

IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling

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Background: In atopic subjects food ingestion drives the production of IgE antibodies that can trigger hypersensitivity reactions. The IL-4 pathway plays a critical role in this response, and genetic polymorphisms in its components have been linked to allergy.

Objective: We sought to test whether an activating mutation in the IL-4 receptor (IL-4R) α chain enhances allergic responses to a food antigen.

Methods: F709 mice, in which the IL-4R α immunoreceptor tyrosine-based inhibitory motif is inactivated, were gavage fed with ovalbumin (OVA). Reactions to OVA challenge and immune responses, including antibody production and T_H2 responses, were assessed.

Results: F709 mice, but not wild-type control animals, sensitized by means of gavage with OVA and either cholera toxin or staphylococcal enterotoxin B, displayed mast cell activation and systemic anaphylaxis on enteral challenge. Anaphylaxis was elicited even in F709 mice enterally sensitized with OVA alone. Bone marrow chimera experiments established that the increased sensitivity conferred by the F709 genotype was mediated mostly by hematopoietic cells but that nonhematopoietic cells also contributed. F709 mice exhibited increased intestinal permeability to macromolecules. The F709 genotype conferred increased OVA-specific IgE but not IgG1

responses, local and systemic T_H2 responses, and intestinal mast cell hyperplasia compared with wild-type mice. Anaphylaxis was abrogated in F709 mice lacking IgE or the high-affinity receptor for IgE (Fc ϵ RI).

Conclusion: Augmented IL-4R α signaling confers increased intestinal permeability and dramatically enhanced sensitivity to food allergens. Unlike anaphylaxis to injected antigens, which in rodents can be mediated by either IgE or IgG antibodies, the food-induced response in F709 mice is solely IgE dependent. (J Allergy Clin Immunol 2010;■■■■:■■■■-■■■■.)

Key words: Food allergy, IL-4, tolerance, anaphylaxis, IgE

Food-mediated allergic reactions are a growing health problem, with an estimated prevalence of 6% in children and 3% to 4% in the adult population.¹⁻³ The cytokines IL-4 and IL-13 play important roles in the induction and effector phases of allergic responses. They bind to and signal through receptors that share the IL-4 receptor (IL-4R) α chain paired with one of 2 alternative subunits (γ c or IL-13 receptor [IL-13R] α 1) to form the type I or type II IL-4Rs, respectively.^{4,5} IL-4R type I, which exclusively binds IL-4, is present predominantly in hematopoietic cells. The type II receptor is expressed on multiple cell types and recognizes both IL-4 and IL-13. Both play distinct roles in the development of immune responses.⁶⁻⁸

A large assortment of independent human studies, including work from our group, has linked polymorphisms in the IL-4/IL-13 axis with atopy.^{4,9-11} We have used knockin murine models to directly test the effects of such mutations on allergic responses in a homogenous genetic background. Targeted disruption of the I4R site of IL-4R α in mice amplifies IgE responses and elicits enhanced allergic responses to inhaled allergens.¹² Mice with a mutation of the immunoreceptor tyrosine-based inhibitory motif at position 709 (Y $\rightarrow$ F) of IL-4R α also have an allergic phenotype with markedly increased IgE levels and susceptibility to allergen-induced airway inflammation.¹³ There is no human equivalent for the F709 mutation. However, the mutation is prototypic of a number of human IL-4R α polymorphisms that promote receptor signaling and are associated with atopy, including IL-4R α I75V and IL-4R α Q576R.^{4,11} The effects of IL-4R α mutations on cell activation by IL-4 and IL-13 extend to nonhematopoietic cells, which do not express the γ c chain of the type I receptor, suggesting that the allergic phenotype of animals harboring these mutations derives from enhanced signaling through both the type I and II receptors.

In the most severely affected patients, food ingestion can provoke systemic anaphylaxis. In animal models both IL-4 and IL-13 can prime end organs for enhanced anaphylactic responses.¹⁴

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Abbreviations used

CT:	Cholera toxin
DNP:	Dinitrophenyl
FITC:	Fluorescein isothiocyanate
HRP:	Horseradish peroxidase
IL-4R:	IL-4 receptor
LPF:	Low-power field
mMCP-1:	Murine mast cell protease 1
OVA:	Ovalbumin
SEB:	Staphylococcal enterotoxin B

A number of studies have demonstrated that anaphylaxis in mice parenterally immunized with antigen and adjuvant and then challenged intravenously can arise through both IgE- and IgG-mediated mechanisms.¹⁵⁻¹⁸ Food allergen ingestion by mice drives robust IgE responses, as demonstrated by Li et al¹⁹ using peanut extracts. Similarly, Brandt et al²⁰ have shown that repeated enteral administration of ovalbumin (OVA) in mice previously primed intraperitoneally with OVA and alum leads to OVA-IgE production and IgE-dependent mast cell activation with increased intestinal permeability and diarrhea. The animals in this “allergic diarrhea” model do not exhibit hypothermia after enteral challenge (parenteral antigen injection is required to elicit systemic responses).²⁰⁻²³ These responses are IgE and mast cell dependent. However, assessment of the relative contribution of IgE antibodies to food hypersensitivity in purely enterally sensitized and enterally challenged animals has been prevented by the fact that ingestion has a tolerizing effect so that it has been generally difficult to induce robust allergic sensitization by means of intestinal immunization alone.

We hypothesized that activating mutations of IL-4R α would enhance the susceptibility of mice to gastrointestinal allergic responses. To test this hypothesis, we studied the responses of F709 mice enterally exposed to OVA either with or without adjuvants (cholera toxin [CT] or staphylococcal enterotoxin B [SEB]) over 9 weeks and then challenged by means of gavage. OVA gavage of sensitized F709 mice triggered intense systemic anaphylaxis. Bone marrow chimera experiments identified hematopoietic cell IL-4R function as the major driver of this effect. These animals had increased serum levels of OVA-specific IgE. Anaphylactic responses could be elicited even in F709 mice sensitized to OVA in the absence of any adjuvant. Unlike active systemic anaphylaxis after immunization by means of injection, which can be IgG mediated and elicited in mice lacking IgE or Fc ϵ RI, the allergic reactions of F709 mice to ingested OVA were completely IgE dependent. Allergen-exposed F709 mice exhibited a T_H2-biased, systemic OVA-specific response along with increased gut expression of T_H2 cytokine transcripts. They had marked intestinal mastocytosis along with increases in intestinal IL-9 transcripts and increased intestinal permeability to macromolecules. Our findings suggest that amplification of IL-4R α signals facilitate allergic sensitization to ingested antigens, impair tolerance, support intestinal mast cell expansion, and drive IgE-dependent anaphylactic responses.

MATERIALS AND METHODS**Animals**

Wild-type (Y709) BALB/c mice were purchased from Taconic Farms (Germantown, NY). Ig γ ^{-/-} (IgE-deficient [IgE^{-/-}])¹⁶ and *C.129.II4rd*^{F709}

(F709) mice were each bred onto a BALB/c background (10 generations). F709 mice have been deposited at JAX Laboratory (strain name: C.129X1-II4ra<tm3.1Tch>/J; stock no.: 012709). F709/IgE^{-/-} mice were generated by crossing F709 mice with IgE^{-/-} mice. All mice were housed in a specific pathogen-free environment and were 6 to 12 weeks old. All experiments were carried out in accordance with the Institutional Animal Care and Use Committee policies and procedures of Children's Hospital Boston.

Sensitization of mice

For sensitization, Y709, F709, IgE^{-/-}, and F709/IgE^{-/-} mice were treated intragastrically with sterile PBS or 5 mg of OVA (Sigma) and 20 μ g of CT in 250 μ L of sterile PBS (saline) on days 0, 1, 2, and 7, followed by weekly sensitization with OVA and CT for 8 more weeks. In the ninth week, mice were challenged with a large dose (150 mg) of OVA. Responses to OVA alone were studied in mice exposed to 5 mg of OVA, as described above, in the absence of adjuvant.

