Absence of $Fc_\epsilon RI$ α Chain Results in Upregulation of FcγRIII-dependent Mast Cell Degranulation and Anaphylaxis

Evidence of Competition between $Fc_\epsilon RI$ and FcγRIII for Limiting Amounts of FcR β and γ Chains

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Abstract

In mouse mast cells, both $Fc_\epsilon RI$ and FcγRIII are αβγ2 tetrameric complexes in which different α chains confer IgE or IgG ligand recognition while the signaling FcR β and γ chains are identical. We used primarily noninvasive techniques (changes in body temperature, dye extravasation) to assess systemic anaphylactic responses in nonanesthetized wild-type, $Fc_\epsilon RI$ α chain −/− and FcR γ chain −/− mice. We confirm that systemic anaphylaxis in mice can be mediated largely through IgG_1 and FcγRIII and we provide direct evidence that these responses reflect activation of FcγRIII rather than FcγRI. Furthermore, we show that FcγRIII-dependent responses are more intense in normal than in congenic mast cell–deficient $Kit^W/Kit^{W\text{-}v}$ mice, indicating that FcγRIII responses have mast cell–dependent and –independent components. Finally, we demonstrate that the upregulation of cell surface expression of FcγRIII seen in $Fc_\epsilon RI$ α chain −/− mice corresponds to an increased association of FcγRIII α chains with FcR β and γ chains and is associated with enhanced FcγRIII-dependent mast cell degranulation and systemic anaphylactic responses. Therefore, the phenotype of the $Fc_\epsilon RI$ α chain −/− mice suggests that expression of $Fc_\epsilon RI$ and FcγRIII is limited by availability of the FcR β and γ chains and that, in normal mice, changes in the expression of one receptor ($Fc_\epsilon RI$) may influence the expression of functional responses dependent on the other (FcγRIII). (*J. Clin. Invest.* 1997. 99:915–925.) Key words: allergy • asthma • FcγRI • IgE • passive cutaneous anaphylaxis

Introduction

In the companion study (1), we showed that fatal active systemic anaphylaxis reactions to ovalbumin were expressed in normal mice, in genetically mast cell–deficient $Kit^W/Kit^{W\text{-}v}$ mice, or in mice which lacked $Fc_\epsilon RI$ because of targeted disruption of the gene encoding the $Fc_\epsilon RI$ α chain ($Fc_\epsilon RI$ α chain −/− mice, reference 2), but not in mice which lacked the FcR γ chain (FcR γ chain −/− mice, reference 3) that is common to FcγRI and FcγRIII, as well as $Fc_\epsilon RI$. We also found that $Fc_\epsilon RI$ α chain −/− mice and mast cell–deficient $Kit^W/Kit^{W\text{-}v}$ mice, but not FcR γ chain −/− mice, could express fatal IgG_1-dependent passive anaphylaxis responses. Taken together with the observation that IgG_1 antibodies bind rather poorly to FcγRI (4), these findings strongly suggested that some of the cardiopulmonary changes, as well as the mortality, associated with active anaphylaxis in the mouse may be mediated by IgG_1 antibodies and FcγRIII.

However, we also found that some of the changes in heart rate (HR)[1] and/or pulmonary function which developed in association with active, or IgG_1-dependent passive, systemic anaphylaxis were significantly greater in $Fc_\epsilon RI$ α chain −/− mice than in wild-type ($Fc_\epsilon RI$ α chain +/+) mice. The explanation for this observation was not immediately apparent. Extensive analysis of the cell and tissue distribution of the $Fc_\epsilon RI$ α chain in mice indicates that it is expressed only in mast cells and basophils (2, 5), whereas the FcR γ chain common to $Fc_\epsilon RI$ and FcγRI/III is expressed in many additional cell types including macrophages, neutrophils, and natural killer cells (3, 6). Moreover, studies in in vitro–derived mouse mast cells indicate that, in the absence of the $Fc_\epsilon RI$ α chain, mast cells can exhibit increased surface expression of FcγRII and/or FcγRIII (2).

Taken together, these findings raise the possibility that, in effector cells which ordinarily express $Fc_\epsilon RI$ (i.e., mast cells and basophils), lack of the $Fc_\epsilon RI$ α chain results in increased expression of FcγRIII and increased sensitivity of these cells to IgG_1- and FcγRIII-dependent activation. This phenomenon would be expected to increase the intensity of the pathophysiological changes associated with FcγRIII-dependent systemic anaphylaxis. On the other hand, fatal active systemic anaphylactic responses associated with cardiopulmonary changes can be elicited in mice that genetically lack mast cells (1, 7–11). This finding would argue against an essential role for enhanced FcγRIII-dependent mast cell activation and mediator production as the mechanism to account for the increased intensity of active anaphylaxis in $Fc_\epsilon RI$ α chain −/− versus +/+ mice.

We pursued the studies presented here to investigate three important issues regarding the pathogenesis of anaphylaxis in mice that were not fully resolved by the companion study (1). First, do FcR γ chain–dependent (and $Fc_\epsilon RI$-independent) anaphylactic reactions reflect the activation of FcγRI or FcγRIII? Second, does the increased intensity of certain physiological changes associated with anaphylaxis in FcγRI α chain

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1. *Abbreviations used in this paper:* BMCMC, bone marrow–derived cultured mast cells; C_{dyn}, dynamic compliance; G_L, lung conductance; HR, heart rate; KLH, keyhole limpet hemocyanin; PCA, passive cutaneous anaphylaxis.

−/− versus wild-type mice reflect increased expression of FcγRIII on effector cells in the absence of the $Fc_\epsilon RI$ α chain? Third, to what extent are the physiological changes associated with FcγRIII-dependent anaphylaxis mast cell–independent?

To investigate directly the role of FcγRIII-dependent effector cell activation in the development of anaphylactic reactions in $Fc_\epsilon RI$ α chain −/− or wild-type mice, we analyzed the responses elicited in these mice, and in genetically mast cell–deficient $WBB6F_1$-*Kit*^{W}/*Kit*$^{W\text{-}v}$ mice (12–14), upon intravenous challenge with an antibody (2.4G2, reference 15) against the external domain of FcγRII/III. We also determined whether the increased surface expression of FcγRII and/or III on mast cells which lack the $Fc_\epsilon RI$ α chain reflects increased association of the common FcR β and γ chains with mast cell FcγRIII in vitro. The results show that FcγRIII-dependent physiological responses, as well as FcγRIII-dependent mast cell activation, are significantly increased in $Fc_\epsilon RI$ α chain −/− versus wild-type mice, but that some of the physiological changes associated with these reactions clearly can occur by mast cell–independent mechanisms. The findings also suggest that availability of the common FcR β and γ chains may limit the surface expression, and, in turn, the function, of $Fc_\epsilon RI$ or FcγRIII on effector cells involved in systemic anaphylactic responses.

Methods

Animals

$Fc_\epsilon RI$-deficient animals ($Fc_\epsilon RI$ α chain −/− mice) were produced by targeted disruption of the α subunit gene (2) and were then backcrossed for four generations with BALB/c mice. Heterozygous animals were then bred to obtain $Fc_\epsilon RI$ α chain −/− and +/+ (wild-type) animals with the same genetic background. Mice deficient for $Fc_\epsilon RI$, FcγRI, and FcγRIII, because of targeted disruption of the gene for the FcR γ subunit (FcR γ chain −/− mice), have been described in detail (3). The FcR γ chain −/− mice used for this study were F_2 offspring of crosses between chimeras and C57BL/6 mice (3). Genetically mast cell–deficient $WBB6F_1$-*Kit*^{W}/*Kit*$^{W\text{-}v}$ mice, which virtually lack tissue mast cells (12–14), and the congenic normal ($WBB6F_1$-+/+) mice were purchased from the Jackson Biological Laboratory (Bar Harbor, ME). Unless otherwise specified, mice were of either gender and were 8–16 wk old at the beginning of the experiments. In individual experiments, mice of approximately the same age (usually, within 2–4 wk) and size were used.

The animal experiments were conducted in accordance with the Beth Israel Hospital's Institutional Animal Care and Use Committee and/or with guidelines prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS Publication No. 86-23, revised 1985). All mice were housed in microisolator cages in facilities supplied with high efficiency particulate-free air. Selected ("sentinel") mice in each room were sampled quarterly (approximately every 3 mo) and were found to be disease-free based on microbiological, parasitological, serological, and pathological examination.

