# Systemic Anaphylaxis in the Mouse Can Be Mediated Largely through $IgG_1$ and FcγRIII

## Assessment of the Cardiopulmonary Changes, Mast Cell Degranulation, and Death Associated with Active or IgE- or $IgG_1$-dependent Passive Anaphylaxis

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## Abstract

**We attempted to elicit active anaphylaxis to ovalbumin, or passive IgE- or $IgG_1$-dependent anaphylaxis, in mice lacking either the $Fc_\epsilon RI$ α chain or the FcR γ chain common to $Fc_\epsilon RI$ and FcγRI/III, or in mice lacking mast cells ($Kit^W/Kit^{W\text{-}v}$ mice), and compared the responses to those in the corresponding wild-type mice. We found that the FcR γ chain is required for the death, as well as for most of the pathophysiological changes, associated with active anaphylaxis or IgE- or $IgG_1$-dependent passive anaphylaxis. Moreover, some of the physiological changes associated with either active, or $IgG_1$-dependent passive, anaphylactic responses were significantly greater in $Fc_\epsilon RI$ α chain −/− mice than in the corresponding normal mice. Finally, while both $Kit^W/Kit^{W\text{-}v}$ and congenic +/+ mice exhibited fatal active anaphylaxis, mast cell–deficient mice exhibited weaker physiological responses than the corresponding wild-type mice in both active and $IgG_1$-dependent passive systemic anaphylaxis. Our findings strongly suggest that while IgE antibodies and $Fc_\epsilon RI$ may influence the intensity and/or kinetics of some of the pathophysiological changes associated with active anaphylaxis in the mouse, the mortality associated with this response can be mediated largely by $IgG_1$ antibodies and FcγRIII. (*J. Clin. Invest.* 1997. 99:901–914.) Key words: allergy • asthma • FcγRI • IgE • passive cutaneous anaphylaxis**

## Introduction

IgE-dependent mast cell activation has long been regarded as one of the essential steps in the development of the bronchoconstriction and other physiological changes associated with active anaphylaxis (1, 2). This hypothesis not only is consistent with clinical observations (1, 2), but has been supported by certain animal studies. For example, the adoptive transfer of IgE antibodies to normal mice (but not to genetically mast cell–deficient mice) primes these animals to express passive systemic anaphylactic reactions to intravenous challenge with specific antigen, and these responses are associated with extensive mast cell degranulation, marked alterations in cardiopulmonary function, and considerable mortality (3). Thus, IgE-dependent mast cell activation can indeed produce many of the pathophysiological changes that are associated with active anaphylaxis (3, 4).

However, several recent findings have called into question the essential role of mast cells or IgE antibodies in murine active anaphylactic responses. Genetically mast cell–deficient $WBB6F_1$-$Kit^W/Kit^{W\text{-}v}$ or $WCB6F_1$-$Mgf^{Sl}/Mgf^{Sl\text{-}d}$ mice, which are virtually devoid of tissue mast cells, can express active anaphylactic responses that are associated with significant physiological changes (5, 6), as well as with mortality rates that are similar to those observed in the identically sensitized and challenged congenic +/+ normal mice (5–9). Moreover, IgE null mice also can express fatal active anaphylaxis responses that are associated with striking airway responses (10). Thus, neither mast cells nor IgE antibodies are essential for the expression of at least some of the features of active systemic anaphylaxis in mice.

Sensitization of mice for active anaphylaxis not only induces an IgE response, but also results in the production of antigen-specific $IgG_1$ antibodies (11, 12). Furthermore, IgG antibodies of the $IgG_1$ isotype, as well as IgE antibodies, can elicit both passive cutaneous anaphylactic reactions (13) and passive systemic anaphylactic responses (14–16) in mice. And it has been shown recently that $IgG_1$ antibodies can also passively sensitize mice for the expression of airway hyperresponsiveness to electrical stimulation in vitro, and for the development of increased numbers of eosinophils and other leukocytes in bronchoalveolar lavage fluid after allergen challenge of the airways in vivo (17). Therefore, we investigated the hypothesis that $IgG_1$ antibodies and FcγR, rather than IgE antibodies and $Fc_\epsilon RI$, can be critical for the expression of the cardiopulmonary changes and mortality associated with active systemic anaphylaxis in mice.

To assess the relative importance of IgE and $Fc_\epsilon RI$ versus $IgG_1$ and FcγR in the pathogenesis of active anaphylaxis in mice, we used mice with targeted mutations of either the $Fc_\epsilon RI$ α chain of the $Fc_\epsilon RI$ (18) or the common γ chain of the $Fc_\epsilon RI$ and the FcγRI/III (19). The $Fc_\epsilon RI$ consists of a single $Fc_\epsilon RI$ α chain, a single β chain, and two disulfide-linked γ chains (18, 20, 21). The $Fc_\epsilon RI$ α chain binds the Fc portion of IgE, whereas the β chain promotes the assembly and cell surface expression of the $Fc_\epsilon RI$ and also helps to amplify signal transduction through the $Fc_\epsilon RI$ receptor's two γ chains (20–22). Mice with targeted disruption of the gene encoding the $Fc_\epsilon RI$ α chain

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($Fc_\epsilon RI$ α chain −/− mice) have normal numbers of tissue mast cells, but do not express $Fc_\epsilon RI$ and do not exhibit detectable increases in vascular permeability when examined for expression of IgE-dependent passive cutaneous or systemic anaphylactic responses (18). However, until this study, the ability of $Fc_\epsilon RI$ α chain −/− mice to express active systemic anaphylaxis, or $IgG_1$-dependent passive systemic anaphylaxis, had not been investigated.

IgG antibodies can bind to three FcγRs, two of which (FcγRI and FcγRIII) share the same γ chain as the $Fc_\epsilon RI$ (19, 20). FcγRI, which consists of an $Fc_\epsilon RI$ α chain and two γ chains, is a high-affinity IgG receptor that binds monomeric IgG (preferentially, $IgG_{2a}$). It is expressed on macrophages and neutrophils and mediates phagocytosis and antibody-dependent cellular cytotoxicity (23, 24). Mouse FcγRII and FcγRIII are low-affinity receptors that bind multimeric IgG (in decreasing order of affinity: $IgG_{2b} > IgG_{2a} > IgG_1$) (20, 24). FcγRII is a single chain receptor expressed on B cells, T cells, and mast cells, on which it acts as an inhibitory receptor (25–27). By contrast, FcγRIII is present on macrophages, neutrophils, and natural killer cells, where it can trigger antibody-dependent cellular cytotoxicity, endocytosis, exocytosis, and release of inflammatory mediators, and on mast cells, where it can trigger degranulation (28).

Mice with targeted mutation of the gene encoding the γ chain of the $Fc_\epsilon RI$ and FcγRI/III (FcRγ chain −/− mice) develop normal numbers of tissue mast cells but express neither $Fc_\epsilon RI$ nor FcγRI/III (19). Accordingly, FcγR −/− mice fail to develop local changes in cutaneous vascular permeability (i.e., passive cutaneous anaphylaxis responses) upon challenge with IgE and specific antigen (19). However, until this study, the ability of FcR γ chain −/− mice to express active systemic anaphylaxis, or $IgG_1$-dependent passive systemic anaphylaxis, had not been investigated.

In this study, we analyzed the ability of $Fc_\epsilon RI$ α chain −/− or FcR γ chain −/− mice, and the corresponding normal mice, to express either active systemic anaphylaxis, or passive IgE- or $IgG_1$-dependent systemic anaphylaxis, as assessed by the extent of mast cell degranulation, the characteristics of the cardiopulmonary responses, and the death rates associated with these responses. We also attempted to elicit and characterize these three forms of systemic anaphylaxis in genetically mast cell–deficient $WBB6F_1$-$Kit^W/Kit^{W\text{-}v}$ mice and the congenic normal ($WBB6F_1$-+/+) mice. The results show that IgE, $Fc_\epsilon RI$, and/or mast cells may contribute to some of the pathophysiology of active systemic anaphylactic responses in the mouse, but that the mortality associated with active anaphylaxis is dependent on the FcR γ chain but not the $Fc_\epsilon RI$. The results also strongly suggest that the mortality associated with active anaphylaxis in the mouse is mediated primarily by $IgG_1$ antibodies and FcγRIII.

## Methods

### *Animals*

The production of mice with targeted mutations that result in failure of production of the α chain of the $Fc_\epsilon RI$ ($Fc_\epsilon RI$ α chain −/− mice) (18) or the FcR γ chain (FcR γ chain −/− mice) (19), and many of the phenotypic characteristics of these mice, have been described in detail. For these studies, we used male and female $Fc_\epsilon RI$ α chain −/− mice (18) that were backcrossed for three generations with BALB/c mice, and used BALB/c mice (purchased from Jackson Biological Laboratory, Bar Harbor, ME) as $Fc_\epsilon RI$ α chain +/+ mice. Male and female FcR γ chain −/− and +/+ mice were generated by breeding the $F_2$ offspring of crosses between chimeras and C57BL/6 mice (19). Genetically mast cell–deficient male $WBB6F_1$-$Kit^W/Kit^{W\text{-}v}$ mice, which virtually lack tissue mast cells (29–31), and the congenic normal ($WBB6F_1$-+/+) male mice, were purchased from the Jackson Biological Laboratory. All mice were 9–23 wk old (18–40 g body wt) at the time of antigen challenge (see below, *Protocols*). In the individual experiments (testing one of the three models of systemic anaphylaxis in the various mutant mice and the corresponding wild-type mice) the mice in the four groups (experimental and control groups of mutant and wild-type animals) varied in mean age by a maximum of 8–34% and in mean body weight by a maximum of 3–28%.

The animal experiments were conducted in accordance with the Beth Israel Hospital's Institutional Animal Care and Use Committee and 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.

### *Reagents*

H 1 DNP-ϵ-26 hybridoma cells, which produce a mouse monoclonal IgE antibody with specificity for DNP (32), were generously provided by Drs. Fu-Tong Liu and David Katz (La Jolla, CA). An ascites preparation containing mouse monoclonal $IgG_1$ antibodies with specificity for DNP (clone U7.6 [reference 33]) was kindly provided by Dr. David M. Segal (Bethesda, MD). Normal mouse IgG (used as a control for mouse monoclonal $IgG_1$ anti-DNP antibodies) was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). $DNP_{30\text{-}40}$ human serum albumin (DNP-HSA), propranolol, and ovalbumin (OVA)[1] were purchased from Sigma Chemical Co. (St. Louis, MO). We also purchased pentobarbital sodium (Anthony Products Co., Arcadia, CA), aluminum potassium sulfate (Fisher Scientific, Pittsburgh, PA), and *Bordetella pertussis* toxin (List Biological Laboratories, Inc., Campbell, CA).

### *Physiological measurements*

Heart rate (HR) and the pulmonary mechanical parameters, dynamic compliance ($C_{dyn}$), and lung conductance ($G_L$), were measured in mice anesthetized with 70–90 mg/kg i.p. of sodium pentobarbital using a plethysmographic method (4, 5, 34). $C_{dyn}$ and pulmonary resistance were calculated from the recordings of volume, flow, and pressure using standard techniques (35). Baseline values of HR, $C_{dyn}$, and $G_L$ were determined 10–20 min after preparation of the animals for recording pulmonary parameters. Baseline values for HR, $C_{dyn}$, and $G_L$ in the six groups of mice tested were similar to those previously reported for $WCB6F_1$-+/+ mice (2), with only minor differences between values for the mutant and the corresponding wild-type mice (Table I).