Measurement of anaphylaxis, symptom scores, and diarrhea

Anaphylaxis was assessed in challenged mice by measuring changes in body temperature and recording symptom scores. Temperature changes were measured with transponders placed subcutaneously 2 days before challenge (Implantable Programmable Temperature Transponder; Biomedic Data Systems, Seaford, Del). Symptom scores were determined according to the criteria shown in Table E1 (available in this article's Online Repository at www.jacionline.org).

Construction of bone marrow chimeras

Recipient mice were irradiated with 2 doses of 600 rads 3 hours apart and were infused immediately with 2.5×10^6 bone marrow cells from congenic donor mice. Mice were treated with co-trimoxazole (Bactrim; Roche, Mannheim, Germany) during a 6-week period of reconstitution and were then subjected to OVA sensitization. Thy1 congenic mice were used, and flow cytometry was used to confirm the effectiveness of bone marrow reconstitution.

Passive anaphylaxis studies

Mice were sensitized intravenously with 4 μ g of anti-dinitrophenyl (DNP)-IgE. Twenty-four hours later, they were challenged intravenously with DNP-HSA, and core body temperature was recorded.

ELISA for murine mast cell protease 1 and total and OVA-specific IgE and IgG1 levels

Murine mast cell protease 1 (mMCP-1), total IgE, OVA-IgE, and OVA-IgG1 ELISAs were performed as previously described.²⁴

Measurement of intestinal permeability

Intestinal permeability was determined as previously described.²⁵ Briefly, mice underwent gavage with 20 mL/kg of 22 mg/mL fluorescein isothiocyanate (FITC)-conjugated dextran (FITC-dextran; molecular mass, 4 kD; Sigma, St Louis, Mo) and 1 mg/mL horseradish peroxidase (HRP; molecular weight, 44 kD; Sigma). Blood was obtained 4 hours later, and the concentration of fluorescein in plasma was determined by means of spectrophotofluorometry by using serially diluted samples of the marker as standard. HRP-captured by anti-HRP on a plate was measured by using the TMB substrate system.

Splenocyte stimulation and ELISAs for IL-4, IL-5, IL-13, and IFN- γ

One million spleen cells were cultured in triplicate with 200 μ g/mL OVA or 0.2 μ g/mL anti-CD3 and anti-CD28 in 1 mL of complete RPMI 1640 with

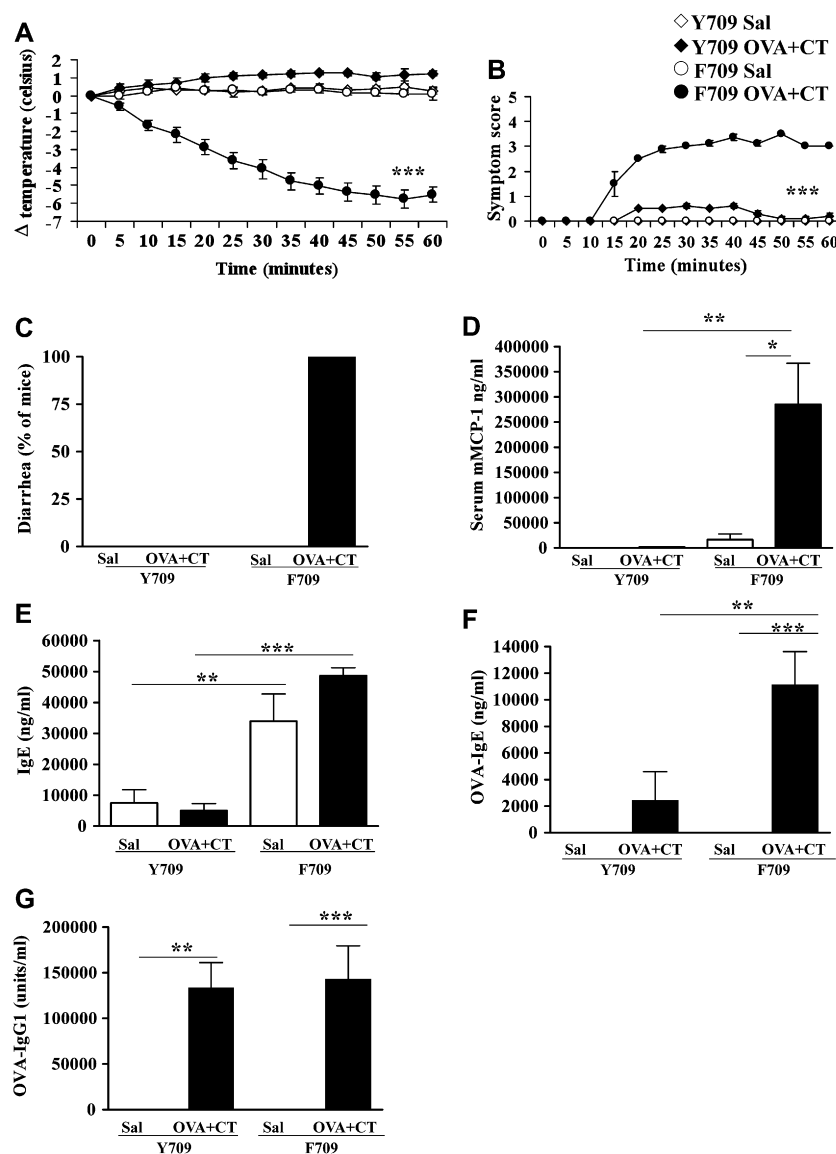


FIG 1. Allergic responses in F709 mice challenged with OVA after enteral sensitization with OVA plus CT. Systemic anaphylaxis was evaluated in mice undergoing oral gavage. Core body temperature (A), symptom score (B), diarrhea (C), serum mMCP-1 levels (D), total IgE levels (E), OVA-IgE levels (F), and OVA-IgG1 levels (G) are shown ($n = 5$ -7 mice). Data are representative of 3 experiments. Sal, Saline. * $P < .05$ and ** $P < .01$, Student t test. *** $P < .001$, ANOVA.

10% FCS. Supernatants were collected at 24, 48, and 96 hours, and levels of IL-4, IL-5, IL-13, and IFN- γ were determined by means of ELISA according to the manufacturer's instructions (IL-4, IL-5, and IFN- γ from BD Biosciences, San Jose, Calif, and IL-13 from R&D Systems, Minneapolis, Minn).

Histologic analysis and enumeration of mast cells

Intestinal mast cells were enumerated by means of microscopic examination of sections of paraffin-embedded jejunal tissue. Tissue sections were stained with chloroacetate esterase. Mast cells were counted in 4 to 5 complete cross-sections of jejunum, as specified in the figure labels.

Quantitative PCR analysis

Quantitative real-time PCR was performed as previously described.²⁶ Expression of IL-4, IL-5, IL-13, IFN- γ , IL-33, IL-10, and IL-9 was calculated relative to glyceraldehyde-3-phosphate dehydrogenase transcripts.

RESULTS

Exposure to oral antigen induces systemic anaphylaxis in F709 mutants but not wild-type Y709 mice

We hypothesized that the F709 mutants, which have enhanced IL-4R signaling, would have exaggerated responses to food allergens. We tested this hypothesis by subjecting F709 mice to enteral sensitization with OVA followed by a gavage challenge. Anaphylaxis was detected by means of noninvasive measurement of body temperature and observation of symptoms (Fig 1, A and B). A dramatic and rapid decrease in core body temperature was observed in F709 mice sensitized with OVA and CT. Enteral challenge of wild-type (Y709) BALB/c mice sensitized by means of this protocol did not elicit significant responses. Similar reactions were elicited in F709 mice sensitized with SEB in place of