ELISA for anti-DNP IgE or IgG antibodies

96-well plates were coated overnight at 4°C with 2 μg/ml anti–mouse IgE (PharMingen, San Diego, CA) or 10 μg/ml anti–mouse IgG (Southern Biotechnology Associates, Birmingham, AL) in PBS, washed three times with PBS, and then saturated for 2 h at 37°C with 1% gelatin in PBS. All subsequent washes (six after each step) were performed with 0.05% (vol/vol) Tween 20 in PBS. Serial dilutions of samples and internal standards were performed in 0.1% gelatin in PBS and incubated overnight at 4°C. Detection of anti-DNP antibodies was performed using 125 ng/ml DNP-biotin (obtained by deactivation of DNP-biocytin succinimidyl ester) (Molecular Probes, Eugene, OR). Detection of IgE or IgG antibodies (for measurement of total IgE or IgG) was performed using a 1:2,000 dilution of goat anti–mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) or a 1:500 dilution of goat anti–mouse IgE (Binding Site, Inc., San Diego, CA). Both antibodies were conjugated to alkaline phosphatase. Revelation of DNP-biotin was performed using horseradish peroxidase-avidin and 3,3′,5,5′-tetramethylbenzidine dihydrochloride (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) as the chromogenic agent. Anti-DNP IgE (Sigma Chemical Co., St. Louis, MO) and anti-DNP IgG_1 (clone U 7.6) (a generous gift from Dr. R. Segal, Bethesda, MD) (16) were used as standards. The linearity range of the assays was typically 0.1–5 ng/ml for anti-DNP IgE and 1–50 ng/ml for anti-DNP IgG. Revelation of alkaline phosphatase–conjugated antibodies was performed using *p*-nitrophenylphosphate as substrate.

Protocols

Sensitization for active systemic anaphylaxis to DNP-HSA. Mice were sensitized according to the protocol of Flores-Romo et al. (17). Mice were injected intravenously with 300 ng of *Bordetella pertussis* toxin (Life Technologies, Gaithersburg, MD), then, 48 h later, they received an intraperitoneal injection of 375 or 630 μg of DNP_{190}-keyhole limpet hemocyanin (KLH) (prepared by incubation of 20 mg/ml KLH with 20 mg/ml 2,4-dinitrobenzene sulfonic acid, sodium salt, and 20 mg/ml K_2CO_3) and 0.5% aluminium hydroxide (Rehydragel; Reheiss, Berkeley Heights, NJ) in PBS. Control animals were injected intravenously with *B. pertussis* toxin and then intraperitoneally with 0.5% aluminum hydroxide without DNP-KLH. Blood samples were obtained by retroorbital puncture 20 d later for determination of serum concentrations of anti-DNP IgE or IgG. 24 h later, mice were injected intravenously with 1 mg $DNP_{30\text{-}40}$-HSA (Sigma Chemical Co.) and 2% Evan's blue dye (Aldrich Chemical Co., Milwaukee, WI) in PBS. To monitor changes in body temperature associated with anaphylaxis (18), rectal temperature was measured before antigen injection and for 40–70 min after antigen injection, using a rectal probe coupled to an analogic thermometer (Yellow Springs Instrument Co., Yellow Springs, OH), as described (2). Animals that did not die from anaphylactic shock were killed 40–70 min after antigen challenge by cervical dislocation. For quantification of Evan's blue dye extravasation, ears were removed, minced, and incubated at 80°C for 3 h in 2 ml of formamide; absorbance was read at 610 nm (19).

Sensitization for IgG_1-dependent passive systemic anaphylaxis. Mouse monoclonal IgG_1 anti-DNP antibodies (clone U7.6, as above, in amounts of 20–1,000 μg, either as a dilution of ascites in PBS or as protein A–Sepharose purified antibodies in PBS) were administered intravenously by tail vein in volumes of ∼ 200 μl/mouse. Control mice received instead normal mouse IgG (Jackson ImmunoResearch Laboratories, Inc.) in PBS (∼ 200 μl/mouse). 1 d (24 h) after injection of IgG_1 anti-DNP or normal IgG, mice were injected intravenously with 1.0 mg $DNP_{30\text{-}40}$-HSA and 2% Evan's blue in PBS. Body temperature was monitored as described above.

Anti-FcγRII/III antibody-induced anaphylaxis. Alternatively, systemic anaphylaxis was induced by the intravenous injection of 100 μg of rat anti-FcγRII/III (2.4G2) antibodies (PharMingen) (15) in 200 μl of PBS. Control mice received an equivalent amount of normal rat IgG (Jackson ImmunoResearch Laboratories, Inc.).

Passive cutaneous anaphylaxis (PCA). We attempted to elicit PCA reactions, essentially as described previously (20), by intradermal injections (with a 30-gauge needle in volumes of 20 μl into the dorsal surface of the ear) of 25 ng anti-DNP IgE in PBS in the left ear, and 1.5 μg anti-DNP IgG_1 in the right ear, followed 24 h later by intravenous injection of 100 μg DNP-HSA in 2% Evan's blue in PBS. Control animals received the same intradermal dose of anti-Dansyl IgE (PharMingen) in the left ear and normal mouse IgG in the right ear. Quantification of Evan's blue dye extravasation was performed 30 min after injection of antigen as described above.

Physiological measurements and histologic studies

In mice, anaphylactic responses can produce a striking diminution in body temperature (2), as well as cardiopulmonary changes. Furthermore, the temperature changes associated with systemic anaphylaxis can be monitored in mice which have been subjected to neither the general anesthesia nor the surgical manipulations that are required to prepare the mice for analysis of the cardiopulmonary changes which are associated with the response (21). Accordingly, in this study, we primarily used changes in body temperature, as well as assessment of death rates, to investigate the intensity of the various anaphylactic responses.

In one set of experiments, we assessed the cardiopulmonary changes and mast cell degranulation associated with 2.4G2 antibody–induced anaphylactic reactions in $Fc_\epsilon RI$ α chain −/− and +/+ mice. HR and the pulmonary mechanical parameters, dynamic compliance (C_{dyn}) and lung conductance (G_L), were measured (by I. Miyajima) in mice anesthetized with 70–90 mg/kg, i.p., of sodium pentobarbital using a plethysmographic method, as described in the companion study (1). HR, C_{dyn}, and G_L were measured at multiple intervals after intravenous injection, via an indwelling jugular vein catheter, of 100 μg of rat 2.4G2 antibody (or, as a control, 100 μg of normal rat IgG) admixed in sterile, pyrogen-free 0.9% NaCl, in a total volume of 100 μl, until death due to 2.4G2 challenge or, after a period of 60 min, cervical dislocation. Changes in HR, C_{dyn}, and G_L after injection of 2.4G2 antibody or normal rat IgG were expressed as percent change versus baseline values (1). The state of mast cell activation was assessed (by I. Miyajima) in 1-μm, Epon-embedded, Giemsa-stained sections, as described in the companion study (1, 22–24). Mast cells were classified as "extensively degranulated" (> 50% of the cytoplasmic granules exhibiting fusion, staining alterations, and/or extrusion from the cell), "moderately degranulated" (10–50% of the granules exhibiting fusion or discharge), or "normal" (10, 20, 23, 24).

Analysis of FcγRIII expression in bone marrow–derived cultured mast cells (BMCMCs)

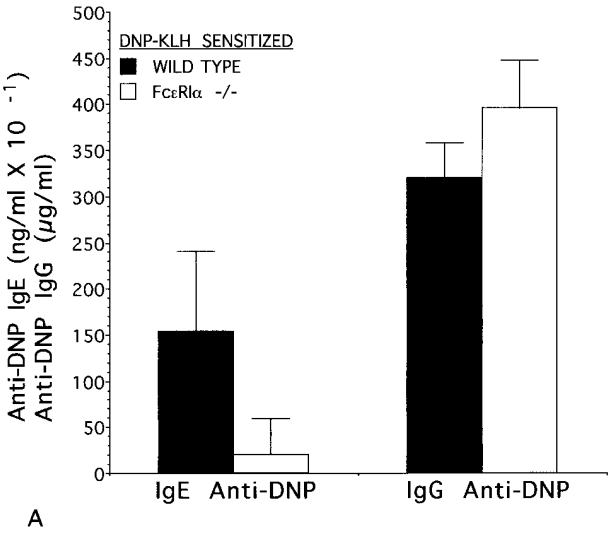
BMCMCs were generated from $Fc_\epsilon RI$ α chain −/− and +/+ mice by culture of femoral bone marrow cells for 3 wk in the presence of IL-3 as described previously (2). For each sample, 1.5×10^7 cells were lysed with 1% digitonin in 150 mM NaCl and 50 mM Hepes, pH 7.4, for 30 min at 4°C at a concentration of 5.0×10^7 cells/ml, then centrifuged for 20 min at 4°C at 14,000 rpm. The supernatants were then immunoprecipitated with either 10 μg of 2.4G2 antibody or with 30 μl of a rabbit antiserum specific for the cytoplasmic portion of either FcγRII or FcγRIII (a kind gift from Dr. M. Daëron, Paris, France). Samples were run on an 8–16% polyacrylamide gel and then transferred to a PVDF membrane. The section containing species with molecular masses > 21,000 D was probed with a monoclonal antibody specific for $Fc_\epsilon RI$ β chain (25), whereas the section containing species with molecular masses < 21,000 D was probed with an antiserum specific for $Fc_\epsilon RI$ γ chain (26).