After establishing baseline levels of physiological parameters, mice were then challenged with antigen as described below (*Protocols*) for assessment of anaphylactic reactions, and HR, $C_{dyn}$, and $G_L$ were measured at multiple intervals until death due to antigen challenge or, after a period of 40 or 60 min, cervical dislocation. Changes in physiological measurements over time are expressed as a percentage of the corresponding baseline value (which was taken as 100%). Time of death was estimated as that time at which no spontaneous respiratory activity was detected when the ventilator was shut off (approximately every 5 min beginning 20 min after antigen challenge). All of the physiological experiments were performed by a single investigator (I. Miyajima).

1. *Abbreviations used in this paper:* $C_{dyn}$, dynamic compliance; $G_L$, lung conductance; HR, heart rate; OVA, ovalbumin.

*Table I. Baseline Values of HR, $C_{dyn}$, and $G_L$ in $Fc_\varepsilon RI$ α Chain −/−, FcR γ Chain −/−, Mast Cell–deficient $Kit^W/Kit^{W-v}$ Mice and Corresponding +/+ Mice*

| Mice | HR | $C_{dyn}$ | $G_L$ |
|---|---|---|---|
| | *beats/min* | *ml·cm$H_2O^{-1}$* | *ml·$s^{-1}$·cm$H_2O^{-1}$* |
| $Fc_\varepsilon RI$ α chain −/− ($n = 39$) | 189±45 | 0.046±0.013 | 1.04±0.52 |
| $Fc_\varepsilon RI$ α chain +/+ ($n = 35$) | 170±33 | 0.046±0.015 | 0.92±0.15 |
| FcR γ chain −/− ($n = 31$) | 190±32* | 0.027±0.008 | 1.29±0.41 |
| FcR γ chain +/+ ($n = 29$) | 209±40 | 0.031±0.009 | 1.50±0.45 |
| $Kit^W/Kit^{W-v}$ ($n = 26$) | 167±22 | 0.034±0.010 | 1.03±0.34 |
| *Kit* (+/+) ($n = 22$) | 179±27 | 0.034±0.011 | 1.02±0.28 |

Values are mean±SD ($n$ = 22–39/group) for mice used in the studies reported in Figs. 1–8. *$P < 0.05$ by Student's $t$ test (two-tailed) vs. values for corresponding wild-type (+/+) mice.

### *Histologic studies*

The presence of tissue mast cells and their state of activation were assessed in 1-μm, Epon-embedded, Giemsa-stained sections (4, 36, 37). Tissues were removed and fixed as described previously (5, 36, 37), either immediately after death induced by antigen (OVA or DNP-HSA) challenge, or after death by cervical dislocation 40–60 min after challenge. Sections of ear skin dermis (5, 37), peribronchial tissues (4, 5), and forestomach (2) were evaluated for mast cell numbers and extent of mast cell degranulation, as described previously. Sections were coded so that the observer (I. Miyajima) was not aware of the identity of individual specimens, and examined at a magnification of 400 by light microscopy. 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" (5, 36, 37). Our assessment of mast cell numbers in dermis of ear skin, peribronchial tissues, and forestomach confirmed the previous reports that the $Fc_\varepsilon RI$ α chain −/− (18) and FcR γ chain −/− (19) mice exhibit essentially normal numbers of tissue mast cells (data not shown).

### *Protocols*

*Sensitization for active systemic anaphylaxis to OVA.* Mice received a single intraperitoneal injection of 0.1 ml of sterile, pyrogen-free 0.9% NaCl containing 100 μg of OVA, 300 ng of *Bordetella pertussis* toxin, and 1.0 mg of aluminum potassium sulfate. Control (sham-immunized) mice received a single intraperitoneal injection of 0.1 ml of the same solution except for the omission of OVA. 18–21 d after OVA or sham immunization, mice were prepared for physiological measurements (see above) and were challenged by a rapid intravenous infusion (via an indwelling jugular vein catheter) of 500 μg of OVA in ~ 50 μl of sterile, pyrogen-free 0.9% NaCl. Approximately 100 μl of tail vein blood was obtained from mice placed under light ether anesthesia on the day before OVA or sham immunization (day −1) and on the day before antigen challenge (days 17–20), and the serum was stored at −80°C before assay for total IgE and IgG using ELISA assays as described in the companion manuscript (38).

*Sensitization for IgE- or $IgG_1$-dependent passive systemic anaphylaxis.* Mouse monoclonal IgE anti-DNP antibodies (~ 20 μg, as a 1:20 dilution of ascites in sterile, pyrogen-free 0.9% NaCl) or mouse monoclonal $IgG_1$ anti-DNP antibodies (~ 400 μg, as a 1:4 dilution of ascites in sterile, pyrogen-free 0.9% NaCl) were administered intravenously by tail vein in volumes of ~ 0.1 ml/mouse. Control mice for the IgE-induced passive anaphylaxis experiments received, instead of IgE anti-DNP antibodies, the same volume of 0.9% NaCl; controls for $IgG_1$-induced passive anaphylaxis experiments received, instead of $IgG_1$ anti-DNP antibodies, 400 μg of normal mouse IgG in the same volume of 0.9% NaCl. 1 d (~ 20–30 h) after passive sensitization (or control injections), the mice were prepared for physiological measurements (see above) and then challenged by a rapid intravenous infusion (via an indwelling jugular vein catheter) of ~ 50 μl of sterile, pyrogen-free 0.9% NaCl containing either 200 μg of DNP-HSA (for experiments assessing IgE-induced passive systemic anaphylaxis) or 1.0 mg of DNP-HSA (for experiments assessing $IgG_1$-induced passive systemic anaphylaxis). We selected the quantities of IgE or $IgG_1$ monoclonal antibodies to use for passive sensitization and the amounts of DNP-HSA to use for challenge based on preliminary dose–response experiments which showed that the chosen amounts of IgE antibodies and DNP-HSA produced fatal anaphylactic responses in the majority of normal mice tested for cardiopulmonary responses to challenge with IgE and specific antigen (Miyajima, I., and S.J. Galli, unpublished data), and on dose–response experiments which showed that the chosen amounts of $IgG_1$ antibodies and DNP-HSA produced marked drops in body temperature in normal mice (see the companion study, reference 38).

### *Statistical analysis*

Differences among the various groups of mice in the time courses of HR, $C_{dyn}$, or $G_L$ responses were examined for statistical significance by ANOVA. Differences in the maximum diminution in $C_{dyn}$ or $G_L$, or the maximum HR responses, and differences in the numbers of mast cells in the tissues of different groups of mice 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 $\chi^2$ test. Differences in the death rates between different experimental groups were examined for statistical significance by Fisher's exact test. Differences in the serum concentrations of total IgE or $IgG_1$ in the different experimental groups in experiments assessing active anaphylaxis were examined for statistical significance by the Mann-Whitney U test, two-tailed. $P < 0.05$ was regarded as significant. Unless otherwise specified, results are expressed as the mean±SEM.

## Results

*Active systemic anaphylaxis is expressed in $Fc_\varepsilon RI$ α chain −/− mice, but not in FcR γ chain −/− mice.* In normal mice ($Fc_\varepsilon RI$ α chain +/+ or FcR γ chain +/+ mice), antigen (OVA) challenge induced an active anaphylaxis response which was associated with the rapid development of tachycardia, diminished $G_L$, and reduced $C_{dyn}$ (Fig. 1). Antigen-challenged, OVA-sensitized $Fc_\varepsilon RI$ α chain −/− mice developed a tachycardia response that was similar in magnitude to that in the identically sensitized and challenged $Fc_\varepsilon RI$ α chain +/+ mice, but which was somewhat more prolonged in duration (Fig. 1 *A*). In addition, in comparison to the responses in OVA-sensitized $Fc_\varepsilon RI$ α chain +/+ mice, antigen challenge of OVA-sensitized $Fc_\varepsilon RI$ α chain −/− mice produced a more prolonged reduction in $G_L$, as well as a significantly greater reduction in $C_{dyn}$ (Fig. 1 *A*). The differences in the physiological responses of the $Fc_\varepsilon RI$ α chain −/− versus +/+ mice are not likely to have reflected differences in the size, age, or gender distribution of the mice expressing active anaphylaxis. Thus, we analyzed active anaphylaxis in four male and two female $Fc_\varepsilon RI$ α chain −/− mice, of 13.8±1.6 wk of age [mean±SEM] and 29.8±0.8 g body weight, versus six male and three female $Fc_\varepsilon RI$ α chain −/− mice, of