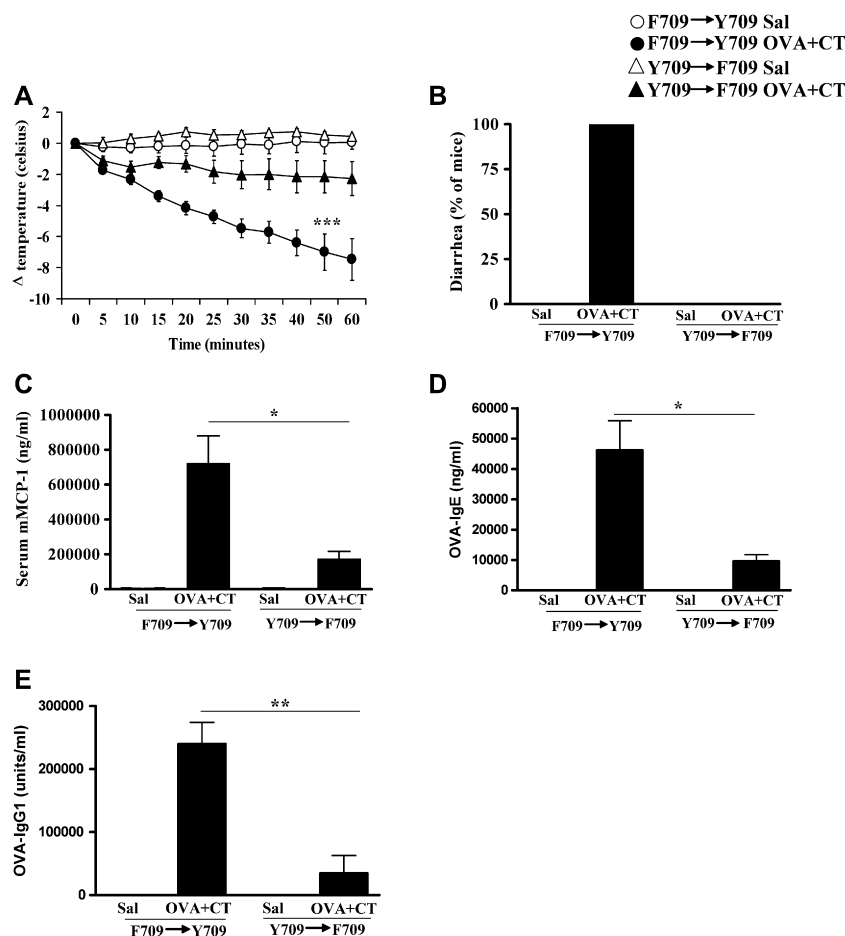


FIG 2. Oral anaphylaxis in bone marrow chimeric mice. Y709/F709 chimeric mice were enterally sensitized and challenged with OVA (Sal) plus CT. Core body temperature (A), diarrhea (B), serum mMCP-1 levels (C), OVA-IgE levels (D), and OVA-IgG1 levels (E) are shown (n = 5-7 mice). *P < .05 and **P < .01, t test. ***P < .001, ANOVA.

CT, as recently described by Ganeshan et al²⁷ (see Fig E1 in this article's Online Repository at www.jacionline.org).

OVA-challenged F709 mice exhibited very obvious signs of anaphylaxis, including decreased activity and periods of inactivity, with no response to prodding or touching. Some mice also exhibited trembling and convulsions (Fig 1, B). All F709 animals and none of the control animals had profuse diarrhea (Fig 1, C). Y709 mice had only minimal symptoms. Levels of mMCP-1, a marker of mast cell degranulation, were increased in OVA-challenged F709 but not Y709 mice, suggesting that mast cell activation drove the physiologic responses (Fig 1, D).

Enterally sensitized F709 mice have increased levels of OVA-specific IgE

Anaphylactic sensitivity to food allergens in human subjects is strongly correlated with the production of high-titer IgE antibodies. Total IgE levels were markedly increased in both control and experimental F709 mice compared with those seen in Y709 mice (Fig 1, E). OVA-IgE responses were detected only in antigen-exposed mice, with levels of $2,459 \pm 2,136$ ng/mL in Y709 mice and $11,159 \pm 2,458$ ng/mL in F709 mice respectively (Fig 1, F). In contrast, and somewhat unexpectedly, anti-OVA

IgG1 responses, which are also T_H2 dependent, were comparable in Y709 and F709 mice treated with OVA and CT (Fig 1, G).

Enhanced anaphylactic sensitivity in F709 mice is conferred primarily by IL-4R effects on hematopoietic cells with a modest contribution from nonhematopoietic cells

Both bone marrow-derived and nonhematopoietic cells, including epithelial cells, express IL-4R. To determine the relative contributions of IL-4R function on hematopoietic versus nonhematopoietic cells to the F709 allergic phenotype, we assessed the responses of bone marrow chimeras. Irradiated Y709 recipients reconstituted with F709 bone marrow had robust anaphylactic responses similar to those observed in F709 animals (Fig 2, A). The induction of anaphylaxis was fatal in at least 2 animals and was accompanied by severe symptoms (data not shown). All of the animals exhibited diarrhea (Fig 2, B) and had increased levels of mMCP-1 (Fig 2, C). In contrast, the development of anaphylaxis in F709 recipients receiving bone marrow from Y709 donors was modest, and only 1 animal had significant decreases in core body temperature (Fig 2, A). Moreover, the presence of diarrhea and the levels of mMCP-1 were also attenuated in these animals.

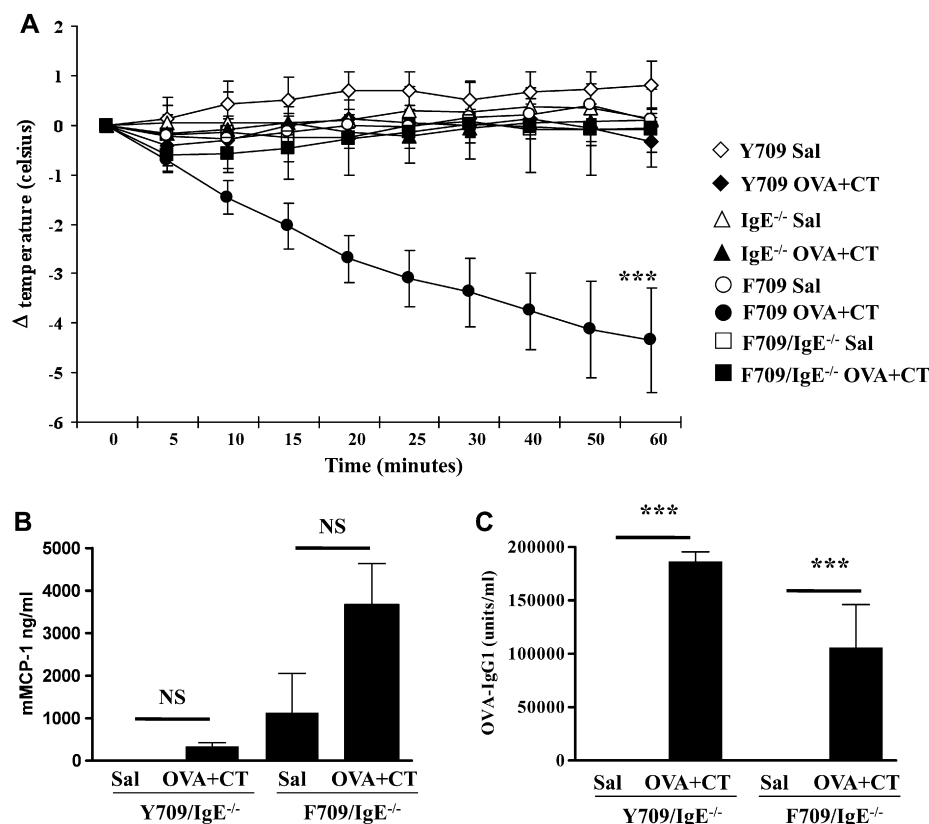


FIG 3. Anaphylaxis and antibody responses in OVA-challenged Y709/IgE^{-/-} and F709/IgE^{-/-} mice enterally sensitized with OVA and CT. **A**, Anaphylaxis was assessed by measuring changes in body temperature. **B** and **C**, After death, the levels of serum mMCP-1 (**Fig 3, B**) and anti-OVA IgG1 (**Fig 3, C**) were determined by means of ELISA ($n = 5-7$ mice). Data are representative of 3 experiments. *NS*, Not significant; *Sal*, Saline. *** $P < .001$, ANOVA.

Determination of antibody responses revealed the presence of enhanced anti-OVA IgE and anti-OVA IgG1 responses in F709 → Y709 mice (**Fig 2, D and E**). These observations indicate that the augmentation of anaphylactic responses in the IL-4R α mutant, F709, is largely mediated by hematopoietic cells, with a modest additional contribution from nonhematopoietic cell types.