Statistical analysis

Differences among the various groups of mice in the time courses of body temperature, HR, C_{dyn}, or G_L responses were examined for statistical significance by ANOVA. Differences in values for Evan's blue dye extravasation into the skin, or in the maximum diminution in C_{dyn} or G_L, or the maximum HR responses, were examined by the Student's *t* test (two-tailed). Differences in the extent of mast cell degranulation in various groups of mice were examined for statistical significance by the χ^2 test. Differences in the death rates between different experimental groups were examined for statistical significance by Fisher's exact test. Differences in the serum levels of anti-DNP IgE or IgG_1 antibodies in the different experimental groups in experiments assessing active anaphylaxis were examined for statistical significance by the Mann Whitney U test. $P < 0.05$ was regarded as significant. Unless otherwise specified, results are expressed as the mean±SEM.

Results and Discussion

Both $Fc_\epsilon RI$ α chain −/− mice and $Fc_\epsilon RI$ α chain +/+ (wild-type) mice exhibit active anaphylaxis to challenge with DNP-HSA. We first wished to confirm, using a different antigen and assessing levels of antigen-specific IgE and IgG antibodies, the finding that active systemic anaphylaxis can be expressed in the absence of the $Fc_\epsilon RI$ α chain (1). In Fig. 1 *A*, we show that both $Fc_\epsilon RI$ α chain −/− and $Fc_\epsilon RI$ α chain +/+ (wild-type) mice developed antigen- (DNP-) specific IgE and IgG antibodies after immunization with 375 μg of DNP-KLH.



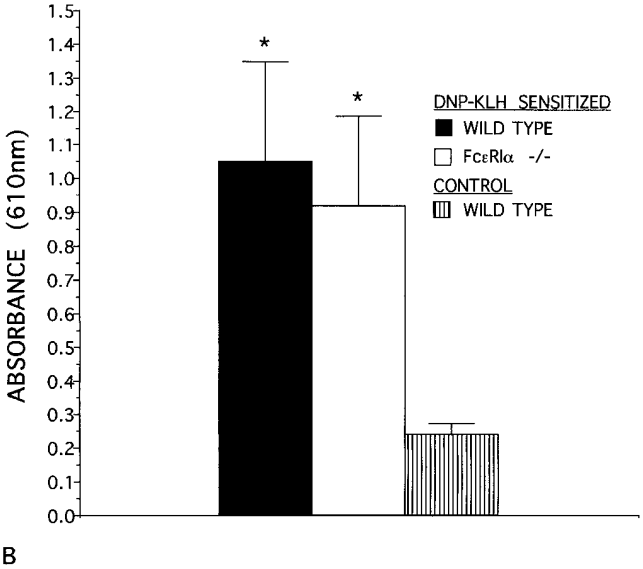


Figure 1. (*A*) Serum levels of anti-DNP IgE or IgG antibodies in $Fc_\epsilon RI$ α chain −/− (*white bars*) or wild-type (*black bars*) mice 20 d after active immunization with 375 μg of DNP-KLH (*DNP-KLH SENSITIZED*) (mean±SD, $n = 3$ per group). (*B*) Extravasation of Evan's blue dye (assessed by absorbance at 610 nm) into the ear skin of mice 30 min after intravenous challenge with 1.0 mg of DNP-HSA; responses in DNP-KLH–immunized (*DNP-KLH SENSITIZED*) wild-type mice (*black bars*) or $Fc_\epsilon RI$ α chain −/− mice (*white bars*) mice are significantly greater than those in sham-immunized (*CONTROL*) wild-type mice (*striped bar*). Data are shown as mean±SD ($n = 3$). *$P < 0.03$ by Student's *t* test (two-tailed) versus data from sham-immunized mice.

The serum levels of IgE anti-DNP antibodies in wild-type mice (15.4±8.7 ng/ml) were greater than those in the $Fc_\epsilon RI$ α chain −/− mice (3.0±3.2 ng/ml), but this difference was not statistically significant ($P > 0.05$ by the Mann-Whitney U test). Fig. 1 *B* shows that the same groups of three $Fc_\epsilon RI$ α chain −/− and three wild-type mice also developed very similar levels of increased cutaneous vascular permeability upon intravenous challenge with 1.0 mg of DNP-HSA and 2% Evan's blue dye (as assessed by quantification of the amount of Evan's blue dye extravasated into the ear skin 60 min after antigen challenge).

We also analyzed serum IgE and IgG anti-DNP antibody levels in two additional groups of three $Fc_\epsilon RI$ α chain −/− mice and three wild-type mice, one group after immunization with 375 μg of DNP-KLH and the other after immunization with 630 μg of DNP-KLH. These DNP-KLH–immunized mice also developed antigen-specific IgE and IgG antibodies, again without statistically significant differences between the values in $Fc_\epsilon RI$ α chain −/− or wild-type mice (data not shown).

All three groups of DNP-KLH–immunized or sham-immunized $Fc_\epsilon RI$ α chain −/− and wild-type mice were examined for changes in body temperature in response to intravenous challenge with 1.0 mg of DNP-HSA. The results for the three groups of actively immunized or sham-immunized mice of the same genotype were very similar, and therefore are presented together in Fig. 2. In comparison to the results obtained in antigen-challenged, nonsensitized control wild-type mice, both wild-type mice and $Fc_\epsilon RI$ α chain −/− mice which had been immunized with DNP-KLH exhibited significant drops in body temperature upon antigen challenge (Fig. 2). The drop in body temperature was more pronounced in $Fc_\epsilon RI$ α chain −/− mice than in the wild-type mice, but the difference did not achieve statistical significance by ANOVA. Antigen challenge resulted in the death of six of the nine wild-type mice and seven of the nine $Fc_\epsilon RI$ α chain −/− mice, versus none of the eight unsensitized wild-type mice ($P < 0.01$ versus the death rate in sensitized mice of either genotype by Fisher's exact test).

$Fc_\epsilon RI$ α chain −/− mice exhibit a greater reduction in body temperature than wild-type mice in association with IgG_1-dependent passive systemic anaphylaxis. The findings presented in Fig. 2 confirm, using a different antigen (DNP-KLH) and a different means of quantification (change in body temperature), the results presented in our companion study (1), which showed that active anaphylaxis responses to ovalbumin were at least as intense in $Fc_\epsilon RI$ α chain −/− mice as in the wild-type ($Fc_\epsilon RI$ α chain +/+) mice. Clearly, the responses to antigen challenge in actively immunized wild-type mice may have reflected the contributions of both IgE and IgG_1 anti-DNP antibodies. By contrast, the available evidence strongly suggests that the responses in $Fc_\epsilon RI$ α chain −/− mice had little or no IgE-dependent component. Thus, $Fc_\epsilon RI$ α chain −/− mice exhibited no detectable enhancement of vascular permeability (as assessed by the extravasation of Evan's blue dye) when challenged for expression of either IgE-dependent passive cutaneous or systemic anaphylaxis (2). Nor did they express detectable changes in HR, C_{dyn}, or G_L, significant mast cell degranulation, or death, in response to challenge for IgE-dependent systemic anaphylaxis (1).

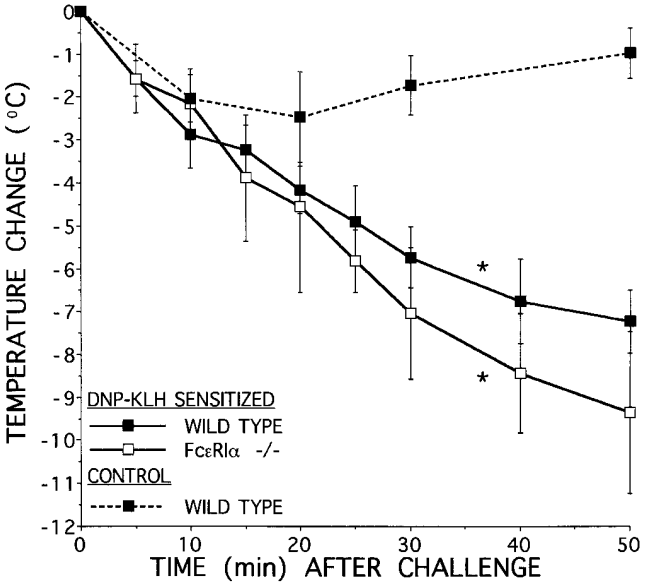


Figure 2. Active systemic anaphylaxis is associated with a drop in body temperature in both $Fc_\epsilon RI$ α chain +/+ (*WILD TYPE*) and $Fc_\epsilon RI$ α chain −/− (*$Fc_\epsilon RI\alpha$ −/−*) mice. DNP-KLH–immunized (*DNP-KLH SENSITIZED*) wild-type mice (*filled squares, solid line,* $n = 9$) and $Fc_\epsilon RI$ α chain −/− mice (*open squares, solid line,* $n = 9$), and sham-immunized (*CONTROL*) wild-type mice (*filled squares, dotted line,* $n = 8$) were challenged with 1.0 mg of DNP-HSA, i.v., 20 d after immunization with DNP-KLH or sham immunization, and the body temperature was recorded at multiple intervals thereafter (see Methods). Data are presented as mean±SD. $^*P < 0.04$ by ANOVA versus data from sham-immunized wild-type mice. The data include results from the mice depicted in Fig. 1.