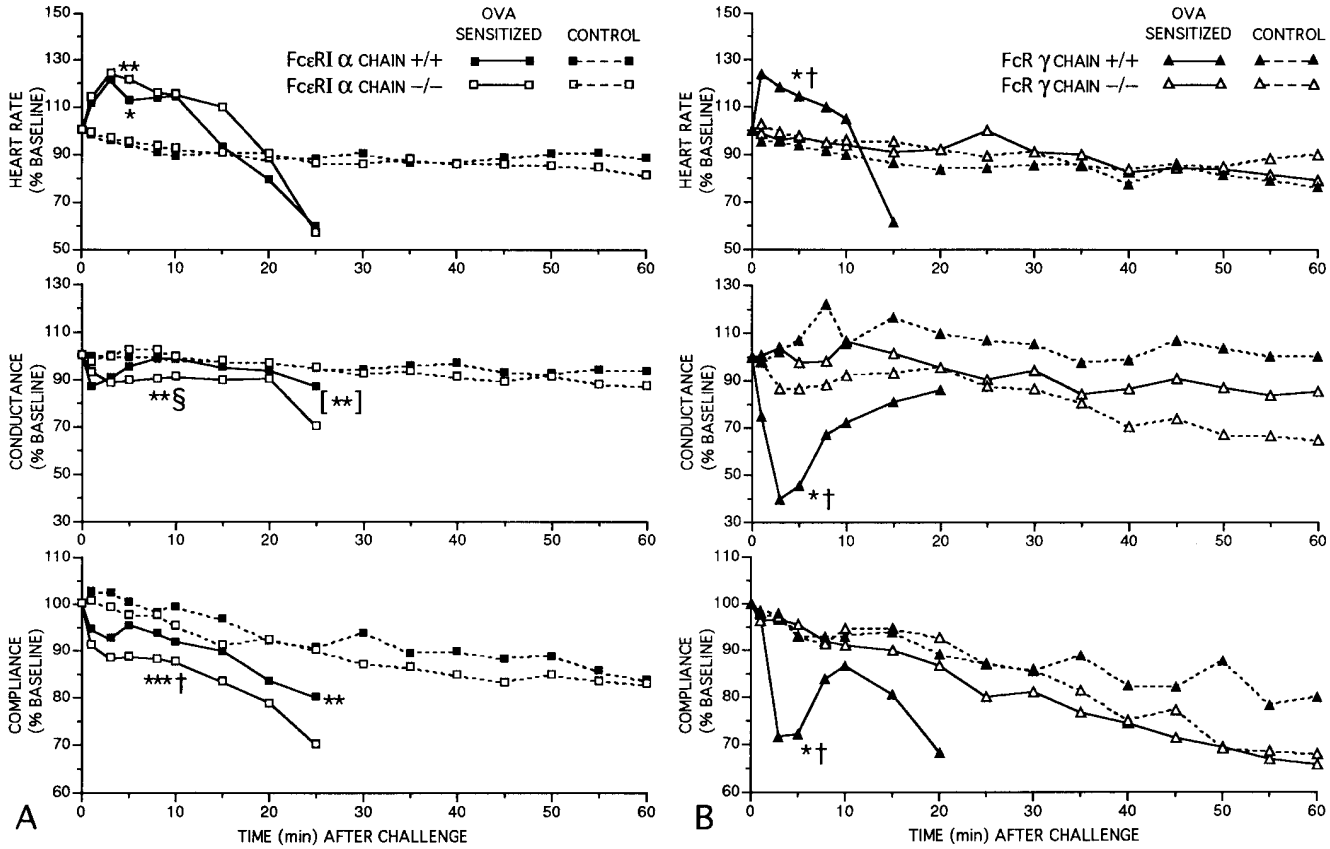


*Figure 1.* Pulmonary $C_{dyn}$, pulmonary $G_L$, and HR in $Fc_\epsilon RI$ α chain −/− mice or FcR γ chain −/− mice (*open symbols*, *A* and *B*, respectively) or the corresponding normal (+/+) mice (*filled symbols*) which had been sensitized with OVA (*OVA-SENSITIZED, solid lines*) or were sham-immunized (*CONTROL, dotted lines*), and then 18–21 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 500 μg of OVA. (*A*) $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice. (*B*) FcR γ chain −/− and corresponding +/+ mice. 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 $C_{dyn}$, $G_L$, or HR were ≤ 20% and usually < 15% of the mean. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ by ANOVA over the first 20 min of the response versus data from nonsensitized (control) mice of the same genotype; $^{[**]}P < 0.01$ by ANOVA over the first 8 min of the response versus data from nonsensitized (control) mice of the same genotype. $^{\S}P < 0.06$, $^{\dagger}P < 0.05$ by ANOVA over the first 15 min (for HR in γ chain +/+ vs. γ chain −/− mice) or 20 min (for all other comparisons) of the response versus data from OVA-sensitized mice of the other genotype. The exact numbers of mice in the various groups ($n$ = 4–9/group) are given in Table II.

15.9±1.8 wk of age and 29.1±1.3 g body weight. Note that in OVA-sensitized mice of either $Fc_\epsilon RI$ α chain +/+ or −/− genotype, the changes in HR reached maximal levels within 5 min of antigen challenge, and that the initial rapid declines in $G_L$ and $C_{dyn}$ also occurred within this same time frame.

As assessed morphologically in 1-μm, Epon-embedded, Giemsa-stained sections (Fig. 2 *A*), antigen challenge of OVA-sensitized $Fc_\epsilon RI$ α chain −/− mice or +/+ mice resulted in extensive degranulation of mast cells in the ear skin, peribronchial tissues, and forestomach, whereas virtually all of the mast cells in the corresponding tissues of OVA-challenged nonsensitized $Fc_\epsilon RI$ α chain −/− mice or +/+ mice appeared normal (i.e., exhibited no morphological evidence of activation). Even though the OVA-sensitized $Fc_\epsilon RI$ α chain −/− mice developed pulmonary changes upon antigen challenge that were significantly greater than those in the identically challenged OVA-sensitized wild-type mice, the extent of mast cell degranulation in the bronchial tissues and forestomach of the OVA-sensitized $Fc_\epsilon RI$ α chain −/− mice was slightly, but significantly, less than that in the corresponding tissues of the OVA-sensitized wild-type mice.

The death rates due to active anaphylaxis were statistically indistinguishable in OVA-sensitized $Fc_\epsilon RI$ α chain −/− mice or +/+ mice (Table II). All but one of the sensitized $Fc_\epsilon RI$ α chain −/− or +/+ mice died, most of them within 30 min of antigen challenge, whereas none of the antigen-challenged nonsensitized mice of either genotype died ($P < 0.01$ for either comparison). Both $Fc_\epsilon RI$ α chain −/− and +/+ mice exhibited increased serum concentrations of total IgE after OVA immunization (day −1 vs. day 17–20 values of 0.77±0.70 vs. 6.41±6.17 μg/ml [mean±SD], $P = 0.006$ for $Fc_\epsilon RI$ α chain −/− mice and 0.68±0.52 vs. 1.70±1.57 μg/ml, $P = 0.082$ for $Fc_\epsilon RI$ α chain +/+ mice). By contrast, serum concentrations of total IgG antibodies exhibited only modest increases in OVA-immunized mice (17 or 57% increases over baseline [day −1] values, from 235±222 to 276±322 μg/ml in $Fc_\epsilon RI$ α chain −/− mice, and from 207±58 to 326±99 μg/ml in the corresponding +/+ mice, respectively).

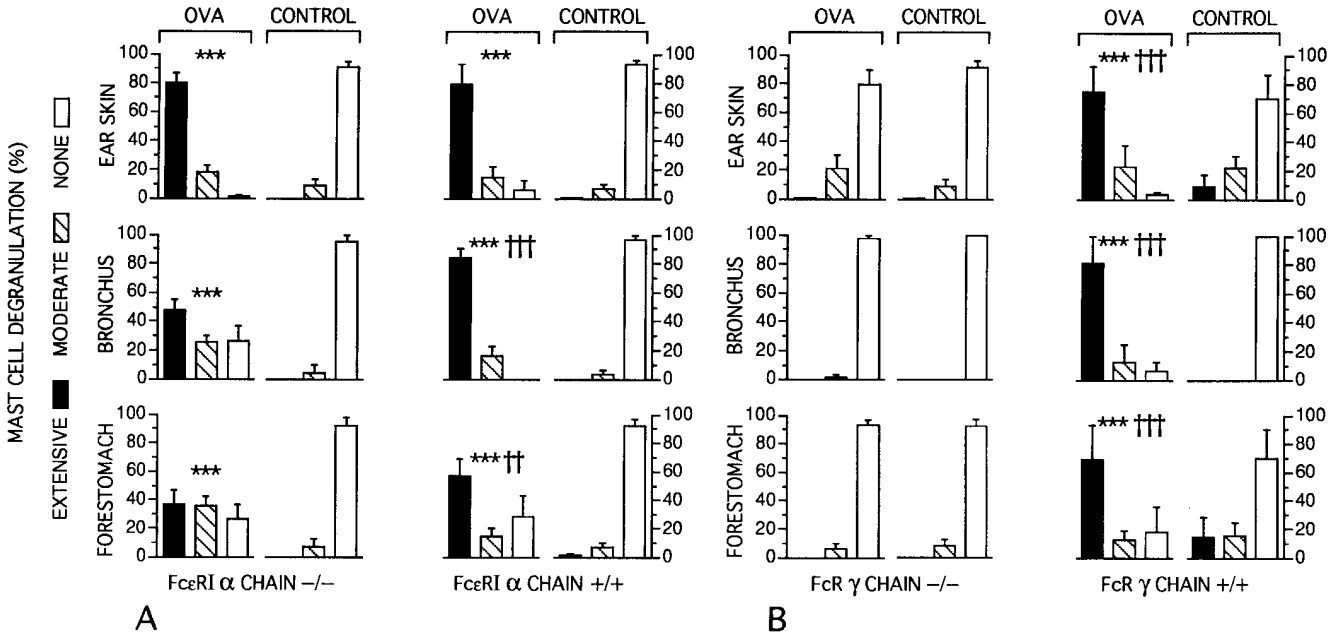


*Figure 2.* Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in OVA-sensitized (*OVA*) or sham-immunized (*CONTROL*) $Fc_\epsilon RI$ α chain −/− or FcR γ chain −/− mice and the corresponding +/+ mice which had been challenged with 500 μg of OVA intravenously. (*A*) $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice. (*B*) FcR γ chain −/− and corresponding +/+ 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. 1, *A* and *B*. ***$P < 0.001$ by the $\chi^2$ test versus data from the same anatomical site in sham-immunized mice of the same genotype. ††$P < 0.01$ and †††$P < 0.001$ by the $\chi^2$ test versus data from the same anatomical site in corresponding OVA-sensitized −/− mice.

OVA-sensitized FcR γ chain +/+ mice also rapidly developed tachycardia in response to intravenous antigen challenge (Fig. 1 *B*), and this response was similar in magnitude to that in the $Fc_\epsilon RI$ α chain +/+ mice (Fig. 1 *A*). The OVA-sensitized FcR γ chain +/+ mice also rapidly developed reductions in $G_L$ and $C_{dyn}$ after antigen challenge, but these responses were even more striking than those in the $Fc_\epsilon RI$ α chain +/+ (or $Fc_\epsilon RI$ α chain −/−) mice (compare Fig. 1, *A* and *B*), perhaps reflecting differences in the genetic backgrounds of the mice (BALB/c for the $Fc_\epsilon RI$ α chain +/+ mice, C57BL/6 × 129 for the FcR γ chain +/+ mice). However, by morphological analysis, the extent of mast cell degranulation in the ear skin, bronchial tissues, and forestomach of the OVA-sensitized FcR γ chain +/+ mice (Fig. 2 *B*) was very similar to that in the corresponding tissues of the OVA-sensitized $Fc_\epsilon RI$ α chain +/+ mice (Fig. 2 *A*).

All of the OVA-sensitized FcR γ chain +/+ mice died within 20 min of intravenous antigen challenge (Table II). By contrast, none of the antigen-challenged OVA-sensitized FcR γ chain −/− mice died (Table II). The OVA-sensitized FcR γ chain −/− mice also developed little or no morphological evidence of mast cell degranulation as a result of OVA challenge (Fig. 2 *B*), nor did these mice develop significant changes in HR, $G_L$, or $C_{dyn}$ (Fig. 1 *B*). Thus, by all of the criteria which we analyzed, FcR γ chain −/− mice were unable to express active anaphylaxis.

The lack of anaphylactic responsiveness in FcR γ chain −/− mice did not appear to reflect a defect in the ability of these mice to generate an IgE antibody response, in that serum concentrations of total IgE in OVA-immunized FcR γ chain −/− mice (31.9±14.9 μg/ml, 10.4 times the baseline level, $P = 0.008$) were even greater than those in the OVA-immunized FcR γ chain +/+ mice (2.96±0.91 μg/ml, 4.8 times the baseline level, $P = 0.016$). However, FcR γ chain +/+ mice exhibited a larger increase in serum concentrations of total IgG (to 474±313 μg/ml, a 260% increase over baseline, $P = 0.016$) than did the FcR γ chain −/− mice (to 520±236 μg/ml, a 37% increase over baseline, $P = 0.22$).

*Table II. Death Rates and Times until Death in OVA- or Sham-immunized Mice Challenged Intravenously with 500 μg of OVA*

| Mice | OVA-immunized | Death rates | Times until death |
|---|---|---|---|
| | | | *min* |
| $Fc_\epsilon RI$ α chain −/− | + | 8/9* | 20, 20, 20, 25, 25, 25, 25, 50 |
| | − | 0/7 | DNA |
| $Fc_\epsilon RI$ α chain +/+ | + | 5/6* | 25, 25, 25, 30, 30 |
| | − | 0/8 | DNA |
| FcR γ chain −/− | + | 0/5‡ | DNA |
| | − | 0/5 | DNA |
| FcR γ chain +/+ | + | 5/5* | 20, 20, 20, 20, 20 |
| | − | 0/4 | DNA |

*DNA*, does not apply. *$P < 0.01$ by Fisher's exact test vs. values from sham-immunized mice of the same genotype. ‡$P < 0.01$ by Fisher's exact test vs. values from corresponding wild-type (+/+) mice in the same treatment group.