Development of anaphylaxis in F709 mice is IgE dependent

In rodents immunized by means of parenteral administration of antigen and adjuvant, systemic anaphylaxis to intravenous antigen challenge can occur in the absence of IgE in a reaction driven by IgG antibodies.^{17,28} To determine the role of IgE in the anaphylactic responses of F709 mice to food antigens, we compared the responses of F709 mice with those of double mutants lacking IgE (F709/IgE^{-/-}). As expected, F709 mice in this experiment again exhibited robust responses to intragastric OVA challenge (**Fig 3, A**). Surprisingly, the development of anaphylaxis was completely abrogated in the absence of IgE in F709/IgE^{-/-} mice (**Fig 3, A**). In contrast to F709 mice, these mice displayed no clinical symptoms and did not exhibit any diarrhea (data not shown). Their mMCP-1 levels (**Fig 3, B**) were only about 1% of those observed in F709/IgE^{+/+} mice (**Fig 1, D**) and were not significantly different from those seen in saline-treated control animals. The dependence of the anaphylactic response on IgE was confirmed in separate experiments in which F709/FceRI^{-/-} double-mutant mice were

sensitized with OVA and SEB (see **Fig E2** in this article's Online Repository at www.jacionline.org). Examination of the levels of OVA-specific IgG1 showed that both groups involved exhibited similar IgG1 responses (**Fig 3, C**). These findings indicate that the presence of a strong IL-4R signal during enteral sensitization has a stronger effect on IgE than on IgG responses and that IgE is required for the expression of systemic anaphylaxis to ingested antigens in animals that have been sensitized enterally.

Sensitization to food allergens in the absence of adjuvant in F709 mice

We hypothesized that increased signaling by IL-4R might enhance allergic sensitization in F709 mice and investigated whether ingestion of OVA alone would provoke responses. Unlike Y709 mice, F709 animals fed OVA alone showed significant responsiveness on challenge (**Fig 4, A**). Diarrhea occurred in some of these animals (**Fig 4, B**), and detectable increases in mMCP-1 levels were present in 5 of 6 mice studied (**Fig 4, C**). OVA-specific IgE responses were also detectable in the majority of animals fed OVA alone (**Fig 4, D**). None of the Y709 mice fed OVA alone generated mMCP-1 or IgE responses. In contrast, both Y709 and F709 mice exhibited enhanced OVA-IgG1 levels (**Fig 4, E**). Such allergic responses to protein antigens ingested in the absence of any adjuvant represents a remarkable and unique feature of the F709 model and strongly indicates that activation of IL-4R α signaling can dampen normal tolerogenic mechanisms.

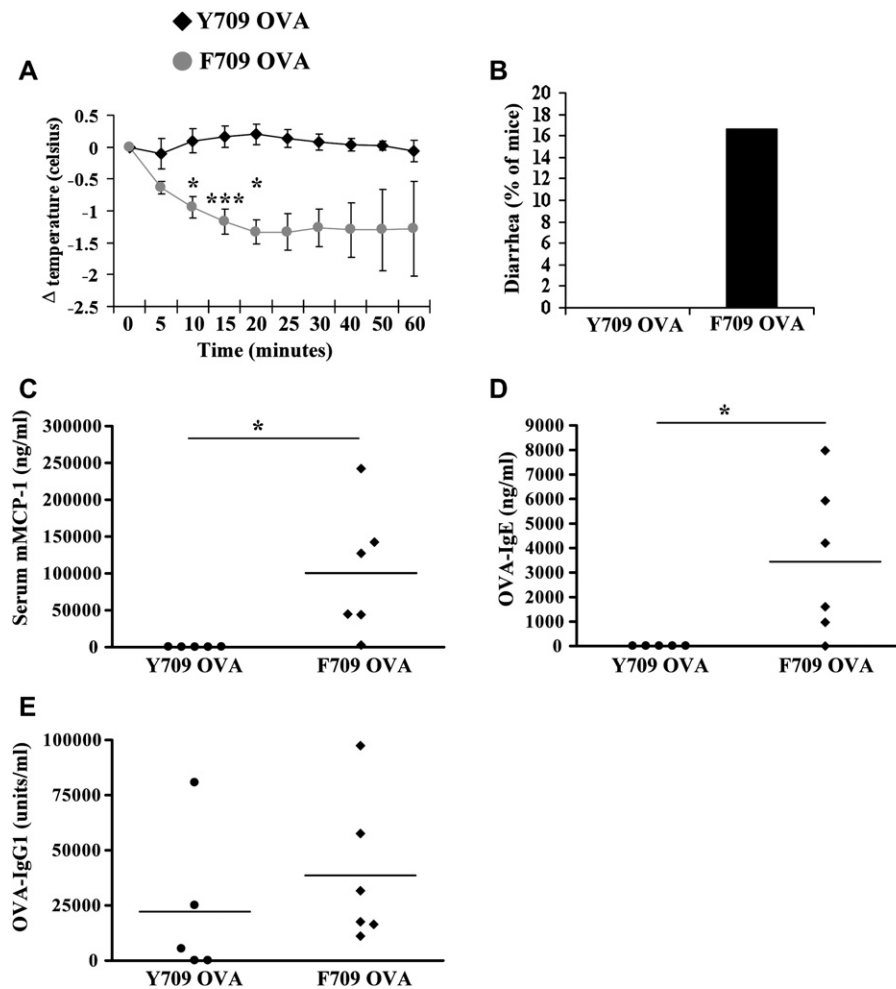


FIG 4. Allergic responses in F709 mice enterally sensitized to OVA in the absence of CT. Mice were sensitized with OVA in the absence of CT and then challenged with OVA. Core body temperature (**A**), diarrhea (**B**), serum mMCP-1 levels (**C**), OVA-IgE levels (**D**), and OVA-IgG1 levels (**E**) are shown ($n = 5-7$ mice). Data are representative of 3 experiments. * $P < .05$ and *** $P < .001$, t test.

Allergen-fed F709 mice have increased numbers of intestinal mast cells

Inspection for chloroacetate esterase reactivity in sections of jejunum revealed the presence of numerous mucosal mast cells both in mice treated with OVA and CT and those receiving OVA alone (Fig 5, A). Enumeration of mast cells showed that OVA- and CT-treated F709 mice had about twice as many mast cells (41 ± 12 cells/5 low-power fields [LPFs]) as Y709 mice (21 ± 4 cells/5 LPFs; Fig 5, B). Similarly, F709 mice receiving OVA alone had 45 ± 15 cells/5 LPFs versus 5 ± 5 cells/5 LPFs for Y709 mice. The F709 animals not only exhibited more mast cells but also had mucosal mast cells high up in the villi, a site that might favor interaction with absorbed food proteins. These results indicate that IL-4R α signaling supports mast cell differentiation in the gut after food allergen exposure and that the intense anaphylactic responses of F709 mice might, in part, be due to the presence of large numbers of these key effector cells. Mast cell expansion was also observed in F709/IgE $^{-/-}$ mice (Fig 5, B), suggesting that IgE antibodies are not required for the recruitment and expansion of intestinal mast cells, as we have previously reported for allergen-induced airway mast cells (see Fig E3 in this article's Online Repository at www.jacionline.org).²⁶ The jejunal mast cells in

F709/IgE $^{-/-}$ mice had a consistently unactivated morphology, whereas many of the mast cells in IgE-sufficient mice exhibited features of degranulation. Levels of mMCP-1 were much higher in F709 than in F709/IgE $^{-/-}$ mice (Fig 3, B, vs Fig 1, D), which is consistent with ongoing IgE-mediated activation.