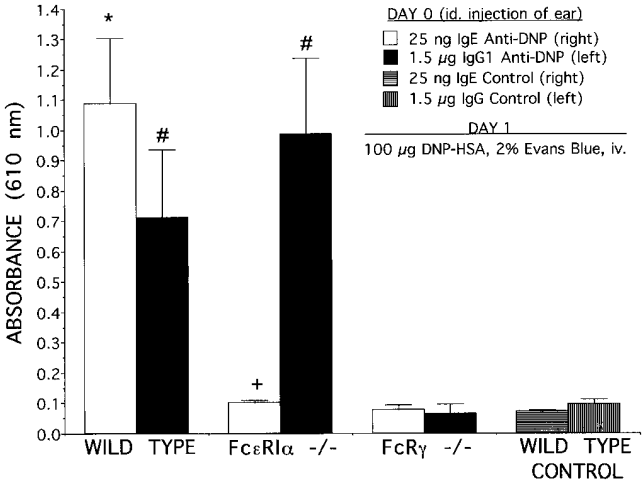


Figure 3. Cutaneous (ear skin) extravasation of Evan's blue dye in mice of various genotypes in response to intravenous challenge with 100 μg of DNP-HSA in 2% Evan's blue. Sites (ears) were injected intradermally 24 h earlier with 25 ng of mouse monoclonal IgE anti-DNP antibodies (*white bars*, right ears) or 1.5 μg of mouse monoclonal IgG_1 anti-DNP antibodies (*black bars*, left ears), or, as controls, 25 ng of a mouse monoclonal IgE antibody of irrelevant antigenic specificity [*IgE Control (right)*, *vertically striped bar*] or 1.5 μg of normal mouse IgG [*IgG Control (left)*, *horizontally striped bar*]. Data are shown as mean±SD ($n = 3$ per group). $^*P < 0.003$ by Student's *t* test (two-tailed) versus data from sites in wild-type mice injected with control IgE; $^+P < 0.004$ versus data from sites in wild-type mice injected with IgE anti-DNP; $^\#P < 0.02$ versus data from sites in wild-type mice injected with control IgG.

Nevertheless, we cannot formally rule out some role for IgE in the active anaphylactic responses detected in $Fc_\epsilon RI$ α chain −/− mice. IgE can interact with receptors other than $Fc_\epsilon RI$ (e.g., FcγRII/III, reference 27) and it is possible that the patterns of expression of such receptors on various effector cells are altered as a consequence of the active immunization procedure. Therefore, we directly investigated whether IgG_1 antibodies might contribute to the drop in temperature associated with systemic anaphylactic responses in mice. We first determined whether our monoclonal IgG_1 anti-DNP antibodies could sensitize mice for the expression of PCA. As shown in Fig. 3, the monoclonal IgG_1 anti-DNP antibodies conferred reactivity for PCA in wild-type mice or $Fc_\epsilon RI$ α chain −/− mice, but not in FcR γ chain −/− mice. By contrast, neither $Fc_\epsilon RI$ α chain −/− mice nor FcR γ chain −/− mice expressed IgE-dependent PCA responses (Fig. 3).

We then performed dose–response experiments in normal mice to assess the ability of our monoclonal IgG_1 anti-DNP antibodies to sensitize mice for passive systemic anaphylaxis. As shown in Fig. 4, normal mice exhibited a drop in body temperature in response to challenge with 1.0 mg of DNP-HSA that was dependent on the dose of IgG_1 anti-DNP antibodies which had been used for passive sensitization 1 d earlier. Maximal and virtually identical responses were elicited in mice which had received either 500 or 1,000 μg of IgG_1 anti-DNP antibodies. We also compared the temperature responses in normal mice which had been injected with either 400 μg of purified IgG_1 anti-DNP antibodies or 400 μg of IgG_1 anti-DNP antibodies as a dilution of ascites, and we found that the responses were essentially identical (data not shown). In all of these experiments, none of the mice died upon antigen challenge.

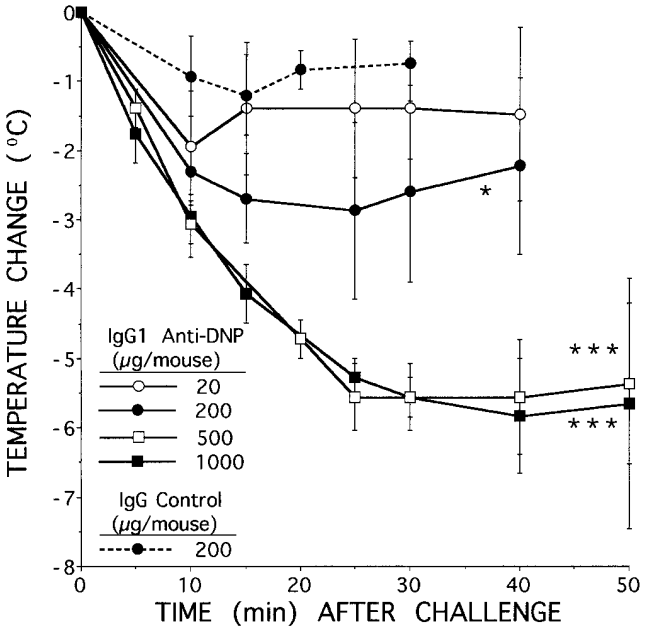


Figure 4. Changes in body temperature in wild-type mice which received various amounts of mouse monoclonal IgG_1 anti-DNP antibodies (*IgG_1 Anti-DNP*) intravenously (as shown) or, as a control, 200 μg of normal mouse IgG (*IgG Control, filled circles, dotted line*), and then 24 h later, were challenged intravenously with 1.0 mg of DNP-HSA. Data are shown as the mean±SD ($n = 3$ per group). $^*P < 0.05$, $^{***}P < 0.001$ by ANOVA versus data from control mice injected with 200 μg of normal mouse IgG.

We then passively sensitized $Fc_\epsilon RI$ α chain −/− mice, FcR γ chain −/− mice, and wild-type mice with 400 μg of IgG_1 anti-DNP (as a dilution of ascites) and, as a control, tested wild-type mice which had received 400 μg of normal mouse IgG. Upon challenge with 1.0 mg of DNP-HSA in 2% Evan's blue, both wild-type and $Fc_\epsilon RI$ α chain −/− mice developed a significant drop in body temperature compared with the response in the control, normal IgG-injected, wild-type mice (Fig. 5). Moreover, the IgG_1-dependent temperature reduction induced by antigen challenge in $Fc_\epsilon RI$ α chain −/− mice was significantly greater (by ∼ 1.0°C at the maximal point of diminution, 50 min after antigen injection) than that in the identically sensitized and challenged wild-type mice ($P < 0.0001$ by ANOVA). By contrast, IgG_1 anti-DNP antibody-injected FcR γ chain −/− mice exhibited no drop in temperature upon antigen challenge (Fig. 5).

