

Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice

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Background: Food-induced anaphylaxis is often a severe allergic reaction characterized by multiorgan dysfunction and a potentially fatal outcome.

Objectives: We sought to investigate the relative contribution of immunoglobulin-dependent effector pathways to anaphylactic responses to food (ie, peanut).

Methods: Wild-type and various mutant mice were sensitized with peanut protein and cholera toxin by means of oral gavage weekly for 4 weeks. Mice were subjected to different cellular depletion and Fc receptor blocking strategies before challenge with peanut 1 week after the last sensitization.

Results: Our data indicate that pathways other than the classical mast cell (MC)–IgE pathway contribute to the full spectrum of anaphylactic reactions to peanut. We show that the single deletion of MCs, basophils, or phagocytes (ie, macrophages) prevents the most significant clinical outcome: death. Remarkably, the combined deficiency of MCs and phagocytes, but not MCs and basophils, averted nearly all clinical and physiological signs of anaphylaxis. Furthermore, blockade of both IgE and IgG1 signaling was necessary to abolish anaphylactic responses to peanut. Although MC responses occurred through IgE and IgG1, phagocyte responses were fully mediated through IgG1.

Conclusions: Peanut-induced anaphylaxis is a process that involves the concerted action of multiple immune effector pathways, and thus interventions targeting a single pathway (eg, MC–IgE) might not be sufficient to fully prevent anaphylactic responses. (*J Allergy Clin Immunol* 2011;127:1552–61.)

Key words: Peanut allergy, anaphylaxis, mast cells, macrophages, basophils, IgE, IgG1, FcεRI, FcγRIII, *Kit*^{W-sh}/*W-sh*

Anaphylaxis is a life-threatening hypersensitivity reaction commonly triggered by allergens, including foods, insect venoms, and medications.^{1,2} Food-induced allergic reactions account for one third to one half of anaphylactic cases in emergency departments worldwide,^{3–5} and fatality registries in the United States implicate peanut as a major culprit of food-induced fatal reactions.⁶ Peanut allergy develops early in life and persists in 80% of patients.^{7,8} The potential lethality of peanut allergy is compounded by the lack of treatments other than epinephrine to rescue patients undergoing anaphylaxis.

Mechanistically, anaphylaxis is thought to involve the massive release of mediators from mast cells (MCs) after allergen interaction with cell-bound IgE. However, numerous studies have demonstrated that anaphylactic responses can occur in mice deficient in MCs,⁹ IgE,¹⁰ or the FcεRIα chain,¹¹ thus arguing for the involvement of alternative pathways. In fact, studies with surrogate allergens (eg, ovalbumin) indicate that the death associated with systemic anaphylaxis, as well as the decrease in body temperature and other physiological changes associated with the reaction, might largely reflect IgG1-dependent activation of FcγRIII.^{11,12}

Presently, the contribution of specific effector cells in IgG-mediated anaphylaxis remains a subject of investigation. Studies in BALB/c mice immunized with goat anti-mouse IgD antiserum and then challenged with goat IgG suggested that macrophages are the major cell type contributing to IgG-mediated responses.¹³ Seemingly at variance, findings in a model of penicillin V–induced anaphylaxis in C57BL/6 mice demonstrated a central role for basophils, thus illustrating the selective contribution of distinct FcγRIII-expressing cells to IgG-mediated anaphylaxis.¹⁴

We have investigated immune effector pathways that mediate systemic anaphylaxis to peanut in mice. Our data demonstrate that effector cells, including MCs, phagocytes (ie, macrophages), and, to a lesser extent, basophils contribute to this process. In fact, absence of any one cell lineage prevented mortality. Remarkably, concomitant deficiency of MCs and phagocytes, but not MCs and basophils, abrogated almost completely a broad range of anaphylactic responses. Furthermore, we show that MCs contribute to anaphylaxis through IgE and IgG1, whereas phagocytes

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Abbreviations used

MC: Mast cell
PAF: Platelet-activating factor
PIA: Peanut-induced anaphylaxis
WT: Wild-type

contribute through IgG1 exclusively, suggesting that in patients with peanut-induced anaphylaxis (PIA), IgG-dependent responses occur through various Fc γ RIII-expressing cells. Collectively, our findings indicate that the full expression of anaphylactic responses to peanut in this model involves cooperation between distinct immune effector pathways.

METHODS

Animals

Female C57BL/6 mice (6–8 weeks old) were purchased from Charles River Laboratories (Ottawa, Ontario, Canada). MC-deficient (WBB6F1/J-Kit^W/Kit^{W-v} and C57BL/6J-Kit^{W-sh}/W^{-sh}) and age-matched congenic wild-type (WT) mice were purchased from Jackson Laboratory (Bar Harbor, Me). IgG1 (C57BL/6)¹⁵ and IgE (BALB/c)-deficient¹⁰ mice were bred in house. The mice were housed in a specific pathogen-free environment and maintained on a 12-hour light-dark cycle. All experiments described in this study were approved by the Animal Research Ethics Board of McMaster University.

Reagents

Ba103 and control rat IgG antibodies were purchased from Hycult Biotechnology B.V. (Uden, The Netherlands) and Sigma-Aldrich (Oakville, Ontario, Canada), respectively. Monoclonal rat IgG2b anti-Fc γ RII/III antibody (2.4G2) specific for the extracellular domains of murine Fc γ RIIB and Fc γ RIII was prepared in house from hybridoma culture supernatants and was a gift from Dr Kathy McCoy, McMaster University, Canada. Clodronate was a gift of Roche Diagnostics GmbH (Mannheim, Germany) and was encapsulated in liposomes in the laboratory of Dr Nico van Rooijen (Amsterdam, The Netherlands). Fluorescein isothiocyanate-conjugated mAbs specific for CD3 (145-2C11) and Fc ϵ RI (MAR-1) and phycoerythrin-Cy7-conjugated mAbs specific for CD11b (M1/70), phycoerythrin-Cy5-conjugated mAbs specific for CD3e (145-2C11) and F4/80 (BM8), and allophycocyanin-conjugated CD117 (2B8) were purchased from eBioscience (San Diego, Calif). Fluorescein isothiocyanate-conjugated anti-IgE (R35-72) and phycoerythrin-conjugated mAbs specific for CD117 (2B8) and CD49b (DX5) were purchased from BD Biosciences (Mississauga, Ontario, Canada). Appropriate isotype controls were used.

