40-43] Most importantly, our conclusion that ingested antigens must be absorbed systemically to induce systemic anaphylaxis suggests that immunization procedures that induce systemic antibody responses should have some capacity to protect against food allergen–induced anaphylaxis and that immunization protocols that induce an IgG response should be as effective as those that induce IgA production at inhibiting systemic responses to food allergens.

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Clinical implications: Induction of a systemic IgG or IgA antibody response against a food allergen should protect against induction of systemic anaphylaxis by ingestion of that allergen.

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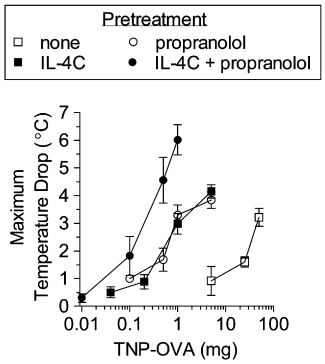

FIG E1. Pretreatment with IL-4C and propranolol increases sensitivity to ingested TNP-OVA. BALB/c mice were injected with 10 μg IgE anti-TNP mAb ± 1 μg IL-4C intravenously. The next day, some of the groups of mice were also injected intravenously with 0.03 mg propranolol or the equivalent volume of saline. Thirty minutes later, all mice were challenged by oral gavage with 10 μg to 50 mg TNP-OVA. Temperatures were followed for 60 minutes. The average maximum temperature drop for each group of mice is shown.