Notably, in contrast to $Fc_\epsilon RI$ α chain −/− or wild-type mice which expressed active systemic anaphylaxis, neither the $Fc_\epsilon RI$ α chain −/− mice nor the wild-type mice which developed a drop in temperature in association with IgG_1-dependent passive systemic anaphylaxis exhibited prominent cutaneous extravasation of Evan's blue dye. Indeed, by visual inspection, it was not possible to detect differences in the Evan's blue dye extravasation in the ears of these mice as opposed to those of control animals which received normal mouse IgG instead of IgG_1 anti-DNP before challenge with DNP-HSA. We did not perform actual measurements of

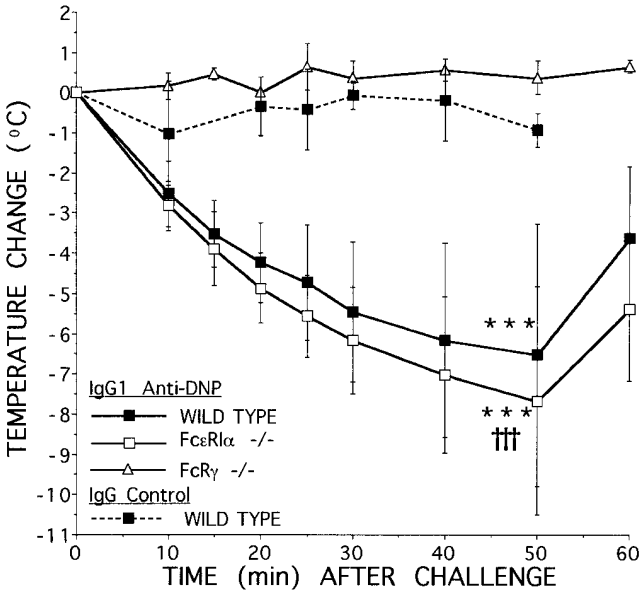


Figure 5. Changes in body temperature after intravenous challenge with 1.0 mg of DNP-HSA, administered 24 h after passive sensitization intravenously with 400 μg of mouse monoclonal IgG_1 anti-DNP antibodies (*IgG_1 Anti-DNP*), in wild-type mice (*WILD TYPE, filled squares, solid line, $n = 23$*), $Fc_\epsilon RI$ α chain −/− mice (*$Fc_\epsilon RI\alpha$ −/−, open squares, solid line, $n = 23$*), and FcR γ chain −/− mice (*FcRγ −/−, open triangles, solid line, $n = 3$*), or administered 24 h after sham passive sensitization of wild-type mice by intravenous injection of 400 μg of normal mouse IgG (*IgG Control, solid squares, dotted lines, $n = 15$*). Data are shown as mean±SD. $^{***}P < 0.0001$ by ANOVA versus data from sham-immunized wild-type mice; $^{\dagger\dagger\dagger}P < 0.0001$ by ANOVA versus data from IgG_1 anti-DNP–immunized wild-type mice.

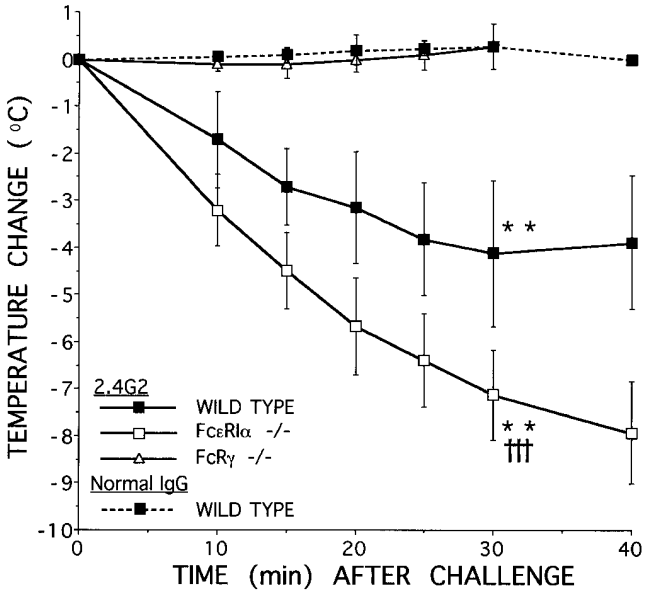


Figure 6. Changes in body temperature in response to intravenous injection of 100 μg of rat monoclonal 2.4G2 antibody (*2.4G2*) in wild-type mice (*WILD TYPE, filled squares, solid line, n* = 6), $Fc_\epsilon RI$ α chain −/− mice (*$Fc_\epsilon RI\alpha$ −/−, open squares, solid line, n* = 9), and FcR γ chain −/− mice (*FcRγ −/−, open triangles, solid line, n* = 3), or in control wild-type mice that were injected intravenously with 100 μg of normal rat IgG (*Normal IgG, solid squares, dotted line, n* = 3). Data are shown as mean±SD. $^{**}P < 0.002$ by ANOVA versus data from control wild-type mice injected with normal rat IgG, $^{\dagger\dagger\dagger}P < 0.0003$ by ANOVA versus data from wild-type mice injected with 2.4G2 antibody.

Evan's blue dye extravasation in these experiments, however, and it is possible that our visual inspection failed to detect small differences in the dye extravasation in the ear skin of control animals versus mice treated to express IgG_1-dependent passive systemic anaphylaxis. Nevertheless, our findings are fully consistent with results presented in the companion study (1), which showed that the cardiopulmonary changes associated with IgG_1-dependent passive systemic anaphylaxis can occur even in the absence of histologic evidence of substantial cutaneous mast cell degranulation.

$Fc_\epsilon RI$ α chain −/− mice exhibit anaphylactic responses to challenge with 2.4G2 anti-FcγRII/III antibodies that are more intense than those in wild-type mice. The FcR γ chain is expressed in both FcγRI and FcγRIII, as well as in $Fc_\epsilon RI$. Although IgG_1 antibodies bind more weakly to FcγRI than to FcγRIII, we wished to investigate whether anaphylactic reactions could be elicited by the direct triggering of FcγRIII, using an approach that does not activate FcγRI. Therefore, we injected mice intravenously with either 100 μg of 2.4G2, a rat monoclonal antibody specific for mouse FcγRII/III, or, as control, the same amount of normal rat IgG. As shown in Fig. 6, injection of 2.4G2 produced a significant drop in temperature in wild-type mice and $Fc_\epsilon RI$ α chain −/− mice, but not in FcR γ chain −/− mice. Moreover, the temperature reduction in the $Fc_\epsilon RI$ α chain −/− mice was significantly greater than that in the wild-type mice ($P < 0.0003$ by ANOVA).

In the companion study (1), we showed that $Fc_\epsilon RI$ α chain −/− mice developed significantly more pronounced reductions in pulmonary G_L and pulmonary C_{dyn} during active systemic anaphylaxis than did the identically sensitized and challenged wild-type mice. We also found that $Fc_\epsilon RI$ α chain −/− mice developed a more pronounced increase in HR, as well as a significantly greater drop in G_L, in association with IgG_1-dependent passive systemic anaphylaxis than did the identically challenged wild-type mice (1). In light of these findings, as well as the results shown in Fig. 6, we evaluated whether the cardiopulmonary changes associated with 2.4G2 antibody–induced anaphylaxis might be greater in $Fc_\epsilon RI$ α chain −/− mice than in wild-type mice.

As shown in Fig. 7, the reduction in G_L induced by 2.4G2 challenge in $Fc_\epsilon RI$ α chain −/− mice was significantly greater than that in the identically challenged wild-type mice, with maximum drops versus baseline (at 5 min after 2.4G2 injection) of 17.2±3.8% in $Fc_\epsilon RI$ α chain −/− mice versus 6.8±1.8% in wild-type mice ($P < 0.05$ by Student's *t* test, two-tailed). Challenge of $Fc_\epsilon RI$ α chain −/− mice with 2.4G2 induced an increase in HR (to a maximum of 19.7±8.8% over baseline at 5 min after 2.4G2 injection) that was significant at the $P < 0.01$ level when compared with the responses in control IgG-injected $Fc_\epsilon RI$ α chain −/− mice, whereas the tachycardia response in the 2.4G2-injected wild-type mice (which reached a maximum of 12.6±9.4% over baseline at 5 min after 2.4G2 injection) was significant at only the $P < 0.06$ level compared with the responses in control IgG-injected wild-type mice. As shown in Table I, challenge with 2.4G2 resulted in the death of all of the wild-type and $Fc_\epsilon RI$ α chain −/− mice tested, whereas none of the mice injected with control IgG died. Death due to 2.4G2 injection tended to occur more rapidly in $Fc_\epsilon RI$ α chain −/− mice than in wild-type mice, with death recorded at 35 min or less after 2.4G2 challenge in four of the six $Fc_\epsilon RI$ α chain −/− mice versus only one of the five wild-type mice.

Notably, the kinetics of the tachycardia response to 2.4G2 injection, as well as the rapid and (in α chain −/− mice) partially reversible early component of the 2.4G2-induced reduction in G_L, more closely resembled the responses observed in active systemic anaphylaxis, or IgE-induced passive systemic anaphylaxis, rather than those seen in IgG_1-induced passive systemic anaphylaxis (compare Fig. 7 to Figs. 1, 3, and 5 in the companion study [1]). In the companion study (1), we found

Table I. Death Rates and Times until Death in Wild-Type ($Fc_\epsilon RI$ α Chain +/+) or $Fc_\epsilon RI$ α Chain −/− Mice Injected Intravenously with Rat 2.4G2 Antibody or, as a Control, Normal Rat IgG

Mice*	Injection	Death rates	Times until death
			min
$Fc_\epsilon RI$ α chain −/−	2.4G2	6/6‡	30, 30, 35, 35, 45, 55
	Normal IgG	0/5	DNA
Wild-type	2.4G2	5/5‡	30, 40, 40, 50, 50
	Normal IgG	0/5	DNA

*In this experiment, female mice of 20–29 g body weight were tested, with mean body weights in the four groups (experimental and control groups of two genotypes) varying by a maximum of 21%. *DNA*, does not apply. ‡$P < 0.01$ by Fisher's exact test vs. values from normal IgG-injected (control) mice of the same genotype.