PIA model

Mice were sensitized as previously described,¹⁶ with slight modifications. Specifically, oral gavages were performed with intragastric feeding needles (191183; DELVO, Zurich, Switzerland). Sensitized mice were challenged with 3.75 mg of crude peanut extract (Greer Laboratories, Lenoir, NC) in 500 μ L of PBS either intraperitoneally or intravenously 1 to 2 weeks after the last gavage.

Anaphylaxis measurements

Mice were monitored for 40 minutes after challenge, and responses were assessed as follows. First, the clinical score was determined. Clinical signs were evaluated by using the scoring system shown in Table I. In some instances mice died or were killed before the 40-minute time point as they reached end-point conditions (≥ 2 seizures, loss of consciousness, or both; moribund condition). Second was measurement of core body temperature, as previously reported.¹⁶ Third was measurement of hematocrit values. Peripheral blood was collected by means of retro-orbital bleeding into heparinized microcapillaries. The tubes were centrifuged for 1 minute by using a

microhematocrit centrifuge. The hematocrit value was expressed as a percentage of cell volume. Fourth was determination of vascular leakage. Mice were injected with crude peanut extract in Evans blue dye intravenously, and footpads were examined for dye extravasation 10 minutes after challenge. Fifth was measurement of intestinal bleeding. The small intestines were removed and examined for macroscopic hemorrhage 40 minutes after challenge. Finally, we performed measurement of histamine values, as previously described.¹⁷

In vivo depletion of cell lineages

For basophil depletion, mice were given an intravenous injection of 100 μ g of the basophil-depleting mAb Ba103¹⁸ or control rat IgG 1 day before peanut challenge. For phagocyte depletion, mice were given an intraperitoneal injection of 0.5 mL of clodronate or PBS liposomes 2 days before challenge, followed by an intravenous injection of 0.2 mL the next day.¹⁹

Blockade of IgG-mediated anaphylaxis

Mice were injected intraperitoneally with 500 μ g of anti-Fc γ RII/III mAb in PBS 24 hours before challenge. We used a dose of anti-Fc γ RII/III mAb previously reported to block Fc γ RII/III-dependent anaphylaxis.^{20–22} However, we validated this dose with dose-response experiments to determine comparable anaphylactic responses to those seen in IgG1-deficient mice (data not shown).

Measurement of peanut-specific IgG1 and IgE levels

Peripheral blood was collected 24 hours before challenge, and levels of serum peanut-specific IgG1 and IgE were measured as previously described.¹⁶

Flow cytometry

Single-cell suspensions from spleen, blood, and peritoneal lavage fluid were preincubated with anti-CD16/32 mAb at 4°C for 15 minutes before incubation with the indicated combination of antibodies. To detect IgE receptors, we incubated cells with anti-CD23 (Fc ϵ RII) at 4°C for 15 minutes and then IgE for 30 minutes to saturate the IgE receptors and then stained them with anti-IgE. Stained cells were analyzed with an LSRII flow cytometer (Becton Dickinson), and the data were analyzed with FlowJo 6.4.2 software (TreeStar, Inc, Ashland, Ore). At least 500,000 events were collected.

Statistical analysis

Statistical analysis was performed with SigmaStat software (Systat Software, Inc, San Jose, Calif). When applicable, results were analyzed with 1- or 2-way ANOVA with repeated measures followed by the Tukey post hoc test. The unpaired Student *t* test (2 tailed) was used when only 2 sets of continuous data were compared. A *P* value of less than .05 was considered statistically significant. Data are expressed as means $\pm$ SDs.

RESULTS

Effect of MC deficiency on the elicitation of anaphylactic responses to peanut

We previously reported that MC-deficient WBB6F1/J-Kit^W/Kit^{W-v} mice sensitized orally manifested no measurable anaphylactic responses after intraperitoneal challenge with peanut.¹⁶ Given that recent studies have described the involvement of other effector cells in anaphylactic reactions to peanut after intravenous challenge,²³ we evaluated the response of Kit^W/Kit^{W-v} mice to this route of challenge (see Fig E1 in this article's Online Repository at www.jacionline.org). We found that Kit^W/Kit^{W-v} mice challenged intravenously exhibited mild clinical signs, a small but significant decrease in temperature, and a modest but

TABLE I. Anaphylactic symptom score table

Score	Symptoms
0	No clinical symptoms
1	Repetitive mouth/ear scratching and ear canal digging with hind legs
2	Decreased activity; self-isolation; puffiness around eyes, mouth, or both; pilar erection; labored breathing
3	Prolonged periods of motionlessness; lying prone on stomach
4	No response to whisker stimuli; reduced or no response to prodding; complete paresis
5	End point (>2 seizures or loss of consciousness, moribund condition); death

significant increase in hematocrit values compared with values seen in control mice. It is noteworthy that hematocrit values in naive Kit^W/Kit^{W-v} mice were lower than in littermate control animals likely because of the anemic phenotype of these mice.²⁴ Histamine was undetectable in Kit^W/Kit^{W-v} mice after challenge. In littermate control animals intravenous challenge elicited more severe anaphylaxis than intraperitoneal challenge. Indeed, the percentage of mice reaching fatal and near-fatal reactions among those challenged intravenously or intraperitoneally was 100% versus 60%, respectively. Core temperatures were monitored in surviving mice; hypothermia was consistently more pronounced in survivors challenged intravenously. Similarly, increases in hematocrit values were more marked after the intravenous challenge. These data indicate that anaphylactic reactions were significantly abrogated but still clearly evident in Kit^W/Kit^{W-v} mice subjected to intravenous challenge.