Enhanced expression of T_H2 cytokines in the intestines of food allergen-exposed F709 mice

We hypothesized that F709 mutants would have enhanced local and systemic T_H2 responses to ingested OVA. Except for IL-10, analysis of jejunal RNA for levels of IL-4, IL-13, IL-5, IL-9, IL-33, and IFN- γ transcripts in Y709 mice exposed to OVA and CT revealed no statistically significant increases in expression of any of these cytokines. In contrast, the relative levels of IL-4, IL-5, IL-13, IL-9, and IL-10 (Fig 6, A-F) were all significantly increased in F709 mice undergoing gavage with OVA and CT in comparison with respective control animals. Similar increases were observed for these cytokines, as well as IL-33, in the group treated with OVA alone (Fig 6). These results indicate that enhanced signaling through IL-4R is permissive for the induction of a local intestinal T_H2 response to food allergens. The intense

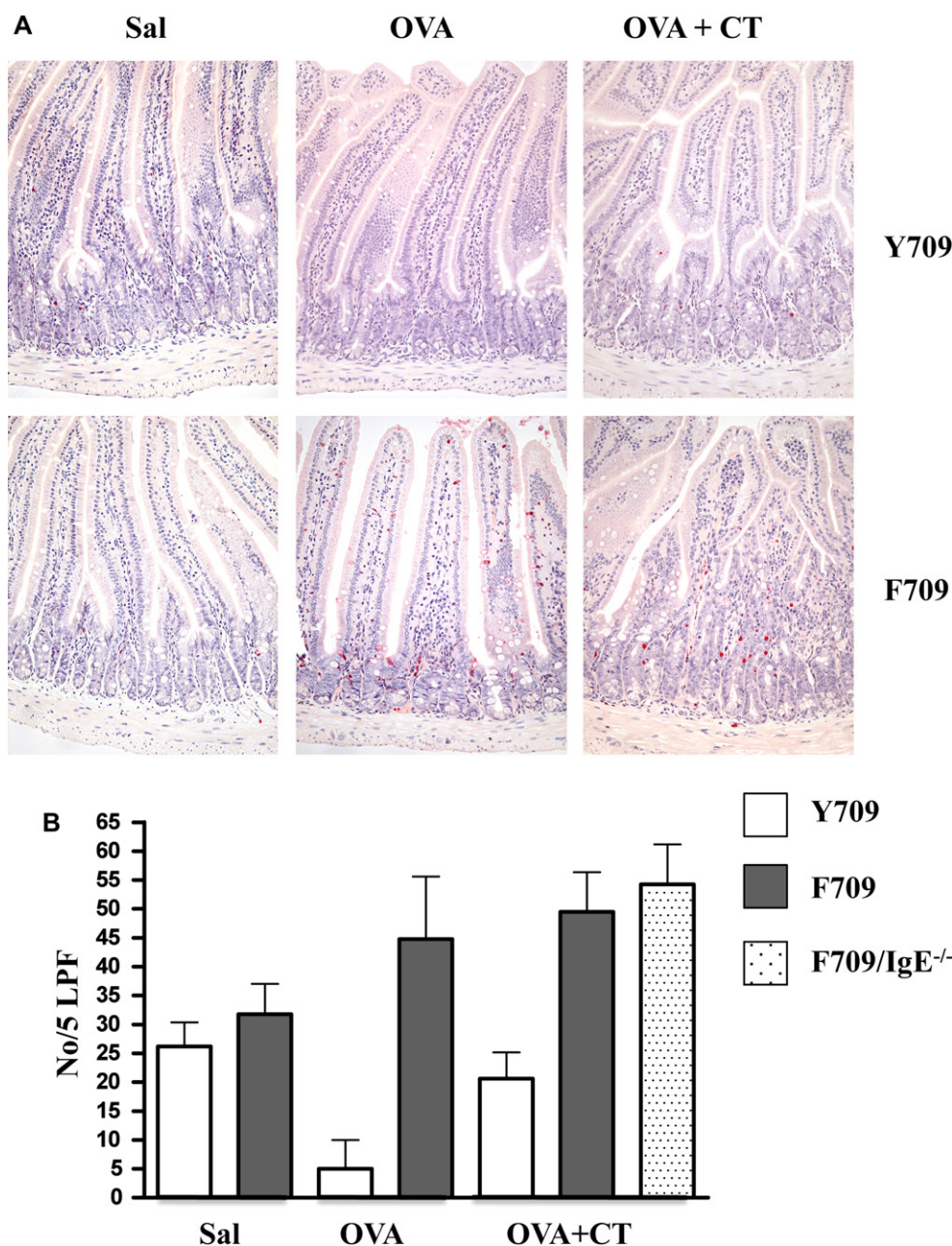


FIG 5. Mast cells in the intestines of OVA-treated F709 mice and Y709 control animals. Mice were treated with OVA and CT or OVA alone. Chloroacetate esterase-reactive cells were identified in sections of jejunum. **A**, Jejunal sections. Mast cells appear red. **B**, Mast cell counts per 5 LPFs ($n = 5-7$ mice). Data are representative of 3 independent experiments. *Sal*, Saline.

induction of IL-9, a cytokine that also supports mast cell development and recruitment, suggests a possible mechanism for the mast cell responses to food allergen observed in the F709 mice.

Systemic T_H2 skewing and T-cell activation in food allergen-treated F709 mice

To test systemic T_H2 responses to food allergens, we measured IL-4, IL-13, and IL-5 levels in the supernatants of spleen cells from saline and CT-, OVA and CT-, and OVA only-treated mice stimulated with OVA (see Fig E4 in this article's Online Repository at www.jacionline.org). As expected, F709 mice mounted strong systemic T_H2 anti-OVA responses, with

markedly more increased splenocyte production of IL-4, IL-13, and IL-5 than either saline-sensitized control animals or OVA-sensitized Y709 mice. Similarly, the levels of serum IL-4 were also significantly enhanced in these animals. These observations confirm that the local T_H2 response to OVA observed in the intestines of F709 mice translates into a strong systemic response.

Target tissues of anaphylaxis of F709 mice exhibit similar sensitivity to mediators of anaphylaxis as do those of Y709 control animals

The abrogation of anaphylaxis in F709/IgE^{-/-} mice and the enhanced levels of OVA-IgE in F709 versus Y709 control animals