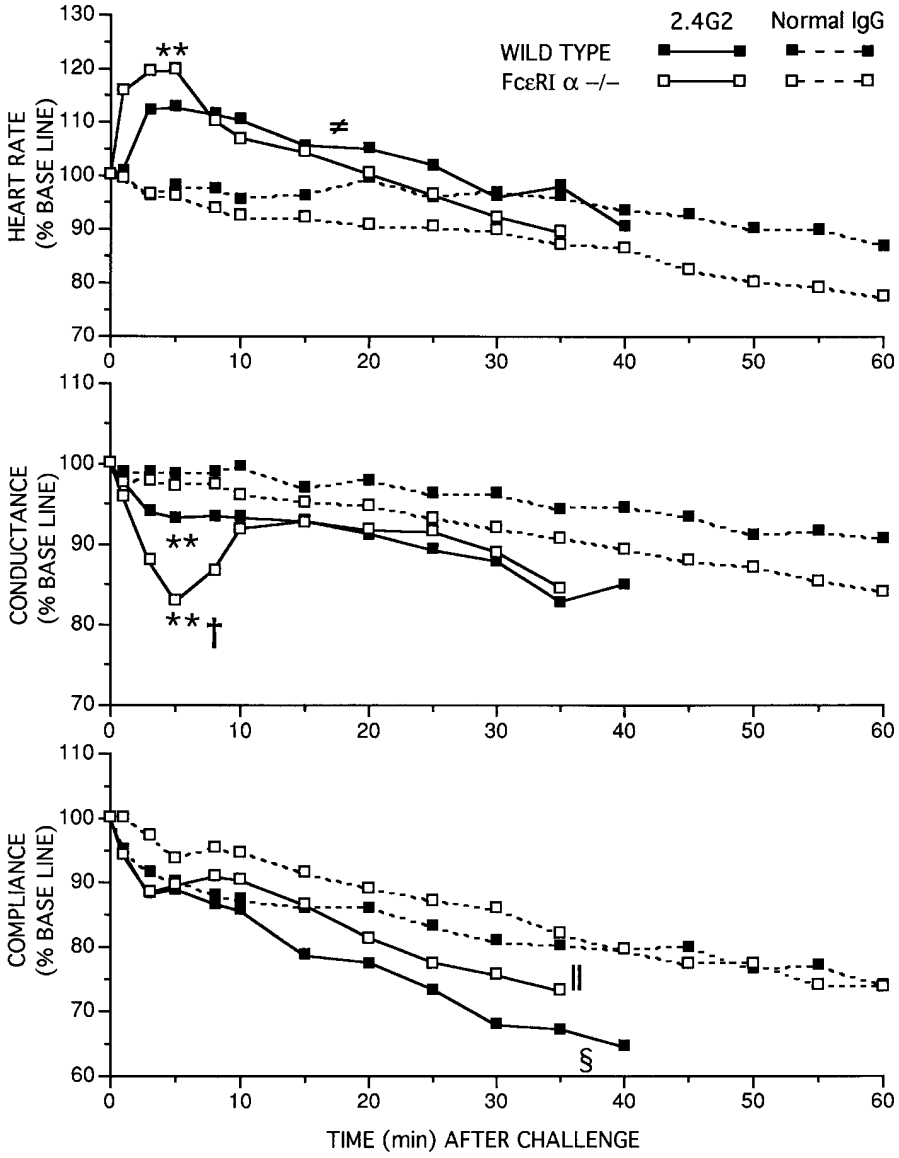


Figure 7. Pulmonary C_{dyn}, pulmonary G_L, and HR in $Fc_\epsilon RI$ α chain −/− mice (*$Fc_\epsilon RI$ α −/−, open symbols*) or the corresponding wild-type mice (*WILD TYPE, filled symbols*) which had been injected intravenously with 100 μg of rat 2.4G2 antibodies (*2.4G2, solid lines*) or 100 μg of normal rat IgG (*Normal IgG, dotted lines*). In groups in which some mice died before the end of the 60-min period of observation, mean values were determined based on data for the surviving mice. For clarity, only mean values are shown. At all intervals in all groups, the SEM for measurements of C_{dyn} or G_L were always < 6% of the mean and measurements of HR were always < 13% of the mean. $^{\S}P < 0.07$ and $^{\|}P < 0.09$ by ANOVA over the first 35 min of the response, and $^{\neq}P < 0.06$ and $^{**}P < 0.01$ by ANOVA over the first 20 min of the response, versus data from normal rat IgG–injected (control) mice of the same genotype. $^{\dagger}P < 0.05$ by ANOVA over the first 20 min of the response versus data from 2.4G2 antibody–injected wild-type mice.

that active, or IgE-induced passive, systemic anaphylactic responses were associated with more extensive mast cell degranulation than was observed in the IgG_1-dependent passive systemic anaphylaxis responses. When tissues from the mice shown in Fig. 7 were analyzed histologically, we found that the extent of mast cell degranulation in the tissues of 2.4G2 antibody–injected $Fc_\epsilon RI$ α chain −/− mice significantly exceeded that detected in the tissues of α chain −/− mice which had been sensitized previously with IgG_1 anti-DNP antibodies and then challenged with DNP-HSA (compare results in Fig. 8 to those in Fig. 4 *A* of the companion study [1]). Moreover, challenge with 2.4G2 antibody produced significantly greater degranulation of mast cells in the bronchial tissues and forestomach of $Fc_\epsilon RI$ α chain −/− mice than in the corresponding tissues of wild-type mice ($P < 0.001$ for either comparison) (Fig. 8).

Taken together, these findings suggest that, under the conditions of our experiments, the cross-linking of FcγRIII by 2.4G2 may represent a stronger trigger for the production of bioactive mediators by effector cells than activation of the cells via IgG_1 anti-DNP–DNP-HSA immune complexes. The results are also consistent with the possibility that mast cell activation can contribute to the intensity and/or kinetics of some of the pathophysiological changes associated with systemic anaphylactic responses, even though mast cells clearly are not required for the expression of the reactions. The more rapid development of physiological changes in 2.4G2 antibody–dependent, versus IgG_1- and antigen-dependent, responses may reflect the difference in the mechanisms of FcγRIII cross-linking in these two models: 2.4G2 antibodies directly induce receptor cross-linking, whereas IgG_1 must first form immune complexes with antigen and then bind to, and induce cross-linking of, FcγRIII.

$WBB6F_1$-+/+ normal mice exhibit anaphylactic responses to challenge with 2.4G2 antibodies that are more intense than those in genetically mast cell–deficient $WBB6F_1$-$Kit^W/Kit^{W\text{-}v}$ mice. To investigate further a possible role for mast cells in FcγRIII-dependent responses, we analyzed the reactions in mice that are virtually devoid of tissue mast cells ($WBB6F_1$-$Kit^W/Kit^{W\text{-}v}$ mice) and the congenic normal (+/+) mice (12–