To further investigate the degree of MC dependence in PIA, we used a genetically different MC-deficient strain, Kit^{W-sh}/Kit^{W-sh}. Oral sensitization led to increased levels of peanut-specific IgE and IgG1 comparable with those observed in WT control animals (Fig 1, A). Kit^{W-sh}/Kit^{W-sh} mice exhibited mild clinical signs (Fig 1, B) and a significant decrease in temperature after either intraperitoneal or intravenous challenge (Fig 1, C). Hemoconcentration was observed after intravenous but not intraperitoneal challenge (Fig 1, D). Increased vascular permeability was ascertained by means of Evans blue extravasation in the footpads (Fig 1, E). Consistent with our findings in Kit^W/Kit^{W-v} mice, anaphylactic responses in WT control animals were more severe after intravenous challenge. In particular, signs of circulatory collapse, including macroscopic intestinal bleeding, were consistently observed only in mice after intravenous challenge (occurrence, 70%; Fig 1, F). As in Kit^W/Kit^{W-v} mice, histamine was undetectable in Kit^{W-sh} mice after challenge (Fig 1, G). Collectively, these data indicate that PIA is substantially mediated by MC. However, MC deficiency does not fully prevent anaphylaxis, thus suggesting the contribution of other cell lineages to this process. Because intravenous challenge resulted in more severe and consistent responses, all subsequent experiments were conducted by using this route.

Role of basophils and macrophages in patients with PIA

Because basophils play a critical role in some types of anaphylaxis,¹⁴ we investigated the contribution of these effector

cells to PIA. We treated peanut-sensitized Kit^{W-sh}/Kit^{W-sh} mice and WT control animals with a basophil-depleting mAb (Ba103) or an isotype control antibody before challenge. Ba103 treatment depleted 80% to 85% of the basophils from the peripheral blood and spleen (blood: control, 2.3 ± 0.48 cells/mm³; Ba103, 0.4 ± 0.11 cells/mm³; spleen: control, 8.4 ± 0.7 cells $\times 10^4$; Ba103, 1.3 ± 0.18 cells $\times 10^4$; Fig 2, A). The depletion was selective because no other cell types, including MCs, were affected (Fig 2, A, and data not shown). Basophil depletion in WT mice prevented death (approximately 38%), end-point conditions (approximately 63%), or both (Fig 2, B). However, absence of basophils did not change the extent of hypothermia (Fig 2, C) or hemoconcentration (Fig 2, D) in either WT or Kit^{W-sh}/Kit^{W-sh} mice, suggesting that basophils contribute only to the most severe expression of anaphylactic responses.

Given that macrophages can mediate systemic anaphylaxis,²⁵ we examined the role of these cells in patients with PIA. We treated peanut-sensitized Kit^{W-sh}/Kit^{W-sh} and congenic control animals with clodronate liposomes before challenge. This treatment almost completely eliminated F4/80⁺ cells from the peritoneal cavity (99.9%) and spleen (95%; Fig 3, A). Phagocyte depletion in WT mice consistently prevented severe clinical signs. Indeed, the absence of phagocytes significantly prevented fatal or near-fatal reactions (12.5% in phagocyte-depleted vs 71.4% in nondepleted mice; Fig 3, B). Decreases in temperature and increases in hematocrit values were consistently milder in phagocyte-depleted mice compared with those seen in nondepleted mice, although these differences did not reach statistical significance. Remarkably, double deficiency of MCs and phagocytes (ie, macrophages) resulted in a nearly complete abrogation of all responses measured (Fig 3, C). Indeed, there was no increase in hematocrit value (Fig 3, D), Evans blue extravasation in the footpads (Fig 3, E), or macroscopic intestinal bleeding in doubly deficient mice (Fig 3, F).

Role of different immunoglobulin isotypes in anaphylactic reactions to peanut

We previously reported that immunoglobulin responses are absolutely required for the elicitation of PIA.¹⁶ However, the relative contribution of different immunoglobulin isotypes to this process has not been formally investigated. To evaluate the role of IgE, we used mice with a homozygous null mutation of the IgE gene, which cannot synthesize IgE but produces other immunoglobulin isotypes normally.¹⁰ Indeed, the levels of peanut-specific IgG1 in IgE-deficient mice were almost identical to those in control mice (data not shown). Despite the IgE deficiency, sensitized mutant mice still displayed mild clinical signs (Fig 4, A), a considerable decrease in body temperature (Fig 4, B), and significantly increased hematocrit values (Fig 4, C). Moreover, histamine levels were lower in IgE-deficient mice than in WT control mice but markedly augmented compared with those in naive mice (Fig 4, D). These findings suggest that the remaining responses observed in these mice might be mediated through IgG1-dependent activation of effector cells.

To examine the involvement of IgG1 in this process, we used mice that lacked the splice donor site in the I γ 1 exon necessary for switch recombination to IgG1.²⁶ Importantly, the frequency of cells switching to IgE is not affected.²⁷ Indeed, we detected equivalent serum levels of peanut-specific IgE in WT and IgG1-deficient mice after sensitization (data not shown). The absence

