

Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis.

I Miyajima, D Dombrowicz, T R Martin, J V Ravetch, J P Kinet, S J Galli

J Clin Invest. 1997;99(5):901-914. <https://doi.org/10.1172/JCI119255>.

Research Article

We attempted to elicit active anaphylaxis to ovalbumin, or passive IgE- or IgG1-dependent anaphylaxis, in mice lacking either the Fc epsilonRI alpha chain or the FcR gamma chain common to Fc epsilonRI and Fc gammaRI/III, or in mice lacking mast cells (KitW/ KitW-v mice), and compared the responses to those in the corresponding wild-type mice. We found that the FcR gamma chain is required for the death, as well as for most of the pathophysiological changes, associated with active anaphylaxis or IgE- or IgG1-dependent passive anaphylaxis. Moreover, some of the physiological changes associated with either active, or IgG1-dependent passive, anaphylactic responses were significantly greater in Fc epsilonRI alpha chain -/- mice than in the corresponding normal mice. Finally, while both KitW/KitW-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 IgG1-dependent passive systemic anaphylaxis. Our findings strongly suggest that while IgE antibodies and Fc epsilonRI 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 IgG1 antibodies and Fc gammaRIII.

Find the latest version:

<https://jci.me/119255/pdf>



Systemic Anaphylaxis in the Mouse Can Be Mediated Largely through IgG₁ and Fc γ RIII

Assessment of the Cardiopulmonary Changes, Mast Cell Degranulation, and Death Associated with Active or IgE- or IgG₁-dependent Passive Anaphylaxis

Ichiro Miyajima,* David Dombrowicz,‡ Thomas R. Martin,§ Jeffrey V. Ravetch,|| Jean-Pierre Kinet,* and Stephen J. Galli*

*Department of Pathology, Division of Experimental Pathology, Beth Israel Deaconess Medical Center-East and Harvard Medical School, Boston, Massachusetts 02215; ‡National Institutes of Health, National Institute of Allergy and Infectious Diseases, Molecular Allergy and Immunology Section, Rockville, Maryland 20852; §Department of Pediatrics and Pulmonary Medicine, The Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115; and ||Laboratory of Molecular Genetics and Immunology, The Rockefeller University, New York 10021

Abstract

We attempted to elicit active anaphylaxis to ovalbumin, or passive IgE- or IgG₁-dependent anaphylaxis, in mice lacking either the Fc ϵ RI α chain or the FcR γ chain common to Fc ϵ RI and Fc γ RI/III, or in mice lacking mast cells (*Kit*^W/*Kit*^{W-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 IgG₁-dependent passive anaphylaxis. Moreover, some of the physiological changes associated with either active, or IgG₁-dependent passive, anaphylactic responses were significantly greater in Fc ϵ RI α chain $-/-$ mice than in the corresponding normal mice. Finally, while both *Kit*^W/*Kit*^{W-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 IgG₁-dependent passive systemic anaphylaxis. Our findings strongly suggest that while IgE antibodies and Fc ϵ 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 IgG₁ antibodies and Fc γ RIII. (J. Clin. Invest. 1997; 99:901–914.) Key words: allergy • asthma • Fc γ RI • 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 WBB6F₁-*Kit*^{W-v} or WCB6F₁-*Mgf*^{Sl}/*Mgf*^{Sl-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 IgG₁ antibodies (11, 12). Furthermore, IgG antibodies of the IgG₁ 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 IgG₁ 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 IgG₁ antibodies and Fc γ R, rather than IgE antibodies and Fc ϵ 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 ϵ RI versus IgG₁ and Fc γ R in the pathogenesis of active anaphylaxis in mice, we used mice with targeted mutations of either the Fc ϵ RI α chain of the Fc ϵ RI (18) or the common γ chain of the Fc ϵ RI and the Fc γ RI/III (19). The Fc ϵ RI consists of a single Fc ϵ RI α chain, a single β chain, and two disulfide-linked γ chains (18, 20, 21). The Fc ϵ RI α chain binds the Fc portion of IgE, whereas the β chain promotes the assembly and cell surface expression of the Fc ϵ RI and also helps to amplify signal transduction through the Fc ϵ RI receptor's two γ chains (20–22). Mice with targeted disruption of the gene encoding the Fc ϵ RI α chain

Address correspondence to Stephen J. Galli, M.D., Department of Pathology, Research North, Room 227, Beth Israel Deaconess Medical Center-East, 330 Brookline Avenue, Boston, MA 02215. Phone: 617-667-5970; FAX: 617-667-3616; E-mail: sgalli@bidmc.harvard.edu

Received for publication 3 June 1996 and accepted in revised form 12 December 1996.