*Table III. Death Rates and Times until Death in Mice Passively Sensitized with 400 μg of Mouse Monoclonal $IgG_1$ Anti-DNP Antibodies or Injected with 400 μg of Control Mouse IgG, 1 d before Intravenous Challenge with 1 mg of DNP-HSA*

| Mice | $IgG_1$ anti-DNP | Death rates | Times until death |
|---|---|---|---|
| | | | *min* |
| $Fc_\epsilon RI$ α chain −/− | + | 6/6* | 25, 30, 30, 30, 30, 40 |
| | − | 1/6 | 50 |
| $Fc_\epsilon RI$ α chain +/+ | + | 5/5* | 25, 30, 30, 30, 30 |
| | − | 1/5 | 60 |
| FcR γ chain −/− | + | 0/5‡ | DNA |
| | − | 0/5 | DNA |
| FcR γ chain +/+ | + | 4/5§ | 30, 30, 40, 40 |
| | − | 0/5 | DNA |

*DNA*, does not apply. §$P < 0.05$; *$P < 0.01$ by Fisher's exact test vs. values from control IgG-injected mice of the same genotype. ‡$P < 0.05$ by Fisher's exact test vs. values from corresponding wild-type (+/+) mice in the same treatment group.

To assess the anaphylactic responsiveness of $Fc_\epsilon RI$ α chain −/− or FcR γ chain −/− mice after sensitization with known quantities of antibodies of defined isotype and antigenic specificity, we examined systemic anaphylactic reactions in mice which had been passively sensitized with mouse monoclonal anti-DNP IgE or $IgG_1$ antibodies.

*$IgG_1$-dependent passive systemic anaphylaxis is expressed in $Fc_\epsilon RI$ α chain −/− mice, but is largely ablated in FcR γ chain −/− mice.* When mice were passively sensitized with a monoclonal anti-DNP $IgG_1$ antibody and then challenged 1 d later with specific antigen, an anaphylactic reaction associated with significant mortality occurred in $Fc_\epsilon RI$ α chain −/− mice and corresponding +/+ mice, as well as in FcR γ chain +/+ mice, but not in FcR γ chain −/− mice (Table III). All but 2 of the 15 deaths in $IgG_1$-sensitized mice occurred within 30–40 min of antigen challenge.

We found that the physiological changes associated with $IgG_1$-dependent passive systemic anaphylaxis responses (Fig. 3, *A* and *B*) were different from those observed in active anaphylaxis (Fig. 1, *A* and *B*). Moreover, in comparison to the findings in mice which expressed OVA-induced active sys-

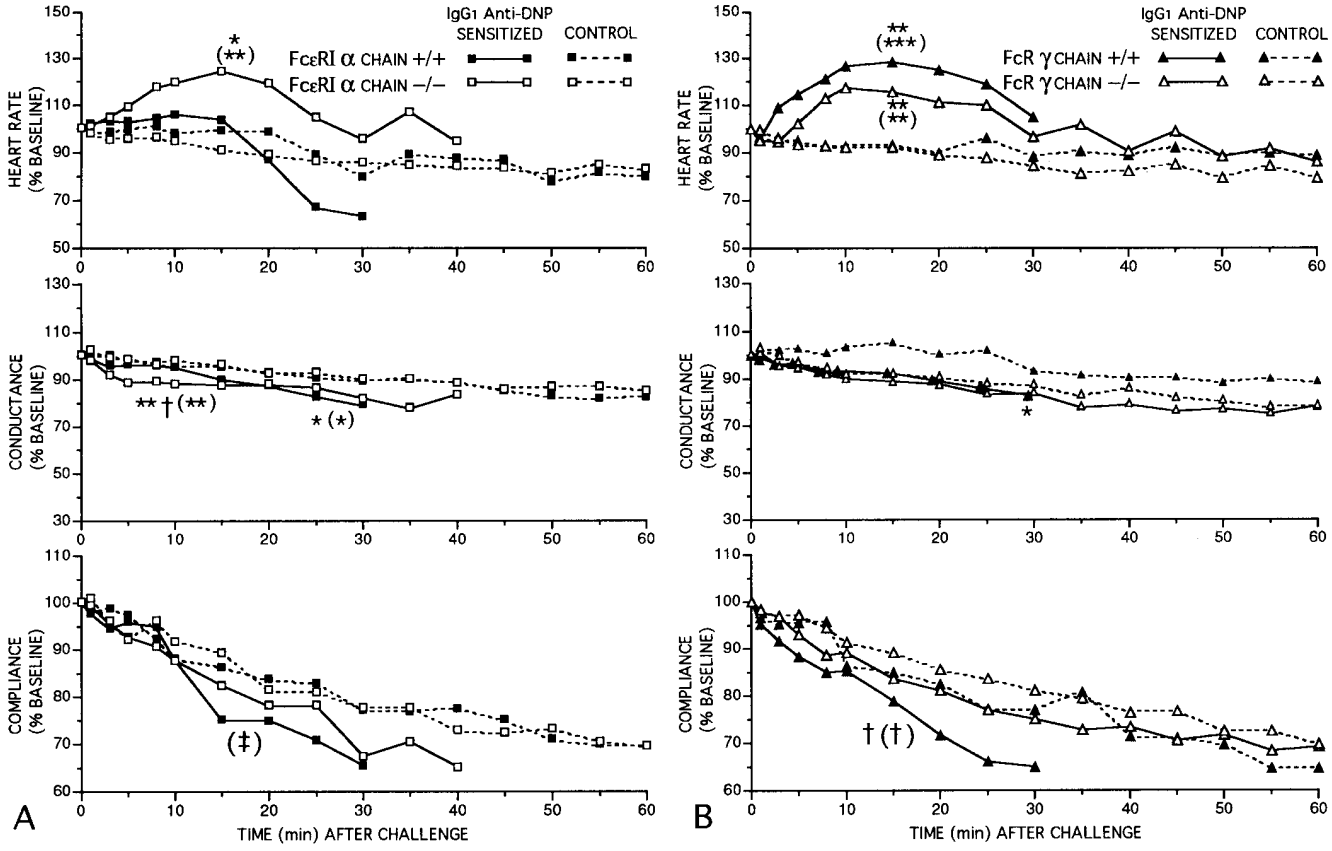


*Figure 3.* Pulmonary $C_{dyn}$, pulmonary $G_L$, and HR in $Fc_\epsilon RI$ α chain −/− mice or FcR γ chain −/− mice (*open symbols*, *A* and *B*, respectively) or the corresponding normal (+/+) mice (*filled symbols*) which had been passively sensitized by an intravenous injection of 400 μg of mouse monoclonal $IgG_1$ anti-DNP antibodies (*$IgG_1$ Anti-DNP SENSITIZED, solid lines*) or injected intravenously with 400 μg of normal mouse IgG antibodies (*CONTROL, dotted lines*), and then 1 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 1.0 mg of DNP-HSA. (*A*) $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice. (*B*) FcR γ chain −/− and corresponding +/+ mice. 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 $C_{dyn}$, $G_L$, or HR were always < 15% and usually < 10% of the mean. *$P < 0.05$ and **$P < 0.01$ by ANOVA over the first 20 min of the response, and $^{(‡)}P \approx 0.08$, $^{(*)}P < 0.05$, $^{(**)}P < 0.01$, and $^{(***)}P < 0.001$ by ANOVA over the first 30 min of the response, versus data from normal mouse IgG-injected (control) mice of the same genotype. †$P < 0.05$ by ANOVA over the first 20 min of the response, and $^{(†)}P < 0.05$ by ANOVA over the first 30 min of the response, versus data from $IgG_1$-sensitized mice of the other genotype. The exact numbers of mice in the various groups ($n$ = 5–6/group) are given in Table III.

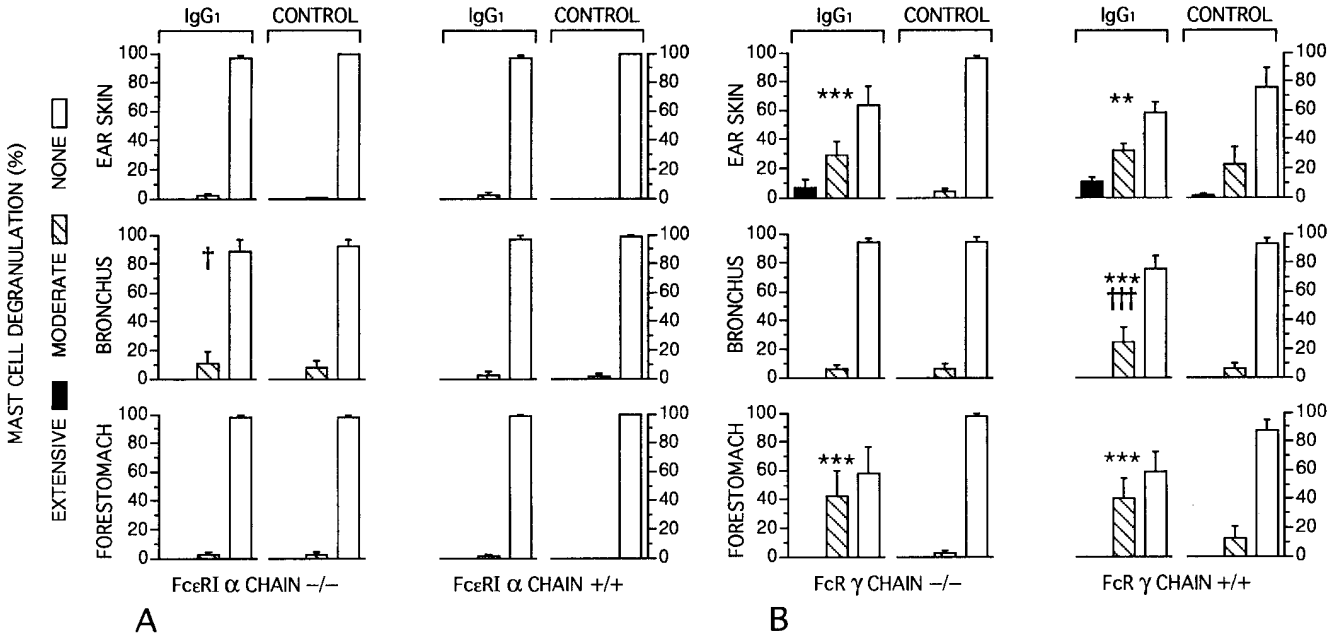


*Figure 4.* Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in $Fc_\epsilon RI$ α chain −/− or FcR γ chain −/− mice, and the respective +/+ mice, which had been passively sensitized by an intravenous injection of 400 μg of mouse monoclonal $IgG_1$ anti-DNP antibodies (*$IgG_1$*) or 400 μg of normal mouse IgG antibodies (*CONTROL*), and then 1 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 1.0 mg of DNP-HSA. (*A*) $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice. (*B*) FcR γ chain −/− and corresponding +/+ 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. 3, *A* and *B*. $^{**}P < 0.01$ and $^{***}P < 0.001$ by the $\chi^2$ test versus data from the same anatomical site in normal mouse IgG-injected (control) mice of the same genotype. $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ by the $\chi^2$ test versus data from the same anatomical site in corresponding $IgG_1$ anti-DNP sensitized mice of the other genotype.

temic anaphylaxis, the responses to $IgG_1$ and antigen were associated with little or no morphological evidence of mast cell degranulation (Fig. 4, *A* and *B*).