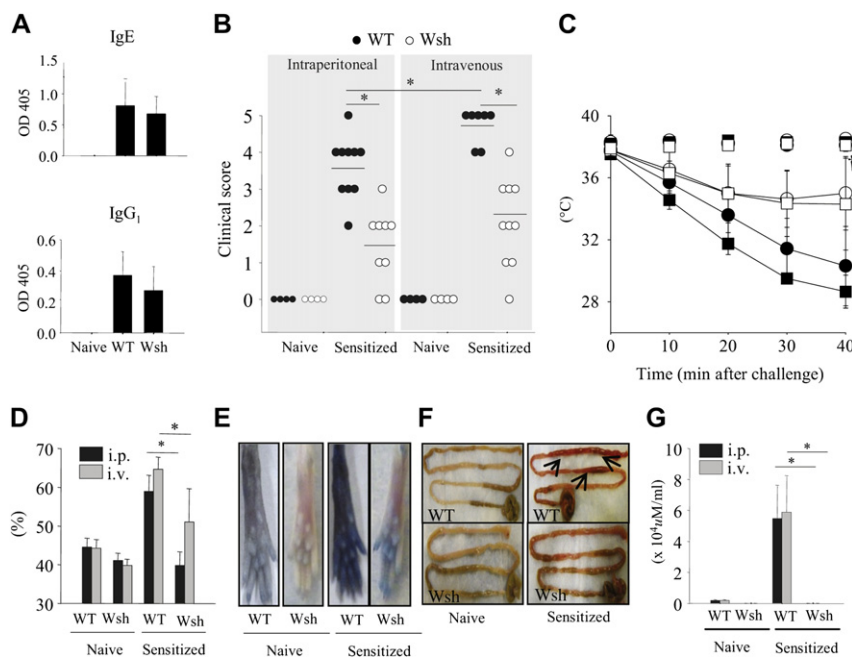


FIG 1. Partial abrogation of PIA in MC-deficient *Kit^{W-sh/W-sh}* (Wsh) mice. **A**, Levels of peanut-specific IgE and IgG1 in serum. **B**, Peak anaphylactic symptom score of each mouse. **C**, Rectal temperature of naive (intraperitoneal: WT, ---●---; Wsh, ---○---; intravenous: WT, ---■---; Wsh, ---□---) and sensitized (intraperitoneal: WT, ---●---; Wsh, ---○---; intravenous: WT, ---■---; Wsh, ---□---) mice. **P* < .05, Wsh intraperitoneal versus corresponding WT control mice. †*P* < .05, Wsh intravenous versus corresponding WT control mice. **D** and **G**, Percentage of hematocrit (Fig 1, D) and histamine levels (Fig 1, G). *i.p.*, Intraperitoneal; *i.v.*, intravenous. **E** and **F**, Representative pictures showing Evans blue extravasation in footpads (Fig 1, E) and intestinal bleeding (Fig 1, F). Data are presented as means ± SDs (4–11 mice per group from at least 3 independent experiments).

of IgG1 did not significantly prevent clinical signs (Fig 4, A) or the initial decrease in temperature. However, it avoided prolonged severe hypothermia. Furthermore, hematocrit (Fig 4, C) and histamine (Fig 4, D) values were significantly lower in IgG1-deficient mice compared with those in WT control mice yet substantially increased compared with those seen in naive mice. These data, along with our observation that FcγRIII-deficient mice are only partly protected from PIA (data not shown), provide evidence of the contribution of IgG1 to this process. To assess the combined contribution of IgE and IgG1 to PIA, we blocked FcγRII/III-dependent responses in IgE-deficient mice. Our data show that mice treated with an anti-FcγRII/III mAb exhibited no measurable anaphylactic responses (Fig 5), hence demonstrating that systemic anaphylactic reactions to peanut are fully mediated by dual IgE and IgG1 actions.

Effect of MCs and other FcγRII/III-expressing cells on anaphylaxis to peanut

To examine the contribution of IgE versus IgG1 to MC-mediated anaphylaxis, we used different approaches. Because engagement of FcγRIV by IgE immune complexes can activate macrophages,²⁸ we assessed the contribution of MCs to IgE-dependent responses by depleting phagocytes (ie, macrophages) in IgG1-deficient mice. Our data show that these mice manifested anaphylactic responses comparable with those seen in nondepleted IgG1-deficient mice (Fig 6), strongly suggesting that the residual anaphylaxis observed in these mice is dependent on

IgE binding to MCs. Given that MC activation can occur after cross-linking of low-affinity IgG receptors (ie, FcγRIII),²⁹ we investigated whether this pathway is relevant in patients with PIA by depleting phagocytes in IgE-deficient mice. Removal of phagocytes (ie, macrophages) did not further reduce anaphylactic reactions in these mice (Fig 6), suggesting that the remaining responses are elicited through IgG1-dependent activation of MCs. To examine whether the concurrent absence of MCs and FcγRII/III signaling (on other effector cells) abrogated PIA, we blocked FcγRII/III signaling in MC-deficient mice. Remarkably, MC-deficient mice treated with an anti-FcγRII/III mAb did not show any indication of anaphylaxis (Fig 7), demonstrating cooperation between MCs and FcγRII/III-expressing cells in patients with PIA.

DISCUSSION

In this study we have investigated immune effector pathways mediating PIA. We previously showed that orally sensitized *Kit^W/Kit^{W-v}* mice subjected to a peanut challenge intraperitoneally did not display any indication of anaphylaxis,¹⁶ suggesting that MCs were essential for this process. However, because recent studies with intravenous challenge have shown that food-induced anaphylaxis can occur in the absence of MCs,²³ we investigated anaphylactic responses in both *Kit^W/Kit^{W-v}* and *Kit^{W-sh}/W-sh* MC-deficient mice challenged either intraperitoneally or intravenously. We confirmed our previous findings in *Kit^W/Kit^{W-v}* mice challenged intraperitoneally but also showed that intravenous

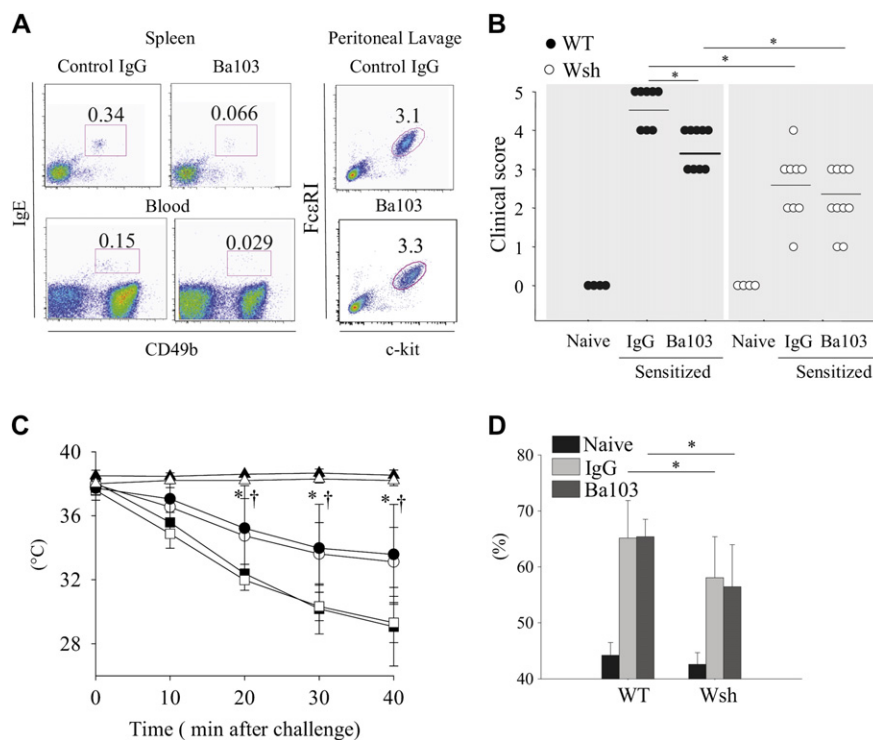


FIG 2. Effect of basophil depletion on peanut-induced anaphylactic reactions. **A**, Percentage of basophils and MCs from sensitized mice after antibody administration. **B**, Peak anaphylactic symptom score of each mouse. **C**, Rectal temperature of naive (WT, $\blacktriangle$; Kit^{W-sh/W-sh} [Wsh], $\triangle$) and sensitized (WT/rat IgG, $\blacksquare$; WT/Ba103, $\square$; Wsh/rat IgG, $\bullet$; Wsh/Ba103, $\circ$) mice. * $P < .05$, Wsh/rat IgG versus corresponding WT control mice. † $P < .05$, Wsh/Ba103 versus corresponding WT control mice. **D**, Hematocrit percentage. Fig 2, C and D, Data are presented as means $\pm$ SDs (4–8 mice per group from at least 2 independent experiments).