(Fc_εRI α chain $-/-$ mice) have normal numbers of tissue mast cells, but do not express Fc_εRI 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_εRI α chain $-/-$ mice to express active systemic anaphylaxis, or IgG₁-dependent passive systemic anaphylaxis, had not been investigated.

IgG antibodies can bind to three Fc_γRs, two of which (Fc_γRI and Fc_γRIII) share the same γ chain as the Fc_εRI (19, 20). Fc_γRI, which consists of an Fc_εRI 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 Fc_γRII and Fc_γRIII are low-affinity receptors that bind multimeric IgG (in decreasing order of affinity: IgG_{2b} > IgG_{2a} > IgG₁) (20, 24). Fc_γRII 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, Fc_γRIII 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_εRI and Fc_γRI/III (FcRγ chain $-/-$ mice) develop normal numbers of tissue mast cells but express neither Fc_εRI nor Fc_γRI/III (19). Accordingly, Fc_γR $-/-$ 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 IgG₁-dependent passive systemic anaphylaxis, had not been investigated.

In this study, we analyzed the ability of Fc_εRI α chain $-/-$ or FcR γ chain $-/-$ mice, and the corresponding normal mice, to express either active systemic anaphylaxis, or passive IgE- or IgG₁-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 WBB6F₁-*Kit*^W/*Kit*^{W-v} mice and the congenic normal (WBB6F₁- $+/+$) mice. The results show that IgE, Fc_εRI, 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_εRI. The results also strongly suggest that the mortality associated with active anaphylaxis in the mouse is mediated primarily by IgG₁ antibodies and Fc_γRIII.

Methods

Animals

The production of mice with targeted mutations that result in failure of production of the α chain of the Fc_εRI (Fc_εRI 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_εRI 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_εRI α chain $+/+$ mice. Male and female FcR γ chain $-/-$ and $+/+$ mice were generated by breeding the F₂ offspring of crosses between chimeras and C57BL/6 mice (19). Genetically mast cell-deficient male WBB6F₁-*Kit*^W/*Kit*^{W-v} mice, which virtually lack tissue mast cells (29–31), and the congenic normal (WBB6F₁- $+/+$) 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 IgG₁ 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 IgG₁ anti-DNP antibodies) was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). DNP₃₀₋₄₀ 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 WCB6F₁- $+/+$ 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 $Fc_{\epsilon}RI \alpha$ Chain $-/-$, $FcR \gamma$ Chain $-/-$, Mast Cell-deficient Kit^W/Kit^{W-v} Mice and Corresponding $+/+$ Mice

Mice	HR	C_{dyn}	G_L
	beats/min	$ml \cdot cmH_2O^{-1}$	$ml \cdot s^{-1} \cdot cmH_2O^{-1}$
$Fc_{\epsilon}RI \alpha$ chain $-/-$ (n = 39)	189 \pm 45	0.046 \pm 0.013	1.04 \pm 0.52
$Fc_{\epsilon}RI \alpha$ chain $+/+$ (n = 35)	170 \pm 33	0.046 \pm 0.015	0.92 \pm 0.15
$FcR \gamma$ chain $-/-$ (n = 31)	190 \pm 32*	0.027 \pm 0.008	1.29 \pm 0.41
$FcR \gamma$ chain $+/+$ (n = 29)	209 \pm 40	0.031 \pm 0.009	1.50 \pm 0.45
Kit^W/Kit^{W-v} (n = 26)	167 \pm 22	0.034 \pm 0.010	1.03 \pm 0.34
Kit (+/+) (n = 22)	179 \pm 27	0.034 \pm 0.011	1.02 \pm 0.28