In those $IgG_1$-sensitized mice which expressed tachycardia in response to antigen challenge, the responses reached maximal levels at ~ 10–15 min after antigen challenge, as opposed to within 5 min or less in the active anaphylactic responses. And challenge with $IgG_1$ and antigen induced gradual and rather modest declines in $G_L$ and $C_{dyn}$, which lacked the rapid, sharp, and partially reversible early drop which developed in normal mice that expressed active systemic anaphylactic responses.

Even though the death rates due to $IgG_1$-dependent anaphylaxis (Table III), as well as the virtual lack of morphological evidence of mast cell degranulation associated with these responses (Fig. 4 *A*), were very similar in $Fc_\epsilon RI$ α chain −/− mice and $Fc_\epsilon RI$ α chain +/+ mice, the $Fc_\epsilon RI$ α chain −/− animals exhibited tachycardia responses and reductions in $G_L$, that were significantly greater than those in the corresponding wild-type mice. These findings are reminiscent of those obtained in animals of the same genotype in the active anaphylactic responses (Fig. 1 *A*). Moreover, the extent of mast cell degranulation in the bronchial tissues of $IgG_1$-sensitized $Fc_\epsilon RI$ α chain −/− mice, while minimal and not significantly different than that in control $Fc_\epsilon RI$ α chain −/− mice which had not been sensitized with $IgG_1$, was significantly greater than that observed in the bronchial tissues of the identically sensitized and antigen-challenged $Fc_\epsilon RI$ α chain +/+ mice (Fig. 4 *A*). As in the case of the active anaphylaxis responses in $Fc_\epsilon RI$ α chain −/− and corresponding wild-type mice, the differences in the responses in the mutant and wild-type mice are not likely to have reflected differences in the age or gender distribution of the animals. Thus, we analyzed $IgG_1$-dependent passive systemic anaphylaxis in five female $Fc_\epsilon RI$ α chain +/+ mice, of 14.8±0.5 wk of age, versus six female $Fc_\epsilon RI$ α chain −/− mice, all of ~ 14 wk of age.

By contrast, $IgG_1$-sensitized FcR γ chain −/− mice failed to develop statistically significant changes in $G_L$ or $C_{dyn}$ upon antigen challenge, whereas significant, albeit rather modest, pulmonary responses developed in the $IgG_1$-sensitized FcR γ chain +/+ mice (Fig. 3 *B*). It may be of some interest, in this regard, that of the three anatomical sites analyzed in mice challenged with $IgG_1$ and antigen, the bronchial tissues were the only location where FcR γ chain +/+ mice exhibited significantly more mast cell degranulation than did the FcR γ chain −/− mice (Fig. 4 *B*). Note, however, that both FcR γ chain +/+ mice and FcR γ chain −/− mice developed a tachycardia response in response to $IgG_1$ sensitization and antigen challenge. Furthermore, even though all of the FcR γ chain −/− mice which had been sensitized with $IgG_1$ anti-DNP antibodies survived intravenous challenge with DNP-HSA (Table III), they exhibited roughly the same modest levels of mast cell activation in the ear skin and forestomach as did the identically sensitized and challenged FcR γ chain +/+ mice (Fig. 4 *B*).

*IgE-dependent passive systemic anaphylaxis requires the $Fc_\epsilon RI$ α chain and FcR γ chain and is associated with cardiopulmonary responses that mimic those in active anaphylaxis.* While tachycardia and declines in $G_L$ and/or $C_{dyn}$ occurred during $IgG_1$-dependent passive systemic anaphylaxis (Fig. 3, *A* and *B*), these changes developed more slowly than in active

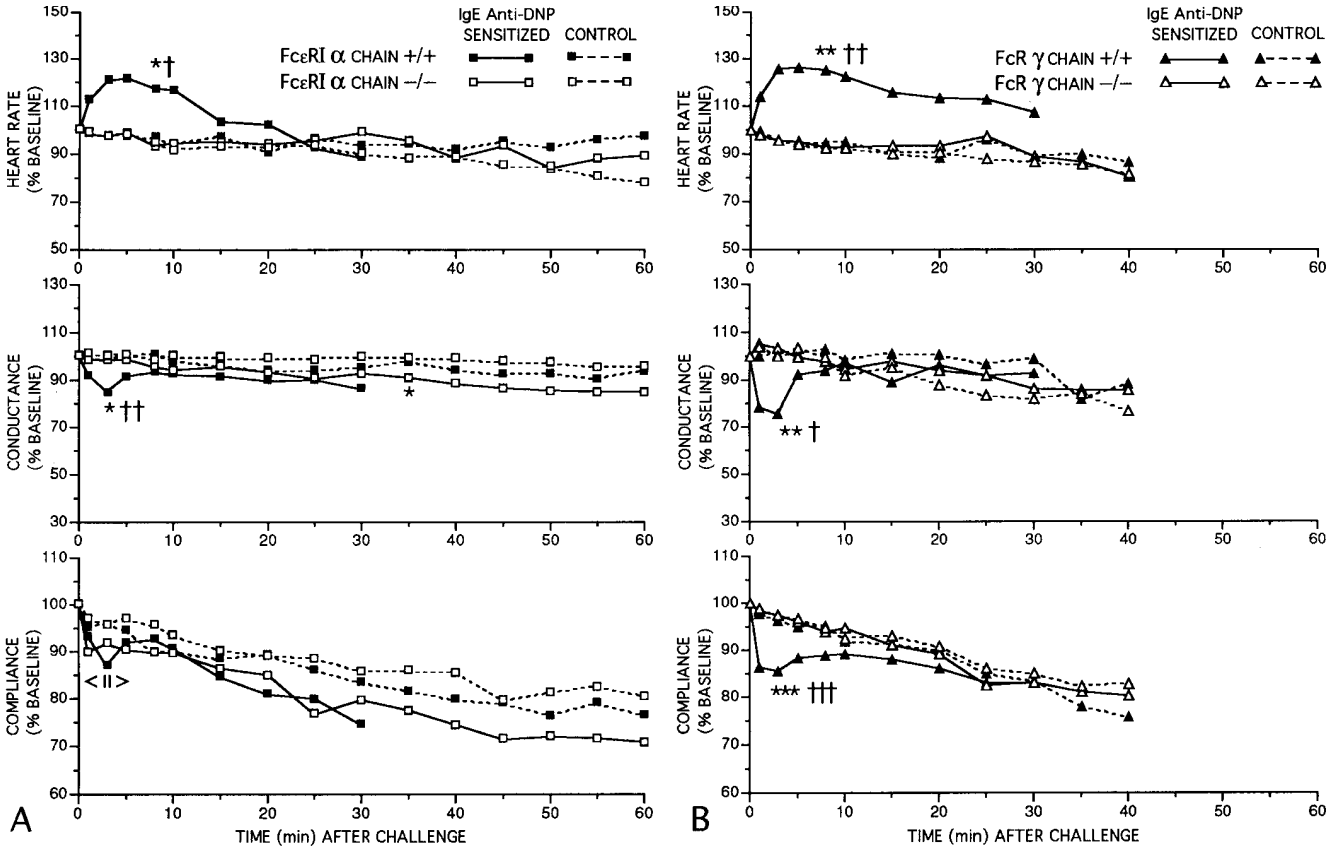


*Figure 5.* Pulmonary $C_{dyn}$, pulmonary $G_L$, and HR in $Fc_\epsilon RI$ α chain −/− mice or FcR γ chain −/− mice (*open symbols*, *A* and *B*, respectively) or the corresponding normal (+/+) mice (*filled symbols*) which had been passively sensitized by an intravenous injection of 20 μg of mouse monoclonal IgE anti-DNP antibodies (*IgE Anti-DNP SENSITIZED, solid lines*) or injected intravenously with 0.9% NaCl (*CONTROL, dotted lines*), and then 1 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 200 μg of DNP-HSA. (*A*) $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice. (*B*) FcR γ chain −/− and corresponding +/+ mice. In groups in which some mice died before the end of the 40- or 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 $C_{dyn}$, $G_L$, or HR were always < 10% of the mean. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ by ANOVA over the first 20 min of the response versus data from nonsensitized, 0.9% NaCl-injected (control) mice of the same genotype; $^{<||>}P \approx 0.06$ by ANOVA over the first 3 min of the response versus data from 0.9% NaCl-injected (control) mice of the same genotype. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, and $^{\dagger\dagger\dagger}P < 0.001$ by ANOVA over the first 20 min of the response versus data from IgE-sensitized mice of the other genotype. The exact numbers of mice in the various groups ($n$ = 4–6/group) are given in Table IV.

systemic anaphylaxis (Fig. 1, *A* and *B*), and the responses were associated with much lower levels of mast cell degranulation (compare Figs. 2 and 4). Therefore, we investigated whether the features of IgE-dependent passive systemic anaphylaxis might more closely resemble those of the active anaphylactic responses than did the characteristics of the $IgG_1$-dependent reactions.

Both $Fc_\epsilon RI$ α chain +/+ mice (Fig. 5 *A*) and, to an even greater extent, FcR γ chain +/+ mice (Fig. 5 *B*) developed tachycardia responses, as well as decreased $C_{dyn}$ and $G_L$, in response to intravenous antigen challenge 1 d after passive sensitization with IgE antibodies. Like the physiological responses associated with active anaphylaxis, both the changes in HR and the initial changes in pulmonary function occurred rapidly after antigen challenge, and these responses, like the active anaphylactic reactions, were associated with morphological evidence of extensive mast cell degranulation (Fig. 6, *A* and *B*). And, as in the case of the active anaphylactic responses (Table II), most of the IgE-sensitized $Fc_\epsilon RI$ α chain +/+ or FcR γ chain +/+ mice died after antigen challenge (Table IV). However, death due to IgE-dependent passive systemic anaphylaxis occurred somewhat later than that due to active anaphylaxis. Thus, all 10 of the mice which succumbed to IgE-dependent passive anaphylaxis died within 30–40 min of antigen challenge (Table IV), whereas 15 of the 18 mice that developed fatal active anaphylaxis died within 20–25 min of antigen challenge (Table II).

As expected, IgE-sensitized, antigen-challenged $Fc_\epsilon RI$ α chain −/− mice or FcR γ chain −/− mice developed neither extensive mast cell degranulation (Fig. 6, *A* and *B*) nor significant cardiopulmonary changes (Fig. 5, *A* and *B*), nor did these mice exhibit significant mortality (Table IV).