challenge elicited significant anaphylactic responses. Anaphylactic responses in Kit^{W-sh/W-sh} mice were similar to those in Kit^W/Kit^{W-v} mice, although modest increases in clinical scores and decreases in core temperatures were observed even in mice challenged intraperitoneally. Consistent with our observations in Kit^W/Kit^{W-v} mice, intravenous challenge induced more severe clinical and biological responses. Thus oral sensitization and intravenous challenge elicit a broad range of anaphylactic reactions, including the most severe ones in MC-deficient mice.

Other investigators have studied mechanisms underlying peanut allergy in murine models in which the oral route is used for both sensitization and challenge. At first glance, the oral route of challenge would be preferred; however, there are some considerations that merit reflection. For example, the amount of peanut antigen necessary to induce objective clinical measurements in mice (eg, decrease in core body temperature) is extraordinarily high (up to 200 mg)^{30,31} and likely not comparable with the amount of peanut antigen to which patients with peanut allergy might be exposed. Indeed, in many cases trace amounts are known to induce severe anaphylactic reactions in humans.⁶ Thus such experimental exposure likely favors distinctive responses that might or might not fully reflect the clinical phenotype observed in these patients (eg, severe reactions, including death).

Furthermore, exposure to peanut by patients with peanut allergy can lead to symptoms that can occur very rapidly after ingestion.³² However, the site or sites of antigen absorption have not been clearly defined. The small intestine, particularly

the jejunum, is thought to be the site of greatest absorption in the gastrointestinal tract.³³ However, it has also been reported that antigen uptake (ie, peanut) occurs in the mouth across the oral or sublingual mucosa, reaching the systemic circulation rapidly after exposure.³² From this perspective, intraperitoneal or intravenous challenge might be viewed not as an unusual route but as a route that facilitates immediate access to the systemic circulation.

The recognition that there are likely different routes of peanut exposure might allow for the investigation of pathways that contribute to PIA because it is increasingly evident that the IgE-dependent pathway is not the sole effector pathway relevant to human disease. Indeed, a study involving systemic exposure to peanut showed the contribution of immunoglobulin-independent activation of the anaphylatoxin C3a to anaphylactic shock.²³ In this study the authors reported that a single intravenous administration of peanut into IL-4/propranolol-pretreated BALB/c mice induced severe shock that was largely independent on MCs. In this system because peanut-induced shock occurred in the absence of adaptive immunity, the contribution of immune pathways involving activation of cells through Ig/Fc receptors could not be investigated. In contrast, in the model we used here, allergic sensitization and effector responses (ie, anaphylaxis) require an adaptive immune system (CD4- and CD40 ligand-deficient mice or μ MT mice do not show any measurable anaphylaxis¹⁶; unpublished data), thus allowing for the examination of antibody-mediated pathways leading to PIA.