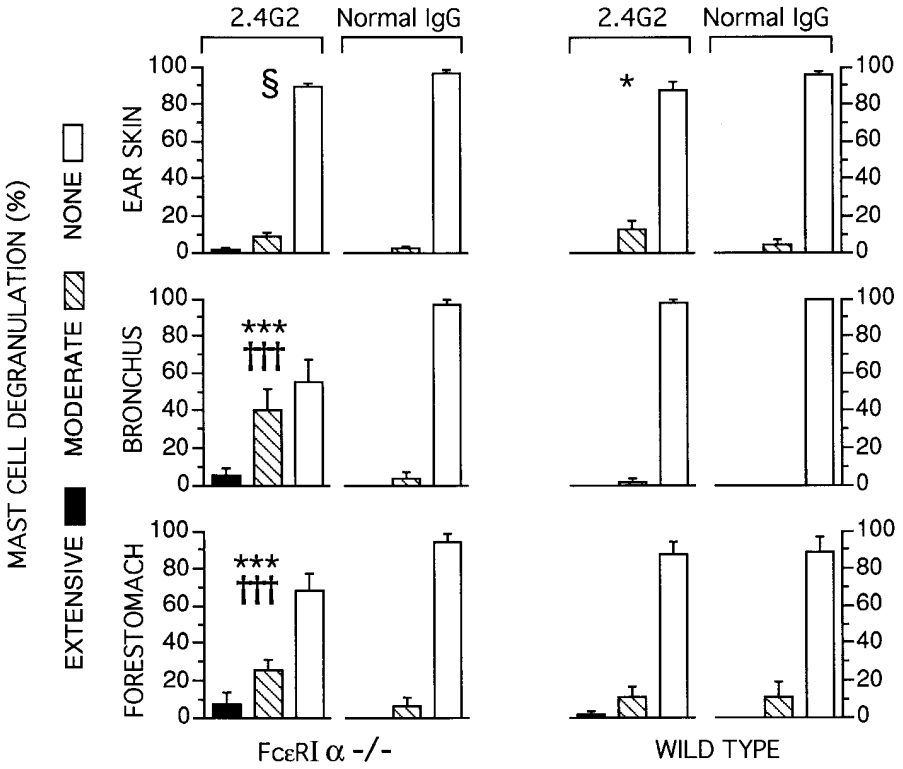


Figure 8. Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in rat 2.4G2 antibody–injected (*2.4G2*) or normal rat IgG–injected (*Normal IgG*) $Fc_\epsilon RI$ α chain −/− ($Fc_\epsilon RI$ α −/−) or corresponding wild-type (*WILD TYPE*) mice. 1-μm-thick, Epon-embedded, Giemsa-stained sections were examined as described in the text to assess the extent of mast cell activation in the tissues. Data, which are expressed as mean±SEM, are from the same mice shown in Fig. 7. $^{\S}P < 0.08$, $^{*}P < 0.05$, $^{***}P < 0.001$ by the χ^2 test versus data from the same anatomical site in normal rat IgG–injected (control) mice of the same genotype. $^{\dagger\dagger\dagger}P < 0.001$ by the χ^2 test versus data from the same anatomical site in corresponding 2.4G2-injected wild-type mice.

14). As shown in Fig. 9 *A*, $Kit^W/Kit^{W\text{-}v}$ mice and the congenic normal mice exhibited statistically indistinguishable drops in body temperature after passive sensitization with 400 μg of monoclonal IgG_1 anti-DNP antibodies and intravenous challenge 1 d later with 1.0 mg of DNP-HSA. By contrast, only the +/+ mice exhibited a drop in body temperature in response to passive sensitization with IgE anti-DNP antibodies and challenge with DNP-HSA (Fig. 9 *A*). None of the mice in this experiment died as a result of IgG_1- or IgE-dependent passive systemic anaphylaxis. These findings indicate that while mast cells may have been essential for the development of IgE-dependent passive anaphylaxis, mast cells made no detectable contribution to the magnitude of the drop in body temperature associated with the IgG_1-dependent responses. This finding stands in contrast to the results obtained in our companion study, which showed that $Kit^W/Kit^{W\text{-}v}$ mice were significantly less susceptible than the congenic +/+ (wild-type) mice to both the cardiopulmonary changes and the mortality associated with passive systemic anaphylactic reactions that were induced by challenge with the same amounts of IgG_1 and specific antigen as used in the present study (1).

One possible explanation for this discrepancy is that anesthetized, surgically manipulated, and artificially ventilated mice, such as those used in the companion study (1), are more likely than nonanesthetized mice to die as a result of IgG_1-induced systemic anaphylaxis, particularly if the responses (such as those in $WBB6F_1$-+/+ mice) are associated with mast cell degranulation. On the other hand, we found that virtually all of the $Fc_\epsilon RI$ α chain −/− or +/+ mice, or FcR γ chain +/+ mice, challenged for IgG_1-dependent passive systemic anaphylaxis in the companion study died within 1 h of antigen challenge, despite developing little or no, or only modest, levels of mast cell degranulation (1). By contrast, in the present study, no deaths occurred in mice of the same genotypes upon challenge with amounts of IgG_1 antibody and antigen that were identical to those used in the study by Miyajima et al. (1).

Whatever the reason(s) for the differences in the mortality associated with IgG_1-dependent passive systemic anaphylaxis in this and the companion study (1), our analysis of $Fc_\epsilon RI$ α chain −/− mice indicated that intravenous challenge with 2.4G2 antibodies produced significantly more mast cell degranulation, as well as more striking cardiopulmonary changes, than did challenge with IgG_1 and specific antigen. Therefore, we compared the responses to intravenous challenge with 2.4G2 antibodies in normal (+/+) and genetically mast cell–deficient ($Kit^W/Kit^{W\text{-}v}$) mice. We found that $Kit^W/Kit^{W\text{-}v}$ mice exhibited a drop in body temperature in response to 2.4G2 challenge, but that the magnitude of this response was significantly less than that in the congenic normal (+/+) mice ($P < 0.0001$ by ANOVA) (Fig. 9 *B*). Moreover, death occurred in 5 of the 12 $WBB6F_1$-+/+ mice challenged with 2.4G2 antibodies, but in none of the 6 identically challenged $Kit^W/Kit^{W\text{-}v}$ mice ($P \approx 0.09$ by Fisher's exact test). These findings show that while 2.4G2 antibody–induced anaphylactic responses can occur in the virtual absence of mast cells, the responses in $Kit^W/Kit^{W\text{-}v}$ mast cell–deficient mice are significantly attenuated compared with those in normal mice. By contrast, FcR γ chain −/− mice were unresponsive to challenge with 2.4G2 antibodies (Fig. 9 *B*).

Mast cells derived from $Fc_\epsilon RI$ α chain −/− mice express higher levels of FcγRIII than do mast cells derived from wild-type mice. One possible explanation for why challenge with 2.4G2 antibodies produced more intense physiological and mast cell degranulation responses in $Fc_\epsilon RI$ α chain −/− mice than in $Fc_\epsilon RI$ α chain +/+ mice would be that mast cells in $Fc_\epsilon RI$ α chain −/− mice exhibit increased expression of FcγRIII

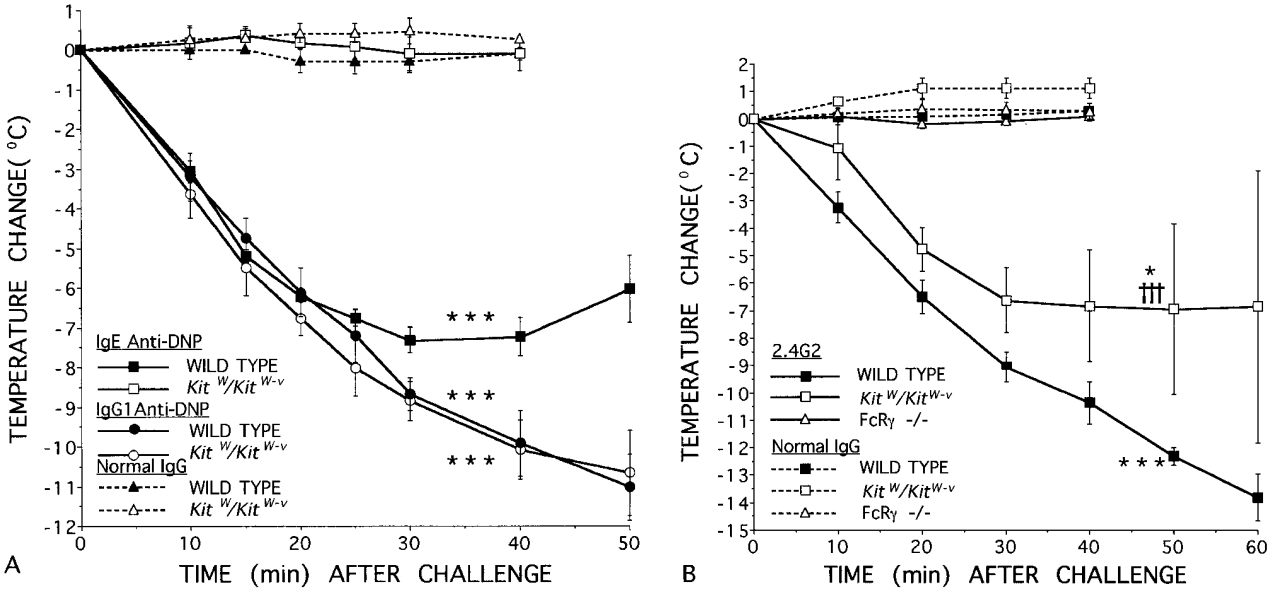


Figure 9. (*A*) Changes in body temperature after intravenous challenge with 1.0 mg of DNP-HSA administered 24 h after passive sensitization intravenously with 20 μg of mouse monoclonal IgE anti-DNP antibodies (*IgE Anti-DNP*), 400 μg of mouse monoclonal IgG_1 anti-DNP antibodies (*IgG_1 Anti-DNP*), or 400 μg of normal mouse IgG (*Normal IgG*) in $WBB6F_1$-+/+ normal mice (*WILD TYPE, filled symbols*) or $WBB6F_1$-*$Kit^W/Kit^{W\text{-}v}$* mast cell–deficient mice (*$Kit^W/Kit^{W\text{-}v}$, open symbols*). Data are shown as mean±SD ($n = 3$ per group). ***$P < 0.0001$ by ANOVA versus data from mice of same genotype that received normal IgG or versus *$Kit^W/Kit^{W\text{-}v}$* mice that received IgE anti-DNP. (*B*) Changes in body temperature after intravenous injection of 100 μg of rat monoclonal 2.4G2 antibody (*2.4G2, solid lines*) or 100 μg of normal rat IgG (*Normal IgG, dotted lines*) in $WBB6F_1$-+/+ normal mice (*WILD TYPE, filled squares*), $WBB6F_1$-*$Kit^W/Kit^{W\text{-}v}$* mast cell–deficient mice (*$Kit^W/Kit^{W\text{-}v}$, open squares*), or FcR γ chain −/− mice (*FcRγ −/−, open triangles*). Data are shown as mean±SD except for *$Kit^W/Kit^{W\text{-}v}$* injected with normal rat IgG ($n = 2$), which are shown as the mean values. *$P < 0.02$, ***$P < 0.0001$ by ANOVA versus data from normal rat IgG–injected mice of the same genotype; $^{\dagger\dagger\dagger}P < 0.0001$ by ANOVA versus data from +/+ mice that had been injected with 2.4G2 antibodies.