*Mast cells are not essential for the expression of active anaphylaxis or $IgG_1$-dependent passive systemic anaphylaxis, but may enhance the intensity of these responses.* To assess the extent to which the cardiopulmonary changes and death associated with active systemic anaphylaxis to OVA, or $IgG_1$-dependent passive systemic anaphylaxis to DNP-HSA, can be mast cell–independent, we attempted to elicit these responses in mast cell–deficient $Kit^W/Kit^{W\text{-}v}$ mice and the congenic normal

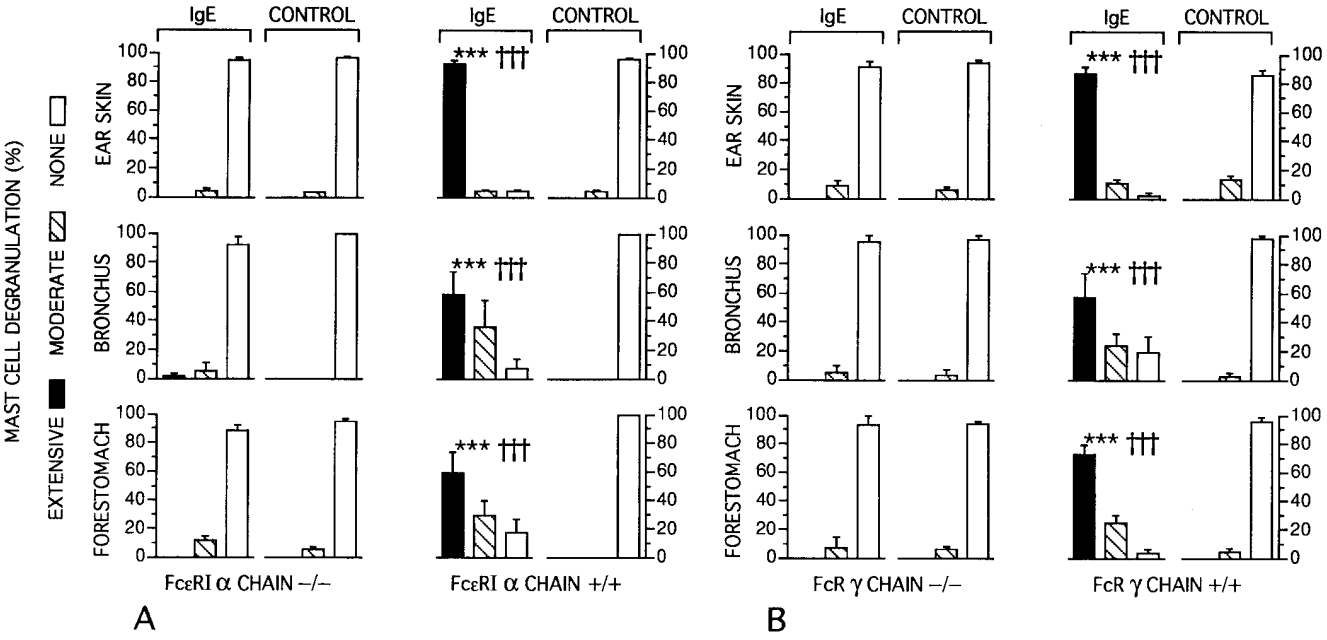


*Figure 6.* Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in IgE anti-DNP-sensitized (*IgE*) or 0.9% NaCl-injected (*CONTROL*) $Fc_\epsilon RI$ α chain −/− or FcR γ chain −/− mice, or the respective +/+ mice, which had been challenged with 200 μg of DNP-HSA intravenously. (*A*) $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice. (*B*) FcR γ chain −/− and corresponding +/+ 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. 5, *A* and *B*. ***$P < 0.001$ by the $\chi^2$ test versus data from the same anatomical site in 0.9% NaCl-injected (control) mice of the same genotype. $^{\dagger\dagger\dagger}P < 0.001$ by the $\chi^2$ test versus data from the same anatomical site in corresponding IgE anti-DNP-sensitized −/− mice.

(+/+) mice. Mice of either genotype exhibited significant increases in serum concentrations of total IgE after OVA immunization (day −1 vs. day 17–20 values of 0.82±0.47 vs. 14.3±15.9 μg/ml [MEM±SD], $P < 0.032$ for $Kit^W/Kit^{W\text{-}v}$ mice and 0.29±0.20 vs. 2.98±0.97 μg/ml, $P < 0.0001$ for the congenic +/+ mice). These results are consistent with our previous finding that both baseline and postimmunization concentrations of total IgE can be as high or higher in $Kit^W/Kit^{W\text{-}v}$ mice than in the congenic +/+ mice (5). These animals, like mice of the other genotypes we analyzed, exhibited much more modest relative increases in serum concentrations of total IgG (day −1 vs. day 17–20 values of 380±180 vs. 631±290 μg/ml for OVA-immunized $Kit^W/Kit^{W\text{-}v}$ mice and 255±98 vs. 296±53 μg/ml for the OVA-immunized congenic +/+ mice. Antigen challenge resulted in high mortality rates in both OVA-immunized $Kit^W/Kit^{W\text{-}v}$ mice and OVA-immunized congenic +/+ mice, but not in the sham-immunized mice of either genotype (Table V).

However, the cardiopulmonary changes associated with ac-

*Table IV. Death Rates and Times until Death in Mice Passively Sensitized with Mouse Monoclonal IgE Anti-DNP Antibodies (20 μg/Mouse) or Injected with Vehicle, 1 d before Intravenous Challenge with 200 μg of DNP-HSA*

| Mice | IgE anti-DNP | Death rates | Times until death |
|---|---|---|---|
| | | | *min* |
| $Fc_\epsilon RI$ α chain −/− | + | 1/6* | 60 |
| | − | 0/5 | DNA |
| $Fc_\epsilon RI$ α chain +/+ | + | 5/6‡ | 30, 30, 40, 40, 40 |
| | − | 0/5 | DNA |
| FcR γ chain −/− | + | 1/6* | 30 |
| | − | 0/5 | DNA |
| FcR γ chain +/+ | + | 5/6‡ | 30, 30, 30, 30, 40 |
| | − | 0/4 | DNA |

*DNA*, does not apply. ‡$P < 0.01$ by Fisher's exact test vs. values from vehicle-injected mice of the same genotype. *$P < 0.05$ by Fisher's exact test vs. values from corresponding wild-type (+/+) mice in the same treatment group.

*Table V. Death Rates and Times until Death in Mast Cell–deficient WBB6F$_1$-$Kit^W/Kit^{W-v}$ ($Kit^W/Kit^{W-v}$) Mice and Congenic Normal Kit (+/+) Mice, Challenged to Express Active Systemic Anaphylaxis to OVA or Passive IgG$_1$-dependent Systemic Anaphylaxis to DNP-HSA**

| Mice | Treatment | Death rates | Times until death |
|---|---|---|---|
| | | | *min* |
| $Kit^W/Kit^{W-v}$ | OVA-immunized | 7/10‡ | 20, 20, 30, 30, 30, 30, 40 |
| | Sham-immunized | 0/5 | DNA |
| *Kit* (+/+) | OVA-immunized | 5/5* | 20, 20, 20, 20, 20 |
| | Sham-immunized | 0/4 | DNA |
| $Kit^W/Kit^{W-v}$ | $IgG_1$ anti-DNP | 1/6 | 40 |
| | Normal mouse IgG | 0/5 | DNA |
| *Kit* (+/+) | $IgG_1$ anti-DNP | 5/6‡ | 50, 50, 50, 55, 60 |
| | Normal mouse IgG | 0/6 | DNA |

*DNA*, does not apply. *$P < 0.05$, ‡$P < 0.01$ by Fisher's exact test vs. values from control (sham-immunized, or normal mouse IgG-injected) mice of the same genotype.