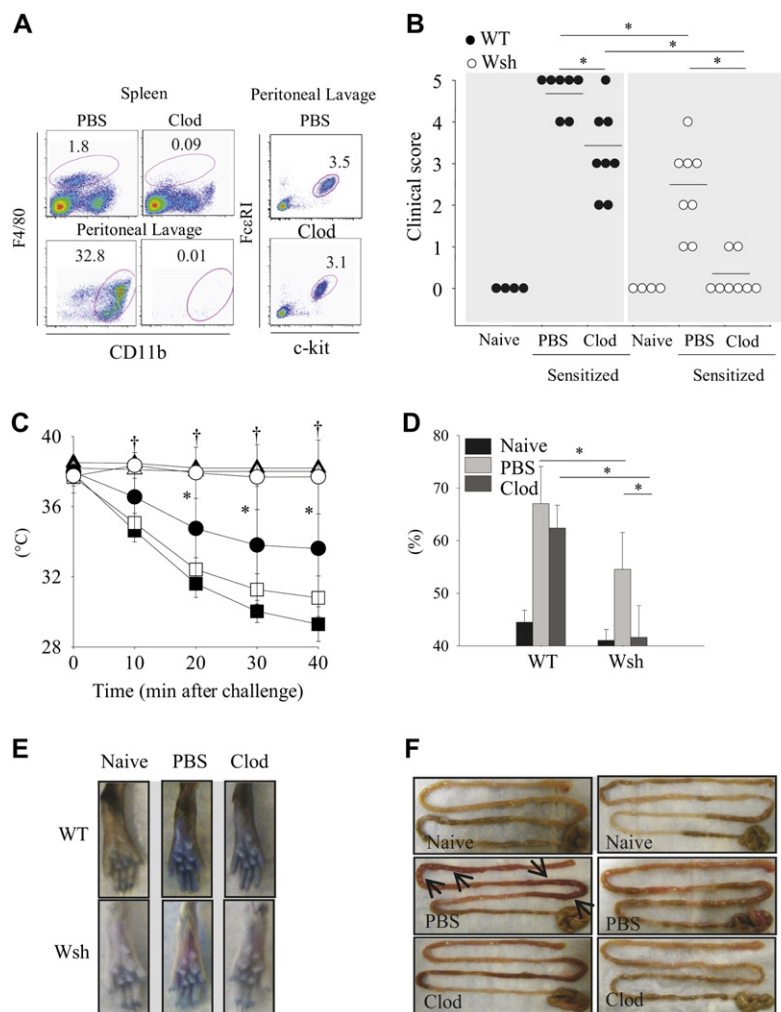


FIG 3. Dual contribution of MCs and phagocytes to PIA. **A**, Percentage of macrophages from sensitized mice after liposome delivery. **B**, Peak anaphylactic symptom score of each mouse. **C**, Rectal temperature of naive (WT, $\blacktriangle$ —; $Kit^{Wsh/Wsh}$ [Wsh], $\triangle$ —) and sensitized (WT/PBS liposomes, $\blacksquare$ —; WT/clodronate liposomes, $\square$ —; Wsh/PBS liposomes, $\bullet$ —; Wsh/clodronate liposomes, $\circ$ —) mice. * $P < .05$, Wsh/PBS liposomes versus corresponding WT control mice. † $P < .05$, Wsh/clodronate liposomes versus corresponding WT control mice. **D**, Hematocrit percentage. **E** and **F**, Representative pictures showing Evans blue extravasation in footpads (Fig 3, E) and intestinal bleeding (Fig 3, F). Fig 3, C and D, Data are presented as means $\pm$ SDs (4–8 mice per group from at least 4 independent experiments). Clod, Clodronate.

Two main pathways leading to systemic anaphylaxis were initially proposed: a classical pathway mediated by IgE, Fc ϵ RI, MCs, histamine, and platelet-activating factor (PAF) and an alternative pathway mediated by IgG, Fc γ RIII, macrophages, and PAF.²⁵ Our data indicate that anaphylaxis was still present in MC- or IgE-deficient mice. Additionally, we found that absence of phagocytes, IgG1, or both or Fc γ RIII (unpublished data) reduced the severity of the reactions. Notably, the concomitant deficiency of MCs and phagocytes abrogated all clinical and physiological responses measured, thus demonstrating the critical involvement of these 2 pathways in PIA. Although our data suggest that activation of phagocytes through Fc γ RIII/IgG1 contributes to anaphylactic responses to peanut, Fc γ RIII-mediated anaphylaxis can occur through phagocyte-independent mechanisms.¹¹ Our observation that IgE-deficient mice depleted of phagocytes exhibited some degree of anaphylaxis likely reflects the

contribution of MCs to Fc γ RIII/IgG-mediated responses. Indeed, increased histamine levels after challenge provide evidence of MC degranulation, which likely occurred through IgG.

Recently, Tsujimura et al¹⁴ showed that basophils are a pre-eminent player in IgG1-induced systemic anaphylaxis to penicillin V in C57BL/6 mice. Importantly, depletion of basophils in WT mice, in a model of active systemic anaphylaxis, did not prevent fatalities but rescued MC-deficient mice from death. However, absence of basophils in the latter mice did not fully prevent hypothermia (peak decrease of approximately 4°C), suggesting that other effector cells contribute to this process. In our study we observed that basophils contribute to the most severe manifestations of PIA. Indeed, the absence of basophils prevented fatal (approximately 38%), life-threatening (end-point condition, approximately 63%), or both types of reactions. However, it did not

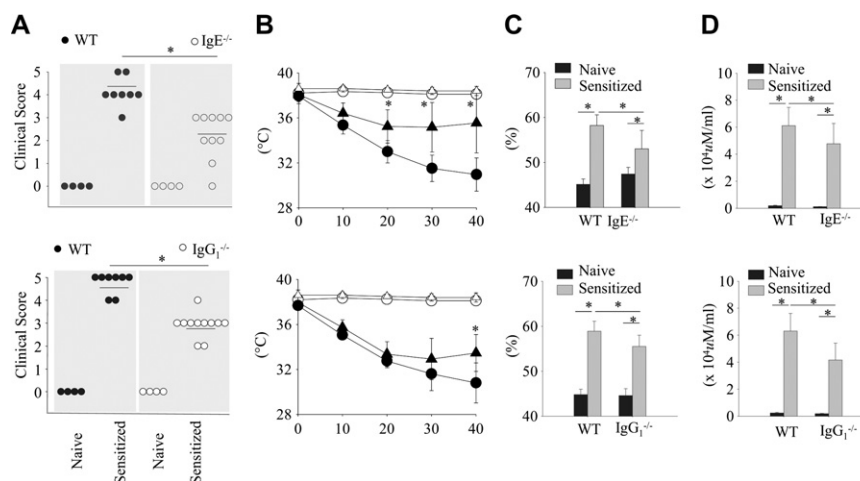


FIG 4. Double, but not single, absence of IgE and IgG1 signaling abolishes PIA. **A**, Peak anaphylactic symptom score of each mouse. **B**, Rectal temperature of naive (WT, —○—; immunoglobulin-deficient, —△—) and sensitized (WT, —●—; immunoglobulin-deficient, —▲—) mice. **P* < .05 relative to corresponding WT control mice. **C** and **D**, Hematocrit percentage (Fig 4, C) and histamine levels (Fig 4, D). Fig 4, B through D, Data are presented as means ± SDs (4-8 mice per group from at least 3 independent experiments).

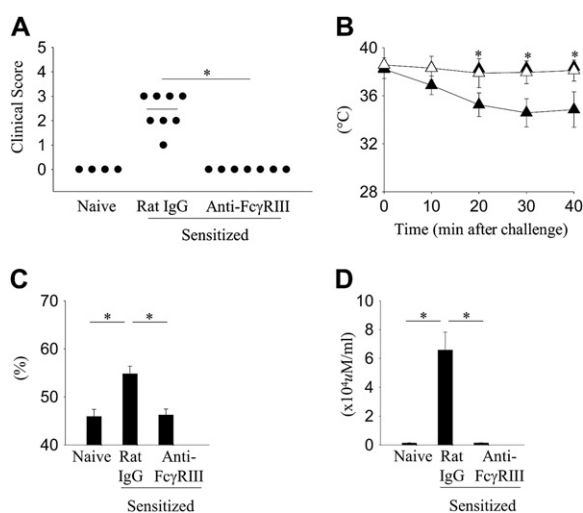


FIG 5. Blockade of IgG1 signaling in IgE-deficient mice abrogates PIA. **A**, Peak anaphylactic symptom score of each mouse. **B**, Rectal temperature of naive (—▲—) and sensitized (rat IgG, —△—; anti-FcγRIII/III, —△—) mice. **P* < .05 relative to corresponding WT control animals. **C** and **D**, Hematocrit percentage (Fig 5, C) and histamine levels (Fig 5, D). Fig 5, B through D, Data are presented as means ± SDs (4-8 mice per group from at least 3 independent experiments).