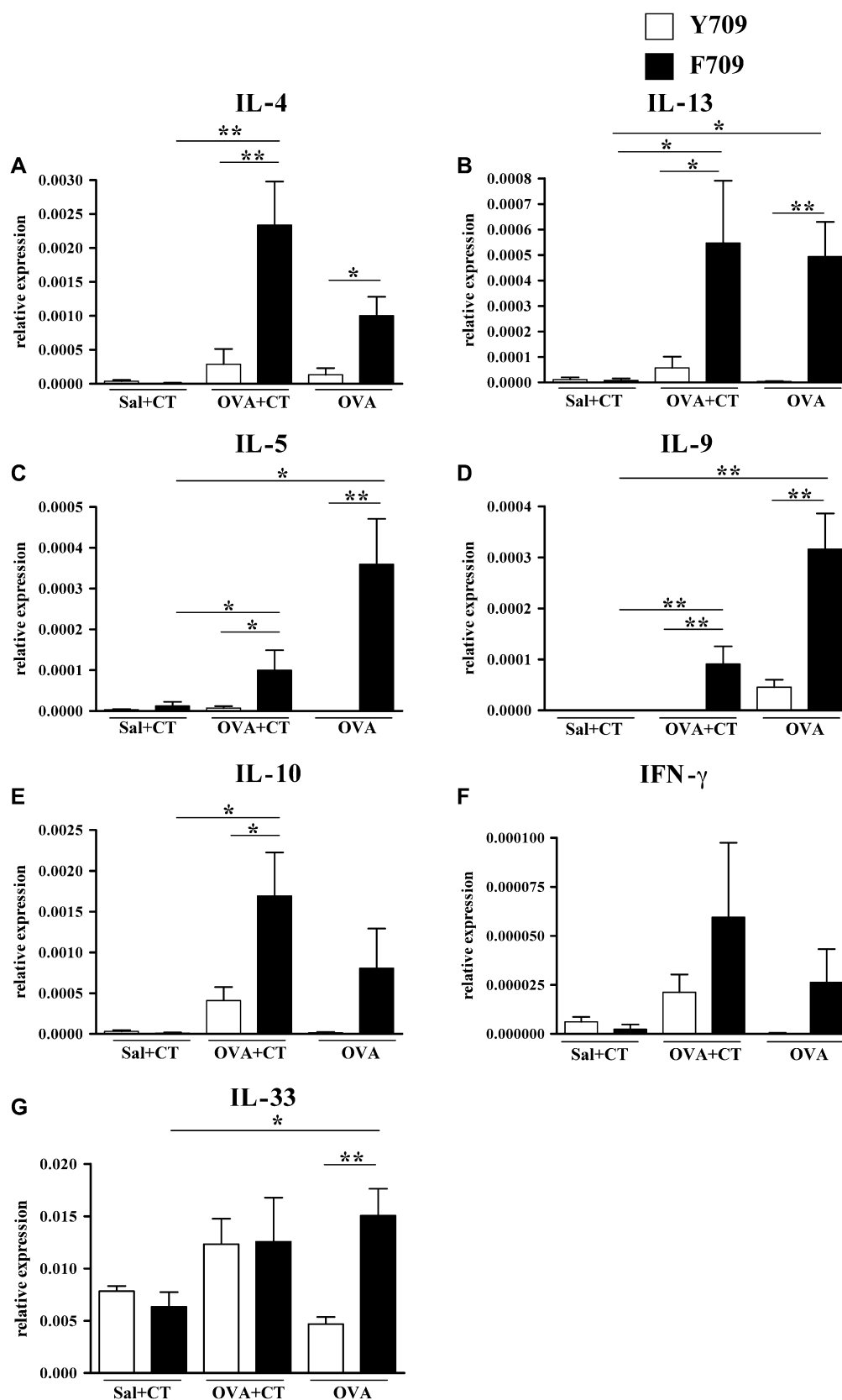


FIG 6. Cytokine transcripts in the intestines of mice enterally sensitized to OVA. Mice were treated with OVA plus CT or OVA alone. RNA from jejunum was analyzed for cytokine transcripts by means of RT-PCR. Expression of the following genes was measured: IL-4 (A), IL-13 (B), IL-5 (C), IL-9 (D), IL-10 (E), IFN- γ (F), and IL-33 (G; $n = 5-7$ mice). * $P < .05$ and ** $P < .01$, t test.

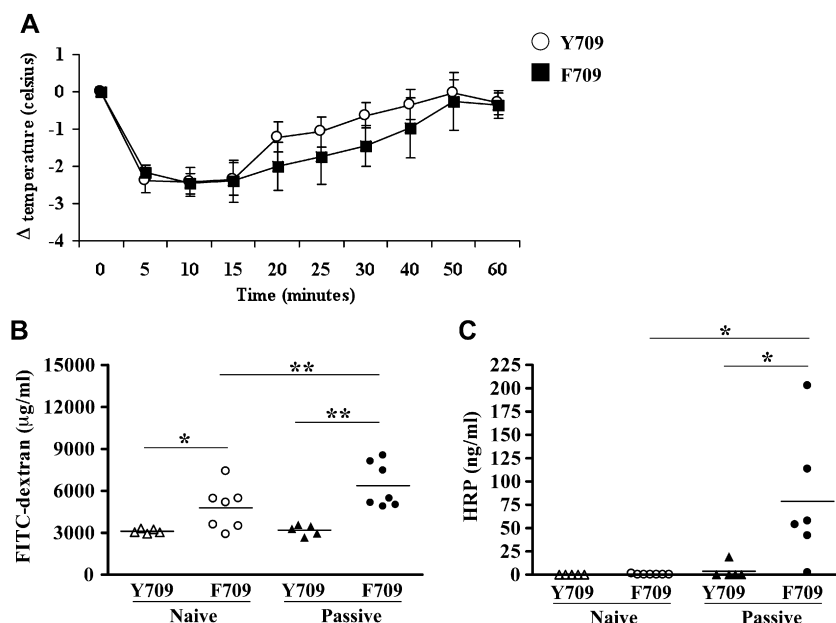


FIG 7. Passive IgE-mediated anaphylaxis and intestinal permeability in F709 mice. **A**, Core body temperature in mice treated with anti-DNP-IgE and challenged with DNP-HSA. **B** and **C**, Plasma levels of FITC-dextran (Fig 7, **B**) and HRP (Fig 7, **C**) in naive mice and mice as treated in Fig 7, **A** ($n = 5-7$ mice). Data are representative of 3 independent experiments. * $P < .05$ and ** $P < .01$, t test.

implicate the intensity of IgE responses as the major determinant of anaphylactic sensitivity. However, an alternative explanation for heightened sensitivity might be an increased sensitivity of target tissues expressing the F709 variant of IL-4R α to mast cell-derived mediators. To distinguish these possibilities, we examined the intensity of passive antigen-specific IgE-mediated anaphylactic responses in Y709 and F709 mice. Sensitization with DNP-specific IgE, followed by challenge with antigen, elicited robust responses with a similar magnitude in Y709 and F709 animals (Fig 7, **A**), suggesting that the increased sensitivity to anaphylaxis in animals harboring the disinhibited IL-4R α variant, F709, is linked to IgE production rather than target tissue sensitivity.

Expression of the F709 form of IL-4R α confers enhanced intestinal permeability in F709 mice

Both the relative ease of sensitization to food allergens delivered enterally and the robust anaphylactic responses exhibited by F709 mice after oral challenge suggested that these animals might have altered intestinal barrier function. This could affect antigen uptake during sensitization, as well as exposure of mast cells to antigen on challenge. Both IL-4 and mast cells have been shown to affect intestinal permeability.²⁹⁻³¹ To determine whether alterations in IL-4R signaling might affect intestinal permeability, we used gavage on F709 mutants, as well as control Y709 mice, with 2 macromolecules, FITC-dextran (4 kd) and HRP (approximately 44 kd), and followed their appearance in the blood. F709 mice exhibited enhanced absorption of FITC-dextran compared with that seen in Y709 animals (Fig 7, **B**), which is consistent with increased intestinal uptake, likely through a paracellular path. In contrast, HRP was barely detectable in the plasma of either Y709 or F709 mice (Fig 7, **C**), suggesting limited permeability for this protein, which likely penetrates the intestinal epithelium through both paracellular and transcellular transport mechanisms. Because mast cell

mediators are known to enhance gut permeability,³¹ we next determined whether uptake of these 2 tracers might be further altered after mast cell activation by IgE-antigen. We therefore followed levels of FITC-dextran and HRP in the blood of mice whose mast cells had been activated by means of intravenous infusion of anti-DNP IgE followed by DNP-HSA. Both FITC-dextran and HRP uptake were significantly enhanced in F709 animals but not Y709 mice after mast cell activation (Fig 7, **B** and **C**), suggesting that enhanced IL-4R signaling renders both paracellular and transcellular pathways of intestinal epithelial antigen uptake more sensitive to mast cell-derived mediators.

DISCUSSION

The findings reported here indicate that augmented IL-4R signaling is singularly effective in promoting both the development of allergic sensitization to ingested protein antigens and the induction of anaphylactic responses on enteral challenge. Studies in human subjects and mice have previously established that IL-4 and IL-13 play central roles in the pathogenesis of gastrointestinal allergic responses.³²⁻³⁸ Genetic polymorphisms affecting the IL-4/IL-13 axis are reported to be correlated with asthma susceptibility,^{4,9-11} and risk associations between polymorphisms in the IL-4, IL-4R α , and IL-13 genes and food allergy have been reported.^{39,40} We therefore hypothesized that augmented IL-4 signaling could predispose to food allergy and tested this in F709 mice in which the sensitivity of IL-4R α signaling is enhanced, resulting in increased IL-4-induced signal transducer and activator of transcription 6 phosphorylation, T_H2 induction, and IgE production.¹³

Our results demonstrate that the F709 genotype confers a very dramatic sensitivity to food allergen and that this phenotype is mediated primarily by IL-4R signaling effects in hematopoietic cells. Mice with this mutation exhibited not only increased susceptibility to immune sensitization, producing both specific IgE antibodies and T_H2 responses, but also a marked intestinal

mast cell expansion, enhanced intestinal permeability, and a capacity to mount intense anaphylactic responses on enteral challenge. Perhaps the single most notable phenotype of these mice with respect to food allergy was their susceptibility both to immune sensitization and to enteral allergen-induced anaphylaxis, even when exposed only to a protein antigen, OVA, in the absence of any immune stimulating adjuvant. Taken together, these observations indicate that IL-4 signaling plays a critical role in food allergy by enhancing allergic sensitization, promoting mast cell expansion and anaphylactic sensitivity, and interfering with the physiologic induction of tolerance to new protein antigens introduced through the gastrointestinal tract.