Values are mean \pm 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- μ m, 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 $Fc_{\epsilon}RI \alpha$ chain $-/-$ (18) and $FcR \gamma$ 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 μ g 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 μ g of OVA in \sim 50 μ l of sterile, pyrogen-free 0.9% NaCl. Approximately 100 μ l 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 IgG₁-dependent passive systemic anaphylaxis. Mouse monoclonal IgE anti-DNP antibodies (\sim 20 μ g, as a 1:20 dilution of ascites in sterile, pyrogen-free 0.9% NaCl) or mouse mono-

clonal IgG₁ anti-DNP antibodies (\sim 400 μ g, as a 1:4 dilution of ascites in sterile, pyrogen-free 0.9% NaCl) were administered intravenously by tail vein in volumes of \sim 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 IgG₁-induced passive anaphylaxis experiments received, instead of IgG₁ anti-DNP antibodies, 400 μ g of normal mouse IgG in the same volume of 0.9% NaCl. 1 d (\sim 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 \sim 50 μ l of sterile, pyrogen-free 0.9% NaCl containing either 200 μ g of DNP-HSA (for experiments assessing IgE-induced passive systemic anaphylaxis) or 1.0 mg of DNP-HSA (for experiments assessing IgG₁-induced passive systemic anaphylaxis). We selected the quantities of IgE or IgG₁ 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 IgG₁ 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 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 concentrations of total IgE or IgG₁ 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 \pm SEM.

Results

Active systemic anaphylaxis is expressed in $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice, but not in $FcR \gamma$ chain $-/-$ mice. In normal mice ($Fc_{\epsilon}RI \alpha$ chain $+/+$ or $FcR \gamma$ 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 $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice developed a tachycardia response that was similar in magnitude to that in the identically sensitized and challenged $Fc_{\epsilon}RI \alpha$ chain $+/+$ mice, but which was somewhat more prolonged in duration (Fig. 1A). In addition, in comparison to the responses in OVA-sensitized $Fc_{\epsilon}RI \alpha$ chain $+/+$ mice, antigen challenge of OVA-sensitized $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice produced a more prolonged reduction in G_L , as well as a significantly greater reduction in C_{dyn} (Fig. 1A). The differences in the physiological responses of the $Fc_{\epsilon}RI \alpha$ 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 $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice, of 13.8 \pm 1.6 wk of age [mean \pm SEM] and 29.8 \pm 0.8 g body weight, versus six male and three female $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice, of