change the extent of hypothermia or increase in hematocrit values. Clearly, hypothermia, hemoconcentration, and severe end points are all related in this system. However, there is no evidence to indicate that the relationships between these outcomes are linear; in fact, it is quite likely that they are not. This is an important issue in a system in which great pathophysiologic changes occur within a short period of time (40 minutes). Moreover, it is very likely that other pathophysiologic changes (eg, hypotension) play a very significant role in fatal or near-fatal responses in this model. These changes might have additive or independent effects and, possibly, a different kinetics. Importantly, our data demonstrate that treatment of MC-deficient

mice with an anti-FcγRII/RIII mAb to temporarily block IgG binding on macrophages/basophils fully abolished anaphylactic responses. Specifically, MCs and phagocytes (ie, macrophages), but not basophils, mediate most of the clinical and physiological signs of anaphylaxis in this model. However, at the far end of the spectrum of severity (mortality), the single elimination of MCs, macrophages, or basophils prevents this outcome, suggesting that anaphylactic death is a catastrophic event that requires the concomitant contribution of these 3 cell types.

We previously showed that peanut-specific antibodies are required for PIA.¹⁶ Here we investigated the relative contributions of IgE and IgG1 to this process. We observed that anaphylaxis is substantially but not completely mediated through IgE and that IgG1-deficient mice manifested some anaphylactic reactions. Importantly, IgE-deficient mice treated with an anti-FcγRII/RIII mAb displayed no measurable reactions at all, hence demonstrating the dual contribution of both IgE and IgG1 to PIA. We should note that involvement of IgG1 in food allergy/anaphylaxis has not been previously reported. Given that IgG1 levels are low in the gastrointestinal tract, it is plausible that within the intestinal mucosa initial responses are primarily mediated through the IgE-MC pathway.³⁴ However, allergen entry into the systemic circulation is likely to result in activation of effector cells through IgG1. This pathway might actively contribute to the development of both more severe and protracted, biphasic, or both reactions. Of relevance, our data indicate that the contribution of IgG1 is most significant at late time points. Thus we hope that our findings will encourage further studies in other murine strains.

The lack of available evidence for the involvement of the IgG1 pathway in human anaphylaxis might merely be a reflection of not having explored this possibility comprehensively. In particular, in reference to food allergy, it is increasingly clear that the IgE pathway is likely not the only immunoglobulin pathway involved. Indeed, approximately 25% of patients do not respond whatsoever to anti-IgE therapy.^{35,36} Moreover, the relationship between levels of food (peanut)-specific IgE and clinical expression, phenotypes, or both is not linear; various studies suggest that there is an adequate relationship for levels of peanut-specific IgE of

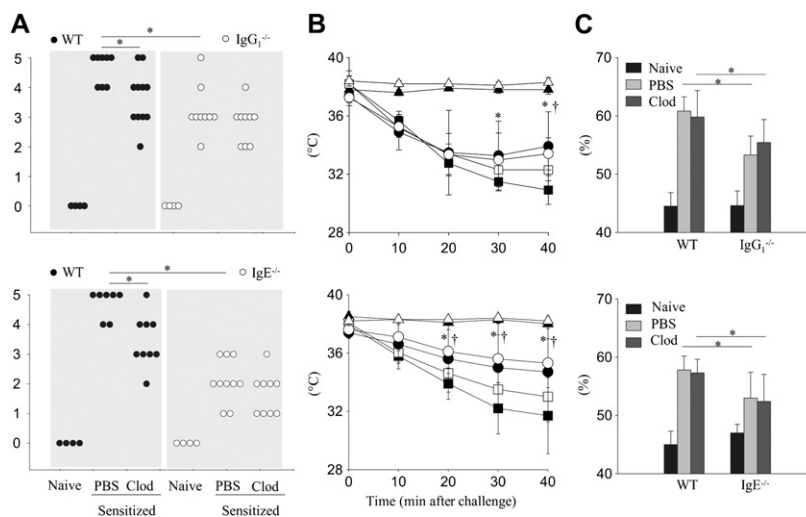


FIG 6. MC-dependent anaphylaxis to peanut occurs through both IgE and IgG1. **A**, Peak anaphylactic symptom score of each mouse. **B**, Rectal temperature of naive (WT, —▲—; immunoglobulin deficient, —△—) and sensitized (WT/PBS liposomes, —■—; WT/clodronate liposomes, —□—; immunoglobulin-deficient/PBS liposomes, —●—; immunoglobulin-deficient/clodronate liposomes, —○—) mice. * $P < .05$, immunoglobulin-deficient/PBS liposomes versus corresponding WT control animals. † $P < .05$, immunoglobulin-deficient/clodronate liposomes versus corresponding WT control animals. **C**, Hematocrit percentage. Fig 6, B and C, Data are presented as means $\pm$ SDs (4-8 mice per group from at least 3 independent experiments). Clod, Clodronate.