A number of groups have developed murine models to study food allergen-mediated hypersensitivity reactions in mice.^{19,20,27,41-47} Studies by Rothenberg et al using "allergic diarrhea" have established a central role for the IL-4/IL-13 axis in the development of food allergy.²⁰⁻²² Our findings confirm and advance this observation by demonstrating that enhanced IL-4R signaling is permissive for purely enteral immune sensitization in the absence of parenteral priming with adjuvant, as is used in the allergic diarrhea protocol. Furthermore, we find that the IL-4R effect also results in the manifestation of vascular responses (hypothermia) on purely enteral challenge (as opposed to intravenous challenge, as in the allergic diarrhea system).

Recently, it was shown that IL-4 can divert developing regulatory T cells to a cytokine profile dominated by IL-9.⁴⁸ IL-9, produced by epithelial and other cells, is an important mast cell growth and differentiation factor and has been implicated in intestinal anaphylaxis in an IgE-dependent manner.^{21,49-51} Forbes et al²¹ have elegantly demonstrated that IL-9-driven mast cell expansion in the intestine leads to enhanced intestinal permeability. This finding suggests a mechanism whereby the enhanced IL-4R α signaling of F709 mice, which is accompanied by high levels of intestinal IL-9 transcripts and markedly enhanced mast cell numbers, might increase the intestinal permeability in these mice. We and others have observed that recruitment of mast cell progenitors to the allergen-challenged lung in sensitized mice is IL-9 dependent.⁵²⁻⁵⁴ Dramatically increased jejunal IL-9 levels were observed in the F709 mutants in our study (Fig 6), suggesting proamplifying effects of the intestinal epithelium on mast cell accumulation. This is further borne out by the finding that although the sensitivity to anaphylaxis is largely mediated by hematopoietic cells, such as mast cells, nonhematopoietic cells also contribute.

IgE antibodies, which in combination with mast cells serve as exquisitely sensitive biological sensors for allergen, have long been implicated as the key trigger of anaphylactic reactions in allergic subjects. Osterfeld et al²³ have shown that reactions to food allergens in parenterally sensitized mice are IgE dependent. Thus, at first glance, our finding that the robust anaphylactic responses expressed by F709 mice in response to food allergen challenge were abrogated in IgE^{-/-} F709 animals might not seem surprising. It is important to note that anaphylaxis in rodents can be driven by completely IgE-independent mechanisms and that the IgG isotype can substitute for IgE.^{16,17,28} Our data show for the first time that IgE is critical in the development of active anaphylaxis to food antigens by using exclusively oral routes for both sensitization and challenge. This is in contrast to our previous findings, which showed that parenterally mediated systemic active anaphylaxis could occur in the absence of IgE.¹⁶

Strait et al have shown that although the IgE pathway is more sensitive, requiring lower threshold levels of antigen for full

activation, IgG-mediated responses, occurring at appropriate antibody to antigen ratios, can nevertheless be lethal.^{17,28} Our observations regarding anaphylactic responses in response to enteral OVA in F709 mice provide important information regarding the relative contributions of IgE and IgG isotypes in the gut. Although OVA-specific IgE responses were markedly higher in F709 mice than in Y709 control animals, IgG1 antibodies to OVA were similar in the 2 groups. This finding indicates a preferential effect of IL-4R α signaling on IgE versus IgG production in intestinal immune responses to food allergens. More interesting, though, was our finding that, in the absence of IgE, the presence of IgG1 responses in both Y709 and F709 animals was insufficient to drive anaphylactic reactions. Neither IgE^{-/-} mice nor F709/IgE^{-/-} double mutants displayed physiologic signs of anaphylaxis on enteral allergen challenge despite having significant anti-OVA IgG1 titers. This observation suggests that either the amount of absorbed antigen in the gut is insufficient to trigger non-IgE pathways or that the local effector cells of anaphylaxis, likely the markedly expanded mast cells, are preferentially IgE responsive. This observation certainly lends support to the concept currently being evaluated in clinical trials that anti-IgE therapy might block food allergy responses.

Our bone marrow chimera data suggest a partial role of heightened target organ responsiveness to mast cell mediators in the anaphylaxis sensitivity of F709 mice. Finkelman et al have demonstrated that administration of IL-4 immediately before anaphylactic challenge results in markedly stronger physiologic reactions because of enhanced tissue sensitivity to platelet-activating factor, histamine, serotonin, and cysteinyl leukotrienes.^{14,55} Nonhematopoietic cells express predominantly the type II IL-4/13 receptor (IL-4R α and IL-13R α 1).

In addition to providing important information regarding potential effects of amplified IL-4 signaling on immune sensitization, mast cell responses, and anaphylaxis, we believe that the F709 mice will provide a powerful tool for investigators studying food allergy. Furthermore, the failure of enteral tolerance in these mice will permit not only investigations of physiologic tolerogenic mechanisms but might additionally provide a practical model for evaluating allergenicity of food proteins in the absence of any immune-stimulating adjuvants. We anticipate that the observations presented in this report, along with the increasing availability of murine strains with alterations in IL-4R function, will lead to fruitful future investigations of the pathogenesis of food allergy.

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Clinical implications: Therapeutic interventions that target the IL-4R pathway might be beneficial in restoring oral tolerance in patients with food allergy.

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METHODS

Animals

FcεRIα^{-/-} mice were obtained from the Jackson Laboratory (Bar Harbor, Me). *C.129.II4ra^{F709/F709}* (F709) mice were bred onto a BALB/c background (ten generations). F709/FcεRIα^{-/-} double-mutant mice were generated by crossing F709 mice with FcεRI^{-/-} mice. All mice were housed in a specific pathogen-free environment and were 6 to 12 weeks old. All experiments were carried out in accordance with the Institutional Animal Care and Use Committee policies and procedures of the University of California, Los Angeles.

Sensitization of mice

F709, FcεRIα^{-/-}, and F709/FcεRIα^{-/-} mice were sensitized according to the protocol of Ganeshan et al.^{E1} Briefly, mice were sensitized weekly by means of intragastric gavage with 100 μg of OVA mixed in 10 μg of SEB

once per week for 8 weeks. Control mice were sensitized with PBS alone. After 8 weeks of sensitization, the mice were challenged with 5 mg of OVA by means of intragastric gavage.

Measurement of anaphylaxis

Anaphylaxis was assessed in challenged mice by measuring changes in body temperature and recording symptom scores. Temperature changes were measured with a rectal temperature probe (RET3) coupled to a Physitemp Thermalert Model TH-5 (Physitemp, Clifton, NJ). After OVA challenge, temperatures were measured every 5 minutes.

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- E1. Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol* 2009;123:231-8, e4.