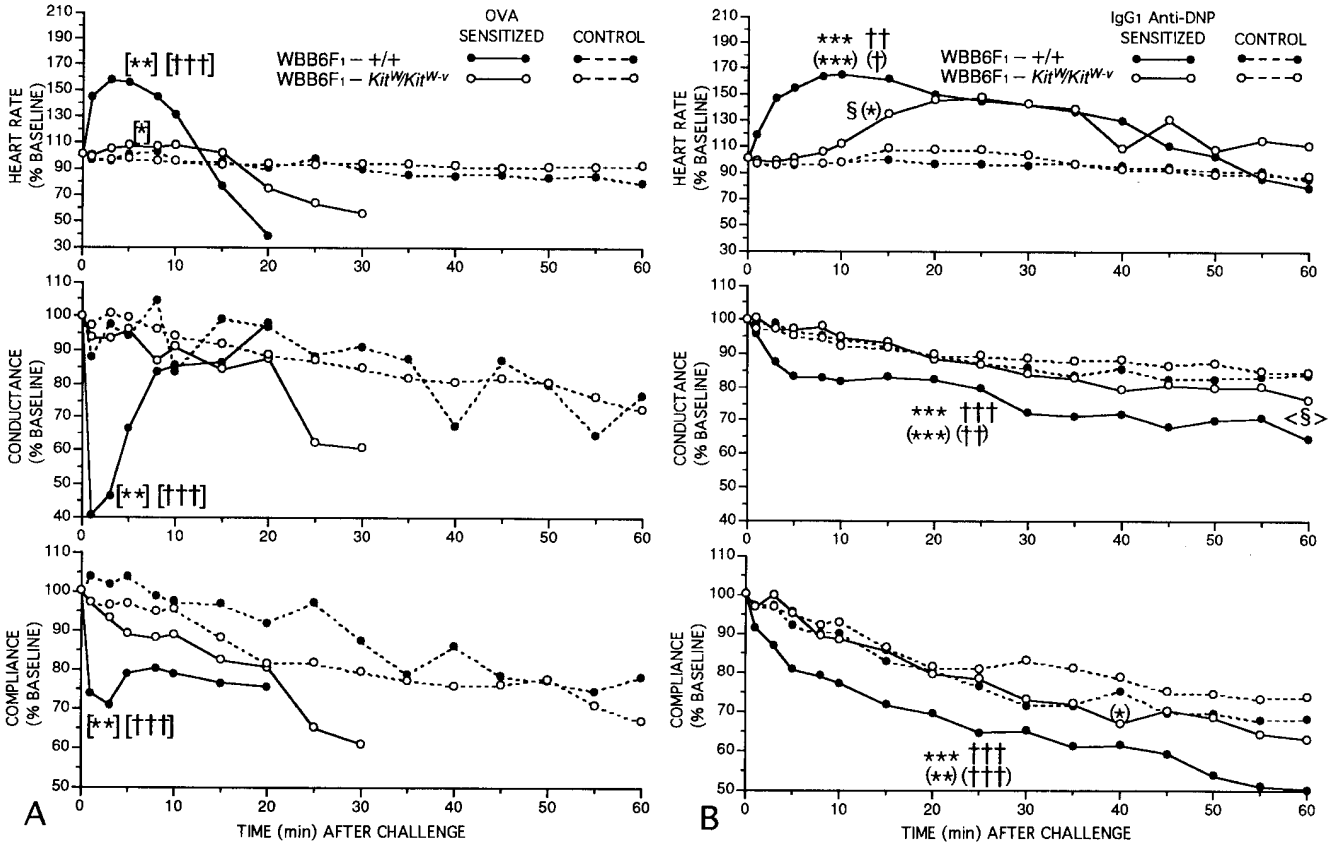


*Figure 7.* (*A*) Pulmonary $C_{dyn}$, pulmonary $G_L$, and HR in WBB6F$_1$-*Kit$^W$/Kit$^{W\text{-}v}$* (*Kit$^W$/Kit$^{W\text{-}v}$*) mice (*open symbols*) or the corresponding normal, WBB6F$_1$-+/+ (+/+) mice (*filled symbols*) which had been sensitized with OVA (*OVA SENSITIZED, solid lines*) or sham-immunized (*CONTROL, dotted lines*), and then 18–21 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 500 μg of OVA. 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 $C_{dyn}$, $G_L$, or HR were always ≤ 29% and usually < 10% of the mean. $^{[**]}P < 0.01$ by ANOVA over the first 8 min of the response versus data from nonsensitized (control) mice of the same genotype. $^{[†††]}P < 0.001$ by ANOVA over the first 8 min of the response versus data from OVA-sensitized mice of the other genotype. The exact numbers of mice in the various groups ($n$ = 5–8/group) are given in Table V. (*B*) Pulmonary $C_{dyn}$, pulmonary $G_L$, and HR in WBB6F$_1$-*Kit$^W$/Kit$^{W\text{-}v}$* (*Kit$^W$/Kit$^{W\text{-}v}$*) mice (*open symbols*) or the corresponding normal, WBB6F$_1$-+/+ (+/+) mice (*filled symbols*), which had been passively sensitized by an intravenous injection of 400 μg of mouse monoclonal IgG$_1$ anti-DNP antibodies (*IgG$_1$ Anti-DNP SENSITIZED, solid lines*) or injected intravenously with 400 μg of normal mouse IgG antibodies (*CONTROL, dotted lines*), and then 1 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 1.0 mg of DNP-HSA. 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 $C_{dyn}$, $G_L$, or HR were always < 16% and usually < 10% of the mean. $^{§}P = 0.07$, $^{***}P < 0.001$ over the first 20 min of the response, $^{(*)}P < 0.05$, $^{(**)}P < 0.01$, $^{(***)}P < 0.001$ over the first 40 min of the response and $^{<§>}P = 0.08$ over the entire 60 min of the response, by ANOVA, versus data from normal mouse IgG-injected (control) mice of the same genotype. $^{††}P < 0.01$, $^{†††}P < 0.001$ by ANOVA over the first 20 min of the response, and $^{(†)}P < 0.05$, $^{(††)}P < 0.01$, $^{(†††)}P < 0.001$ by ANOVA over the first 40 min of the response, versus data from IgG$_1$-sensitized mice of the other genotype. The exact numbers of mice in the various groups ($n$ = 5–6/group) are given in Table V.

tive systemic anaphylaxis to OVA were quite different in the wild-type mice, which rapidly developed both striking tachycardia and marked declines in $G_L$ and $C_{dyn}$, and the *Kit$^W$/Kit$^{W\text{-}v}$* mice, which developed only a slight (albeit significant) elevation in HR and slow, modest declines in $G_L$ and $C_{dyn}$ (which were not even statistically significant in comparison to values for the sham-immunized *Kit$^W$/Kit$^{W\text{-}v}$* mice) (Fig. 7 *A*). WBB6F$_1$-+/+ mice exhibiting active anaphylaxis to OVA also developed extensive mast cell degranulation (Fig. 8 *A*), to levels similar to those observed in association with OVA-induced active systemic anaphylaxis in the wild-type mice of the other genotypes tested (compare Fig. 2, *A* and *B*).

When challenged for IgG$_1$-induced passive systemic anaphylaxis, WBB6F$_1$-+/+ mice exhibited responses which in some respects were even stronger than those of FcR γ chain +/+ mice (Figs. 3 *B* and 4 *B*). The WBB6F$_1$-+/+ mice exhibited a strong tachycardia response, which peaked at ∼ 10 min, and developed substantial and significant declines in $G_L$ and $C_{dyn}$. The mast cell degranulation which developed in these mice was also more extensive (Fig. 8 *B*) than that in FcR γ chain +/+ mice which had been challenged for IgG$_1$-dependent passive systemic anaphylaxis (Fig. 4 *B*), but was less striking than that in WBB6F$_1$-+/+ mice which had been challenged for active anaphylaxis to OVA (compare Fig. 8 *A*).

Although IgG$_1$-dependent passive systemic anaphylaxis was expressed in *Kit$^W$/Kit$^{W\text{-}v}$* mice, the responses were substan-

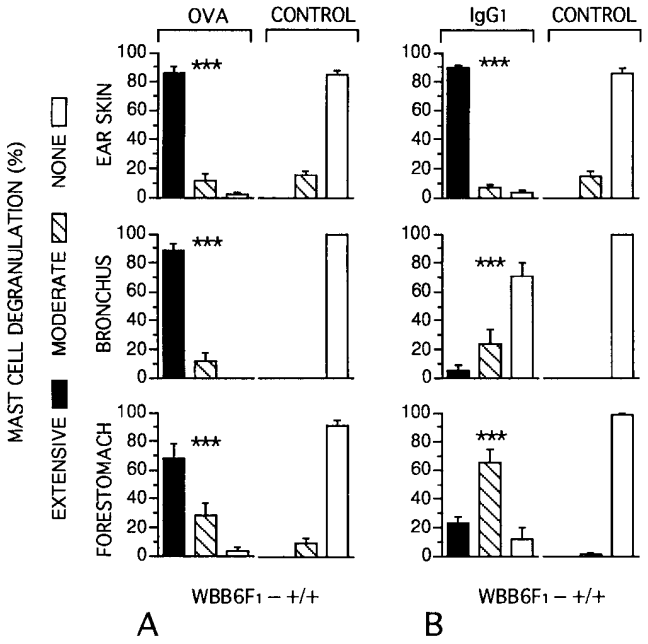


*Figure 8.* (*A* and *B*) Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in (*A*) OVA-sensitized (*OVA*) or sham-immunized (*CONTROL*) WBB6$F_1$-+/+ (+/+) mice which had been challenged with 500 μg of OVA intravenously or (*B*) in WBB6$F_1$-+/+ (+/+) mice which had been passively sensitized by an intravenous injection of 400 μg of mouse monoclonal $IgG_1$ anti-DNP antibodies (*$IgG_1$*) or 400 μg of normal mouse IgG antibodies (*CONTROL*), and then 1 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 1.0 mg of DNP-HSA. 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. ***$P < 0.001$ by the $\chi^2$ test versus data from the same anatomical site in control mice of the same genotype.

tially weaker than those in the congenic +/+ mice. Thus, the *$Kit^W$/$Kit^{W\text{-}v}$* mice developed tachycardia more slowly (and to lower maximum levels of HR) than the congenic +/+ mice and developed only small declines in $G_L$ and $C_{dyn}$. Furthermore, only one out of six *$Kit^W$/$Kit^{W\text{-}v}$* died as a result of $IgG_1$-dependent passive anaphylaxis, as opposed to five out of six of the congenic +/+ mice (Table V).

In confirmation of our previous findings (4, 5), WBB6$F_1$-+/+ mice challenged for IgE-dependent systemic anaphylaxis to DNP-HSA developed strong responses, associated with a rapid elevation of HR, rapid, large, and partially reversible drops in $G_L$ and $C_{dyn}$, and extensive mast cell degranulation, whereas the *$Kit^W$/$Kit^{W\text{-}v}$* mice gave no detectable responses (data not shown). Five out of six WBB6$F_1$-+/+ mice challenged for IgE-dependent systemic anaphylaxis died (at 40–60 min after antigen challenge) versus zero out of four antigen-challenged control +/+ mice which had been injected with vehicle instead of IgE ($P < 0.01$ by Fisher's exact test). By contrast, only one out of six *$Kit^W$/$Kit^{W\text{-}v}$* mice challenged for IgE-dependent systemic anaphylaxis died versus zero out of four antigen-challenged control *$Kit^W$/$Kit^{W\text{-}v}$* mice which had been injected with vehicle instead of IgE (NS [$P > 0.05$] by Fisher's exact test).

Table VI summarizes the characteristics of the physiological responses, mast cell degranulation, and death rates associated with the three types of anaphylactic reactions in the $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice, the FcR γ chain −/− and corresponding +/+ mice, and the mast cell–deficient *$Kit^W$/$Kit^{W\text{-}v}$* and congenic +/+ mice. The table does not summarize the intensity of the cardiopulmonary responses in the mice of each genotype, as all of the key conclusions of our study are based on comparisons of responses of mutant and corresponding wild-type mice of the same genetic backgrounds. However, the figures depicting the cardiopulmonary responses illustrate differences in the intensity of the responses in wild-type mice of different genetic backgrounds, generally in the order WBB6$F_1$ > FcR γ chain +/+ (C57BL/6 × 129) > $Fc_\epsilon RI$ α chain +/+ (BALB/c). These differences, and perhaps also the differences in the extent of mast cell degranulation among the various wild-type mice, may be related to the differences in the genetic backgrounds of these animals.

## Discussion

In the models tested, mice devoid of only $Fc_\epsilon RI$, or lacking both $Fc_\epsilon RI$ and FcγRI/III, exhibited very distinct patterns of defects in their ability to express active systemic anaphylaxis or IgE- or $IgG_1$-dependent passive systemic anaphylaxis. $Fc_\epsilon RI$ α chain −/− mice that lacked $Fc_\epsilon RI$ nonetheless expressed active systemic anaphylactic responses that were associated with extensive mast cell degranulation and a high mortality rate. These results indicate that even though IgE-dependent mast cell degranulation can result in the expression of fatal passive systemic anaphylactic responses that are associated with significant cardiopulmonary changes (references 4 and 5 and Table VI), a functional $Fc_\epsilon RI$ is not required for either the mast cell degranulation or the mortality associated with active systemic anaphylaxis.

Indeed, the cardiopulmonary changes associated with active systemic anaphylactic reactions in the $Fc_\epsilon RI$ α chain −/− mice were even more intense and/or more prolonged than those in the corresponding wild-type mice. In vitro findings indicate that, in the absence of the $Fc_\epsilon RI$ α chain, mast cells can exhibit increased expression of FcγRIII (see companion study, reference 38). Accordingly, one possible explanation for the increased magnitude and/or duration of cardiopulmonary responses during active systemic anaphylaxis in $Fc_\epsilon RI$ α chain −/− versus the corresponding +/+ mice is that the $Fc_\epsilon RI$ α chain −/− mice exhibited increased levels of FcγRIII-dependent mast cell signaling and mediator production. However, if this indeed occurred during our experiments, it was not reflected in higher levels of mast cell degranulation in the tissues of $Fc_\epsilon RI$ α chain −/− versus corresponding wild-type mice, at least as could be assessed by histological analysis. Alternatively, the $Fc_\epsilon RI$ α chain −/− and corresponding +/+ mice may have differed in some other aspect(s) of their response to active immunization and/or antigen challenge which affected the intensity of their physiological responses. However, whatever the explanation for the more severe cardiopulmonary changes associated with active anaphylaxis in $Fc_\epsilon RI$ α chain −/− mice than in the corresponding wild-type mice, the results clearly illustrate that the physiological changes, as well as the death, associated with active systemic anaphylaxis can occur in the complete absence of the $Fc_\epsilon RI$ α chain.