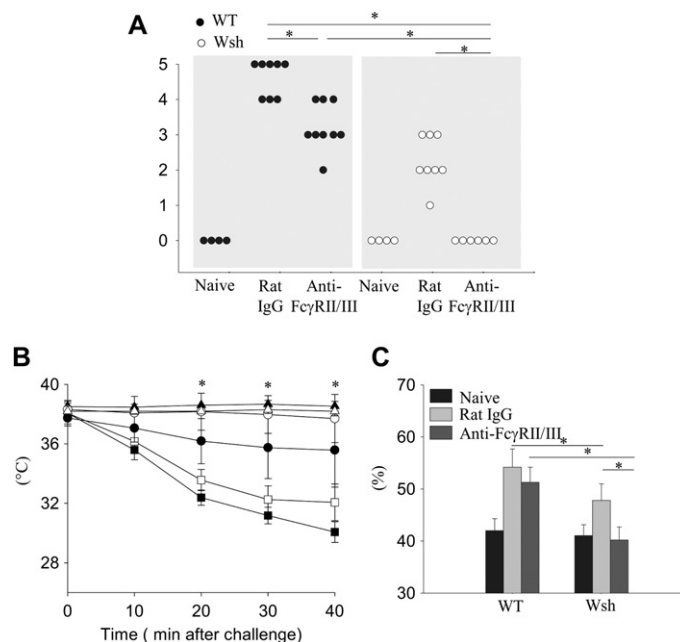


FIG 7. Synergism between MCs and other FcγRIII-expressing cells in the elicitation of PIA. **A**, Peak anaphylactic symptom score of each mouse. **B**, Rectal temperature of naive (WT, —▲—; Kit^{W-sh/W-sh} [Wsh], —△—) and sensitized (WT/rat IgG, —■—; WT/ anti-FcγRII/III, —□—; Wsh/rat IgG, —●—; Wsh/anti-FcγRII/III, —○—) mice. * $P < .05$, Wsh/anti-FcγRII/III versus Wsh/rat IgG. **C**, Hematocrit percentage. Fig 7, B and C, Data are presented as means $\pm$ SEMs (3-9 mice per group from at least 2 independent experiments).

greater than 15 kU/L but poor for levels of less than this.³⁷⁻³⁹ Lastly, Scott-Taylor et al⁴⁰ have recently reported increased levels of food-specific IgG1 in 23 children with multiple food allergies compared with those seen in 20 healthy control subjects. Interestingly, levels of food-specific IgG1 in sensitized children correlated with cytokine responses as an indication of T_H2

polarization, a central marker in atopy. As the authors state, compared with IgE, “much less is known about the role of other immunoglobulin classes.”

A clinically relevant observation in our study is that anaphylaxis can occur in the absence of histamine, indicating that other vasoactive mediators mediate PIA. In human subjects circulating

PAF levels correlate with the severity of anaphylactic reactions to peanut. In mice blockade of PAF activity significantly reduces the severity and duration of peanut-induced anaphylactic reactions, whereas isolated H1/H2 pharmacologic blockade has no effect.¹⁷ Other studies have demonstrated that blockade of PAF partially inhibits IgE-mediated anaphylaxis but fully prevents IgG-mediated anaphylaxis, suggesting a greater role of PAF in IgG than in IgE-dependent anaphylaxis. Furthermore, macrophages and basophils contribute to IgG-mediated anaphylaxis by producing PAF.^{13,14} These observations suggest that PAF, which is produced largely by macrophages and basophils activated through the IgG pathway, might substantially contribute to the severity of PIA. This notion would explain why the single elimination of MCs, macrophages, or basophils prevented end-point events.

Our data support the conjecture that PIA is a complex process in which distinct immune pathways act in concert to bring about a full range of pathological responses. Our findings suggest that whereas the classical pathway plays a prominent role, progressive recruitment of additional pathways expand clinical expression, culminating ultimately in death. Thus therapeutic strategies to fully prevent peanut-associated anaphylactic reactions might need to target several pathways or alternatively shared components within these pathways. This construct might have clinical and therapeutic implications to human subjects because there is no reason to think that the complexity of food-induced anaphylaxis in human subjects is any less than in mice.

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Key Messages

- Single depletion of MCs, macrophages, or basophils prevents death from PIA. Deletion of both MCs and phagocytes, but not MCs and basophils, fully prevents anaphylactic reactions.
- Both IgE and IgG1 signaling contribute to peanut-induced anaphylactic reactions. MC responses occurred through IgE and IgG, whereas phagocyte responses were fully mediated through IgG1.

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Correction

With regard to the April 2011 article entitled "House dust mite sublingual immunotherapy: Results of a US trial" (*J Allergy Clin Immunol* 2011;127:974-81.e7), Mary Morris, MD, was inadvertently omitted as an author. Dr Morris should have been included as the second-to-last author in the author list. She is affiliated with Allergy Associates of LaCrosse, Onalaska, Wisconsin, and discloses that she is a shareholder and medical director of Allergychoices.

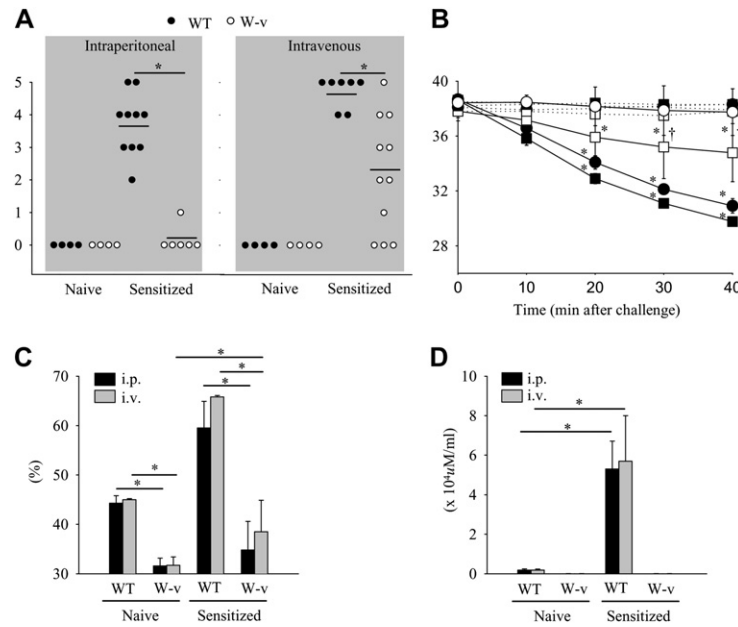


FIG E1. Partial abrogation of PIA in MC-deficient *Kit^W/Kit^{W-v}* (*W-v*) mice after intravenous but not intraperitoneal challenge. **A**, Peak anaphylactic symptom scores of each WT and *W-v* mouse. **B**, Rectal temperature of naive (intraperitoneal: WT, —●—; *W-v*, —○—; intravenous: WT, —■—; *W-v*, —□—) and sensitized (intraperitoneal: WT, —●—; *W-v*, —○—; intravenous: WT, —■—; *W-v*, —□—) mice. * $P < .05$ relative to corresponding naive mice. † $P < .05$ relative to corresponding WT control animals. **C** and **D**, Hematocrit percentage (**Fig E1, C**) and histamine levels (**Fig E1, D**). **Fig E1, B** through **D**, Data are presented as means $\pm$ SDs (4-11 mice per group from at least 2 independent experiments).