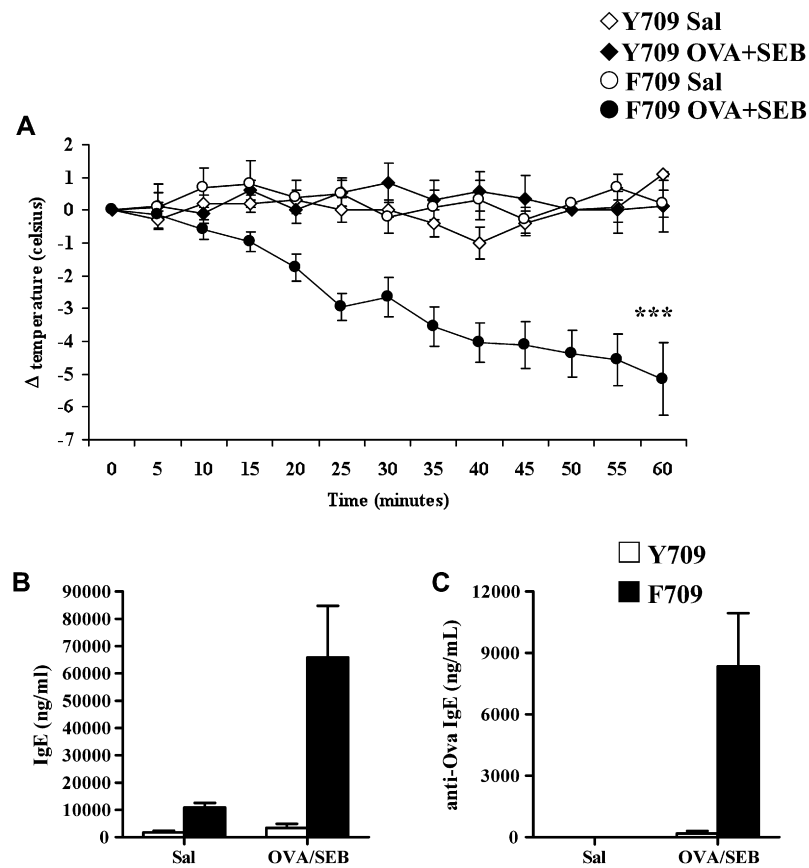


FIG E1. Allergic responses in F709 mice enterally sensitized to OVA and SEB. Mice were sensitized and challenged with OVA plus SEB, as described in the Methods section. Core body temperature (**A**), total IgE levels (**B**), and anti-OVA IgE levels (**C**) in the sera are shown ($n = 5-7$ mice). Data are representative of 3 independent experiments. *Sal*, Saline *** $P < .001$, ANOVA.

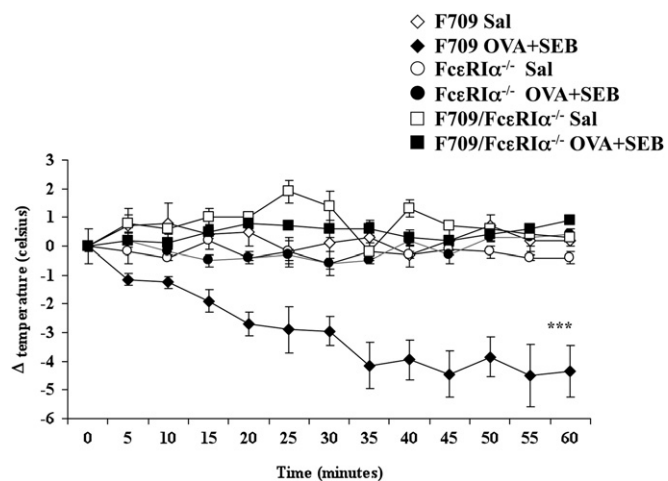


FIG E2. Evaluation of anaphylaxis in F709/FcεRIα^{-/-} mice. F709, FcεRIα^{-/-}, and F709/FcεRIα^{-/-} mice were treated with OVA plus SEB, as in Fig E1. The development of anaphylaxis was assessed by measuring changes in body temperature, as described in the Methods section (n = 5-7 mice). Data are representative of 3 independent experiments. ***P < .001, ANOVA.

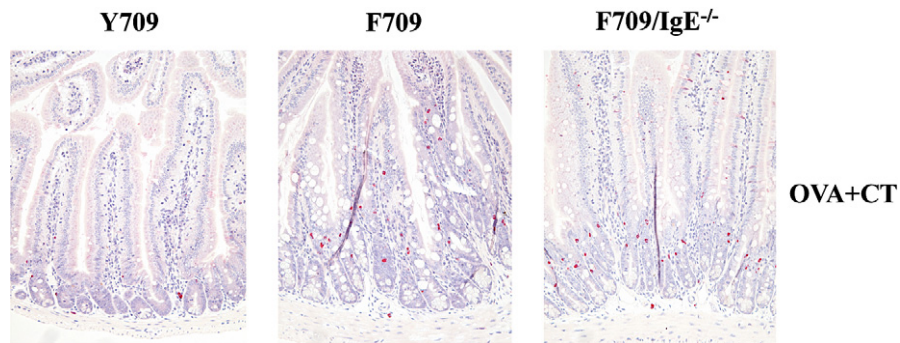


FIG E3. Mast cells in the intestines of OVA-treated F709 mice and F709/IgE^{-/-} mice. Mice were treated with OVA and CT. Chloroacetate esterase-reactive cells were identified in sections of jejunum. Mast cells appear red. Data are representative of 3 experiments.

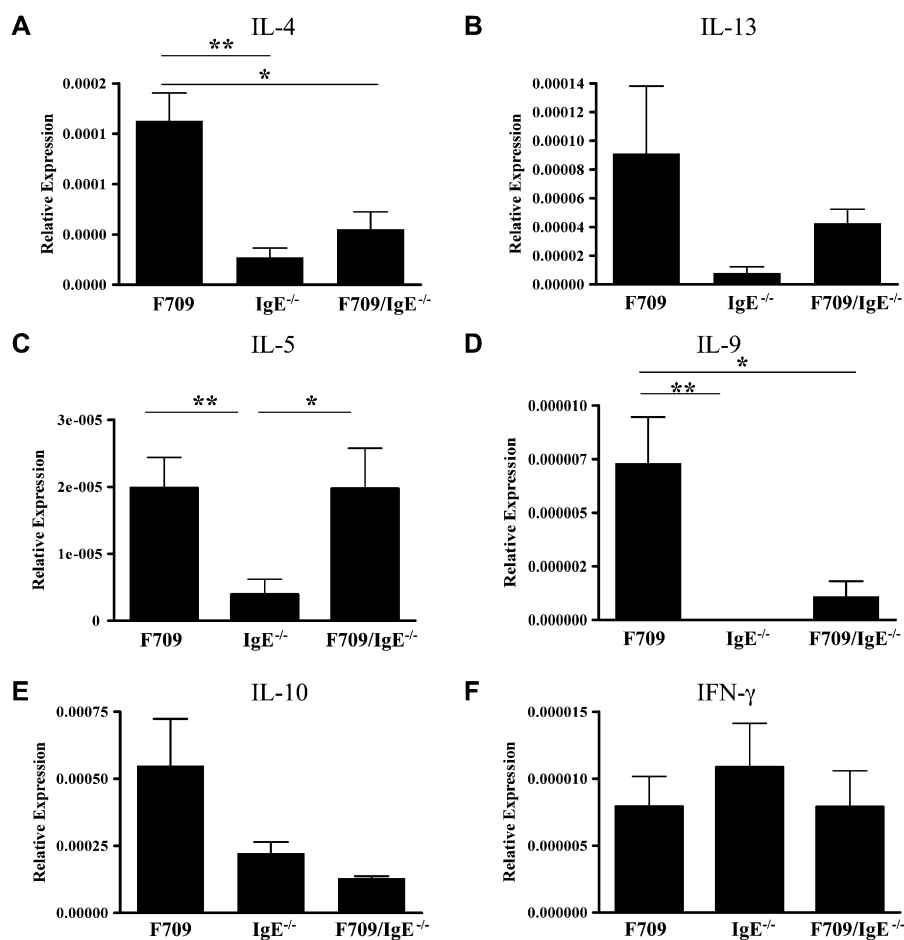


FIG E4. Systemic T_H2 responses to OVA in F709 mice after enteral sensitization. Mice were treated with OVA plus CT or OVA alone. Splenocytes from mice were cultured with OVA, and supernatant levels were determined for IL-4 (A), IL-13 (B), IL-5 (C), IL-9 (D), IL-10 (E), and IFN-γ (F) levels were evaluated in the sera of treated mice (n = 5-7 mice). **P* < .05 and ***P* < .01, *t* test.

TABLE E1. Rubric for assessing symptom score in anaphylactic animals

Score	Symptom
0	No symptoms
1	Repetitive mouth/ear scratching and ear canal digging with hind legs
2	Decreased activity; self-isolation; puffiness around eyes, mouth, or both
3	Periods of motionlessness for more than 1 min; lying prone on stomach
4	No response to whisker stimuli; reduced or no response to prodding
5	End point: tremor; convulsion; death

The development of systemic anaphylaxis was evaluated in mice sensitized with either OVA or saline (both in the presence of CT) and then challenged with OVA. Immediately after challenge, mice were examined for symptoms of anaphylaxis and assessed according to the symptom score shown.