In contrast to their ability to express all of the features of active systemic anaphylaxis reactions that we analyzed, $Fc_\epsilon RI$

*Table VI. Characteristics of Active, or IgE- or $IgG_1$-dependent Passive, Systemic Anaphylactic Reactions in $Fc_eRI$ α Chain −/− Mice, FcR γ Chain −/− Mice, and Mast Cell–deficient $Kit^W/Kit^{W-v}$ Mice, and the Corresponding Normal (+/+) Mice*

| Feature | Type of anaphylaxis | Mice | | | | | |
|---|---|---|---|---|---|---|---|
| | | $Fc_eRI$ α +/+ | $Fc_eRI$ α −/− | FcRγ +/+ | FcRγ −/− | *Kit* (+/+) | $Kit^W/Kit^{W-v}$ |
| Tachycardia (time to peak) | Active | Yes (3 min) | Yes (3 min) | Yes (1 min) | No | Yes (3 min) | ± (10 min) |
| | IgE, passive | Yes (5 min) | No | Yes (5 min) | No | Yes (3 min) | No |
| | $IgG_1$, passive | No | Yes (15 min) | Yes (15 min) | Yes (10 min) | Yes (10 min) | Yes (25 min) |
| ↓ $G_L$ (rapid early ↓) | Active | Yes (Yes) | Yes (Yes) | Yes (Yes) | No | Yes (Yes) | ± (No) |
| | IgE, passive | Yes (Yes) | No | Yes (Yes) | No | Yes (Yes) | No |
| | $IgG_1$, passive | Yes (No) | Yes (No) | Yes (No) | No | Yes (±) | ± (No) |
| ↓ $C_{dyn}$ (rapid early ↓) | Active | Yes (Yes) | Yes (Yes) | Yes (Yes) | No | Yes (Yes) | ± (No) |
| | IgE, passive | *± (±) | No | Yes (Yes) | No | Yes (Yes) | No |
| | $IgG_1$, passive | ± (No) | No | Yes (No) | No | Yes (No) | ± (No) |
| Extent of mast cell degranulation | Active | ‡+++ | ++ | +++ | − | +++ | DNA |
| | IgE, passive | +++ | − | +++ | − | +++ | DNA |
| | $IgG_1$, passive | − | − | + | + | ++ | DNA |
| Mortality rate | Active | 5/6 | 8/9 | 5/5 | 0/5 | 5/5 | 7/10 |
| | IgE, passive | 5/6 | 1/6 | 5/6 | 1/6 | 5/6 | 1/6 |
| | $IgG_1$, passive | 5/5 | 6/6 | 4/5 | 0/5 | 5/6 | 1/6 |

*±, minimal response; ‡−, not statistically significant at $P < 0.05$ at any site vs. values for control (nonsensitized) mice, and < 25% extensive or moderate degranulation in all three sites. +, statistically significant at $P < 0.001$ in two sites vs. values for control (nonsensitized) mice, and 25–50% extensive or moderate degranulation in two or three sites. ++, statistically significant at $P < 0.001$ at all sites vs. values for control (nonsensitized) mice, and > 50% extensive or moderate degranulation in two or three sites. +++, statistically significant at $P < 0.001$ at all sites vs. values for control (nonsensitized) mice, and > 80% extensive or moderate degranulation in two or three sites. *DNA*, does not apply (no mast cells present).

α chain −/− mice completely lacked the ability to express IgE-dependent passive systemic anaphylactic responses, whether judged by histological evidence of mast cell degranulation, the development of cardiopulmonary changes, or mortality. These findings are fully consistent with the observation that $Fc_eRI$ α chain null mice also fail to develop either the augmented vascular permeability, or the marked drop in body temperature, associated with these responses (18), and indicate that most, and perhaps all, of the pathophysiology of IgE-dependent anaphylactic responses occur by $Fc_eRI$-dependent mechanisms, rather than by interactions between IgE and FcγRII/III or other receptors that can interact with IgE (39).

Unlike mice that had a selective deficiency of $Fc_eRI$, mice that lacked the FcR γ subunit were profoundly deficient in their ability to express all three types of systemic anaphylactic responses. FcR γ chain −/− mice exhibited no detectable active systemic anaphylactic responses or IgE-dependent passive anaphylactic reactions, whether these responses were assessed by cardiopulmonary changes, mast cell degranulation, or mortality. These latter results are consistent with the observations that FcR γ chain −/− mice failed to express detectable IgE-dependent passive cutaneous anaphylaxis responses in vivo, or significant IgE-dependent mast cell degranulation in vitro (19). However, the findings in this study are the first to indicate that all (or virtually all) of the cardiopulmonary changes, and the mortality, associated with either IgE-dependent passive systemic anaphylaxis or active systemic anaphylaxis in mice also require the signal transduction function of the FcR γ chain.

The FcR γ chain represents a component of both FcγRI and FcγRIII. We found that $IgG_1$ antibodies, which bind much more preferentially to FcγRIII than to FcγRI (23), can sensitize $Fc_eRI$ α chain −/− mice, $Fc_eRI$ α chain +/+ mice, or FcR γ chain +/+ mice, but not FcR γ chain −/− mice, for passive systemic anaphylaxis responses. Thus, while we have not formally ruled out the possibility that FcγRI contribute to active anaphylaxis in mice, it appears likely that FcγRIII, not FcγRI, are critical for the expression of this response. Additional, and more direct, evidence supporting this conclusion is presented in the companion study (38). Further support for the hypothesis that it is FcγRIII, not FcγRI, which are critical for the mast cell degranulation and perhaps other components of the pathogenesis of $IgG_1$-dependent systemic anaphylactic response has been provided by a report of the generation and initial characterization of FcγRIII α chain −/− mice (40), which appeared while the present manuscript was in review. FcγRIII α chain −/− mice, unlike the corresponding wild-type mice, failed to exhibit IgG-dependent peritoneal mast cell degranulation in vitro or IgG-dependent passive cutaneous anaphylaxis (as assessed by extravasation of Evan's blue dye into the ear skin) in vivo (40). However, the ability of such mice to express active or passive systemic anaphylactic reactions was not examined (40).

Given the potential complexity of the pathogenesis of active anaphylactic responses, it is perhaps not surprising that the features of the $IgG_1$-dependent passive systemic anaphylactic responses and active anaphylactic reactions which we studied were not fully identical. Although the $IgG_1$-dependent reactions were associated with a high mortality rate, the tachycardia observed in these responses developed much more slowly than in IgE-dependent passive, or OVA-induced active, systemic anaphylactic responses. Moreover, $G_L$ and $C_{dyn}$ diminished very gradually during the passive, $IgG_1$-dependent responses, without the rapid and partially reversible early reductions noted in the active or IgE-dependent passive responses.

Immunization for active anaphylaxis may have been associated with many changes (e.g., in levels of antigen-specific immunoglobulins of different isotypes, numbers of various effector cells, levels of FcR expression by these cells, and local concentrations of immunomodulatory cytokines) which were not mimicked in mice that had been passively sensitized with $IgG_1$ antibodies. Thus, it may be quite difficult to ascertain the reason(s) for the observed differences in the physiological changes associated with antigen challenge in animals sensitized for active as opposed to passive, $IgG_1$-dependent anaphylaxis. It is possible that some of these differences are related to the much more modest levels of mast cell activation that were observed in association with the $IgG_1$-dependent passive responses, as opposed to the active or passive IgE-dependent reactions. However, it has been demonstrated already that significant pulmonary changes (4, 5), and death (4, 5, 7–9), can occur during active anaphylactic responses which are elicited in the virtual absence of mast cells.

These observations, as well as the results of our histological analysis (Fig. 4 *A*), suggest that the pulmonary changes which develop during $IgG_1$-dependent passive anaphylaxis can also occur independently of any critical contribution of the mast cell. On the other hand, the cardiopulmonary changes associated with $IgG_1$-dependent passive systemic anaphylaxis, as well as the mortality associated with this response, were greater in $WBB6F_1$-+/+ (normal) mice than in the congenic mast cell–deficient $WBB6F_1$- $Kit^W/Kit^{W\text{-}v}$ mice (Fig. 7 *B*). These findings suggest that $IgG_1$-dependent mast cell degranulation can, under some circumstances, contribute to the intensity of $IgG_1$-dependent passive systemic anaphylactic responses.

When taken together, do our results (and those presented in the companion study, reference 38) prove that the pathophysiology of active anaphylaxis in mice is solely dependent on signaling mediated by the FcR γ chain? Not at all. While FcR γ chain −/− mice completely failed to express active anaphylaxis to OVA challenge and exhibited neither mortality nor pulmonary changes in response to challenge with $IgG_1$ antibodies and specific antigen, $IgG_1$-sensitized FcR γ chain −/− mice did express a statistically significant tachycardia response upon antigen challenge (Fig. 3 *B*). Moreover, these FcR γ chain −/− mice also developed modest, but statistically significant, levels of mast cell degranulation in the ear skin and forestomach, responses that were statistically indistinguishable from those in the $IgG_1$-sensitized and antigen-challenged FcR γ chain +/+ mice (Fig. 4 *B*).

These results indicate that sensitization with $IgG_1$ and challenge with specific antigen can induce both tachycardia responses and small amounts of mast cell degranulation independently of FcγRI/III- (or $Fc_\epsilon$RI-) dependent signaling. Mast cells can be activated by anaphylatoxins (C3a, C5a), peptides that are generated as a result of complement activation (41). Moreover, we have found that tachycardia is a much more sensitive indicator of mast cell–dependent passive systemic anaphylactic reactions than are changes in $G_L$ or $C_{dyn}$ or mortality (5, and Miyajima, I., and S.J. Galli, unpublished data). Accordingly, it is possible that the weak tachycardia response to antigen challenge in $IgG_1$-injected FcR γ chain −/− mice reflected complement activation by anti-DNP $IgG_1$–DNP-HSA immune complexes. Nevertheless, it should be emphasized that FcR γ chain −/− mice exhibited neither pulmonary changes nor increased mortality upon challenge with $IgG_1$ and specific antigen. Therefore, we can conclude that, no matter what $IgG_1$-dependent but FcR-independent processes may have occurred in our experiments, the development of the most clinically significant consequences of $IgG_1$-dependent passive anaphylaxis reactions required adequate signaling through the FcR γ chain.

If virtually all of the pathophysiology of both active and $IgG_1$-dependent passive systemic anaphylaxis responses reflects the signaling function of the FcR γ chain, and if both active and $IgG_1$-dependent passive systemic anaphylaxis responses can be expressed in mice that lack the $Fc_\epsilon$RI, can we conclude that IgE and mast cells have no role in active anaphylaxis in the mouse? Again, not without qualification. Many of our findings, particularly those derived from analyses of mast cell–deficient $Kit^W/Kit^{W\text{-}v}$ mice and the congenic normal mice, are consistent with the possibility that mast cell degranulation contributed to the rapid and partially reversible diminutions of $G_L$ and $C_{dyn}$, and to the rapid onset of tachycardia, that typically occurred during the active or IgE-dependent passive systemic anaphylactic responses. In some circumstances (e.g., in $WBB6F_1$-+/+ mice), mast cell degranulation may also contribute to the intensity of the cardiopulmonary changes and mortality associated with $IgG_1$-dependent passive systemic anaphylaxis. Mast cell degranulation during active anaphylaxis may have occurred primarily via IgE and $Fc_\epsilon$RI in normal mice, but by $IgG_1$-dependent mechanisms in $Fc_\epsilon$RI α chain −/− mice that express increased levels of mast cell activation via FcγRIII (16, and see companion study [38]). However, no matter what role IgE and/or mast cells played in active systemic anaphylaxis to OVA, the findings reported here, as well as previous work (4, 5, 7–10), indicate that neither IgE nor mast cells are required for the mortality associated with this reaction.

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