compared with those in the wild-type mice. Previously, we showed that BMCMCs of $Fc_\epsilon RI$ α chain −/− mouse origin exhibited increased surface expression of FcγRII/III (as detected by binding of 2.4G2 antibodies) compared with wild-type BMCMCs (2). However, this experiment did not discriminate between levels of expression of the two FcγR (FcγRII and FcγRIII) that are detected by the 2.4G2 antibody, which recognizes the highly conserved extracellular domains of FcγRII/III (15). Therefore, we generated BMCMCs of $Fc_\epsilon RI$ α chain −/− mouse and wild-type ($Fc_\epsilon RI$ α chain +/+) mouse origin, and then used antibodies specific for FcγRII and FcγRIII, as well as 2.4G2, in an attempt to immunoprecipitate FcR γ chains and FcR β chains that might be associated with the cells' FcγR.

As shown in Fig. 10, anti-FcγRIII or 2.4G2 antibodies, but not anti-FcγRII antibodies, precipitated β and FcR γ chains from the BMCMCs. However, the amounts of β or FcR γ chains precipitated from the $Fc_\epsilon RI$ α chain −/− BMCMCs greatly exceeded those precipitated from the wild-type (+/+) BMCMCs. It has been reported that the FcR β chain can associate with FcγRIIIα, as well as $Fc_\epsilon RI$, in transfected cells (28). Our results now show that the FcR β chain can also associate with FcγRIII in nontransfected, bone marrow–derived mast cells. The demonstration that $Fc_\epsilon RI$ α chain −/− BMCMCs express increased amounts of FcγRIII in comparison with wild-type BMCMCs is consistent with other lines of evidence which show that FcγRIII expression is increased when FcR γ chains and FcR β chains are not incorporated into $Fc_\epsilon RI$ and therefore are available for FcγRIII (2,6). Taken together with the recent observation that the β chain can increase the intensity of signal transduction via the $Fc_\epsilon RI$ (29), these findings are compatible with at least two mechanisms by which lack of the $Fc_\epsilon RI$ α chain could result in increased FcγRIII-dependent

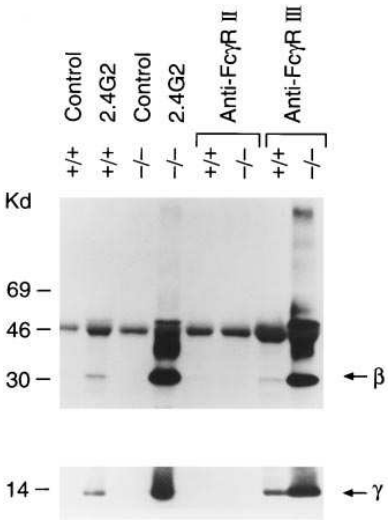


Figure 10. BMCMCs of $Fc_\epsilon RI$ α chain −/− mouse origin (−/−) express increased amounts of FcR β chain (*β*) and FcR γ chain (*γ*) in association with FcγRIII than do BMCMCs of $Fc_\epsilon RI$ α chain +/+ (wild-type) mouse origin (+/+). Lysates of BMCMCs were immunoprecipitated with either a rat 2.4G2 antibody against FcγRII/III (*2.4G2*), normal rat IgG (*Control*), or polyclonal rat antibodies specific for the cytoplasmic domains of FcγRII (*Anti-FcγRII*) or FcγRIII (*Anti-FcγRIII*). Samples run on an 8–16% polyacrylamide gel were transferred to a PVDF membrane which then was probed separately with a monoclonal antibody specific for FcR β chain (*β*) and an antiserum against FcR γ chain (*γ*).

mast cell activation: increased expression of FcγRIII on the cell surface and/or increased association of the β chain with FcγRIII.

Conclusions. In this study, we have resolved three important questions concerning the pathogenesis of systemic anaphylaxis responses in mice. First, what is the relative importance of FcγRI versus FcγRIII in FcR γ chain–dependent systemic anaphylactic reactions? Second, does increased surface expression of FcγRIII on mast cells which lack the $Fc_\epsilon RI$ α chain reflect increased association of the common FcR β and γ chains with FcγRIII, and does this have pathophysiological significance in vivo? Third, to what extent are the pathophysiological changes in FcγRIII-dependent systemic anaphylaxis mast cell–independent? To address these issues, we used measurements of body temperature to detect and quantify systemic active or passive anaphylactic reactions, or responses to injections of an antibody against FcγRII/III, in nonanesthetized mice which were subjected to neither surgical manipulations nor artificial ventilation.

We found that $Fc_\epsilon RI$ α chain −/− mice can express active systemic anaphylactic reactions, as well as IgG_1-dependent passive systemic anaphylaxis. We also showed that the intensity of some of the physiological changes associated with responses to IgG_1 and specific antigen or intravenous challenge with 2.4G2 anti-FcγRII/III antibodies was greater in $Fc_\epsilon RI$ α chain −/− mice than in the wild-type animals. These observations thus confirm and extend those in our companion study, which showed that anesthetized, surgically manipulated, and artificially ventilated $Fc_\epsilon RI$ α chain −/− mice challenged to express active or IgG_1-dependent passive systemic anaphylaxis can develop changes in HR, C_{dyn}, and G_L which are at least as large as, and in some cases greater than, those in the corresponding wild-type mice (1).

In addition, we found that the previously reported increased surface expression of $Fc_\epsilon RII$ and/or III by mouse mast cells which lack the $Fc_\epsilon RI$ α chain (2) reflects the increased association of the common FcR β and γ chains with FcγRIII. This finding clearly may have in vivo relevance, in that the mast cell degranulation, as well as the pathophysiological changes, associated with responses to intravenous challenge with 2.4G2 antibodies was significantly greater in $Fc_\epsilon RI$ α chain −/− mice than in wild-type mice. Although these observations were made by comparing the responses in $Fc_\epsilon RI$ α chain −/− mice with those in the corresponding wild-type mice, the findings strongly suggest that the expression of $Fc_\epsilon RI$- or FcγRIII-dependent effector cell function in normal mice may be significantly affected by the availability of limiting amounts of the common FcR β and/or γ chains. Thus, approaches which result in diminished expression of $Fc_\epsilon RI$ α chain may result in enhanced FcγRIII-dependent effector function.

Finally, we observed that the responses induced by 2.4G2 antibody challenge in normal (+/+) mice were significantly stronger than those observed in the congenic mast cell–deficient $Kit^W/Kit^{W\text{-}v}$ mice. In other words, although FcγRIII-dependent pathophysiological changes can occur in the virtual absence of mast cells, mast cells may contribute to the intensity of these reactions.

Thus, we have shown that the direct activation of FcγRIII with 2.4G2 antibodies, by a mechanism that does not also activate FcγRI, can produce physiological changes that are very similar to those observed in active anaphylaxis. This finding in normal mice, when taken together with the results obtained in $Fc_\epsilon RI$ α chain −/− mice, strongly supports the hypothesis that the death associated with active systemic anaphylaxis in the mouse, as well as the drop in body temperature and other physiological changes associated with the reaction, may largely reflect IgG_1-dependent activation of FcγRIII. Our findings also indicate that mast cell activation can contribute to the intensity of FcγRIII-dependent responses, especially in $Fc_\epsilon RI$ α chain −/− mice, but that other effector cells must have important roles in the pathogenesis of these reactions. Monocytes and macrophages represent attractive candidate additional effector cells in these settings, since only these cells and natural killer cells are known to express FcγRIII in the mouse and since monocytes/macrophages represent potential sources of many biologically active mediators (3, 6).

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