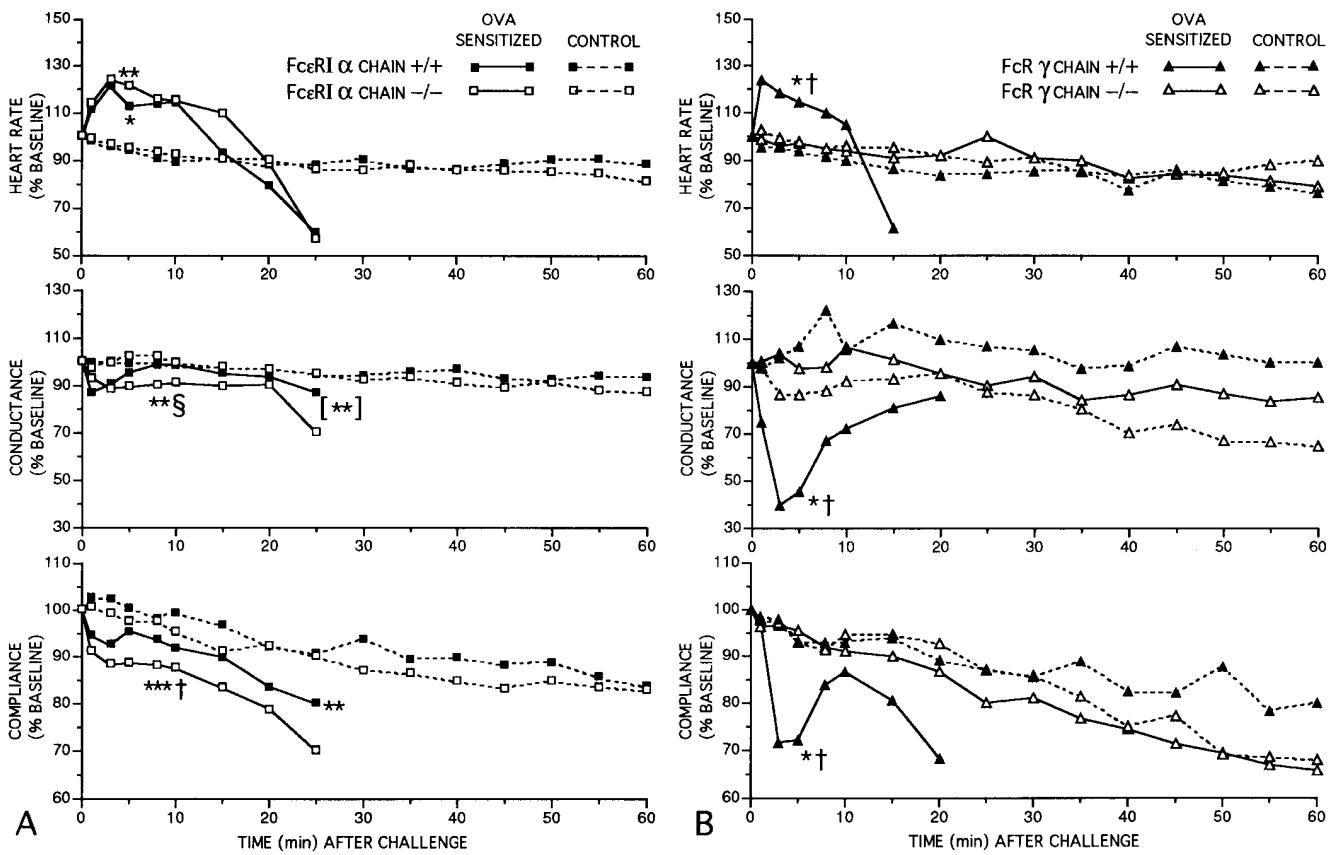


Figure 1. Pulmonary C_{dyn} , pulmonary G_L , and HR in $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice or $FcR \gamma$ chain $-/-$ mice (open symbols, A and B, respectively) or the corresponding normal (+/+) mice (filled symbols) which had been sensitized with OVA (OVA-SENSITIZED, solid lines) or were sham-immunized (CONTROL, dotted lines), and then 18–21 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 500 µg of OVA. (A) $Fc_{\epsilon}RI \alpha$ chain $-/-$ and corresponding +/+ mice. (B) $FcR \gamma$ 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 $\leq 20\%$ 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. § $P < 0.06$, † $P < 0.05$ 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 \pm 1.8 wk of age and 29.1 \pm 1.3 g body weight. Note that in OVA-sensitized mice of either $Fc_{\epsilon}RI \alpha$ 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 $Fc_{\epsilon}RI \alpha$ 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 $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice or +/+ mice appeared normal (i.e., exhibited no morphological evidence of activation). Even though the OVA-sensitized $Fc_{\epsilon}RI \alpha$ 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 $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice was slightly, but signifi-

cantly, 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 $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice or +/+ mice (Table II). All but one of the sensitized $Fc_{\epsilon}RI \alpha$ 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 $Fc_{\epsilon}RI \alpha$ chain $-/-$ and +/+ mice exhibited increased serum concentrations of total IgE after OVA immunization (day –1 vs. day 17–20 values of 0.77 \pm 0.70 vs. 6.41 \pm 6.17 µg/ml [mean \pm SD], $P = 0.006$ for $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice and 0.68 \pm 0.52 vs. 1.70 \pm 1.57 µg/ml, $P = 0.082$ for $Fc_{\epsilon}RI \alpha$ 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 \pm 222 to 276 \pm 322 µg/ml in $Fc_{\epsilon}RI \alpha$ chain $-/-$ mice, and from 207 \pm 58 to 326 \pm 99 µg/ml in the corresponding +/+ mice, respectively).

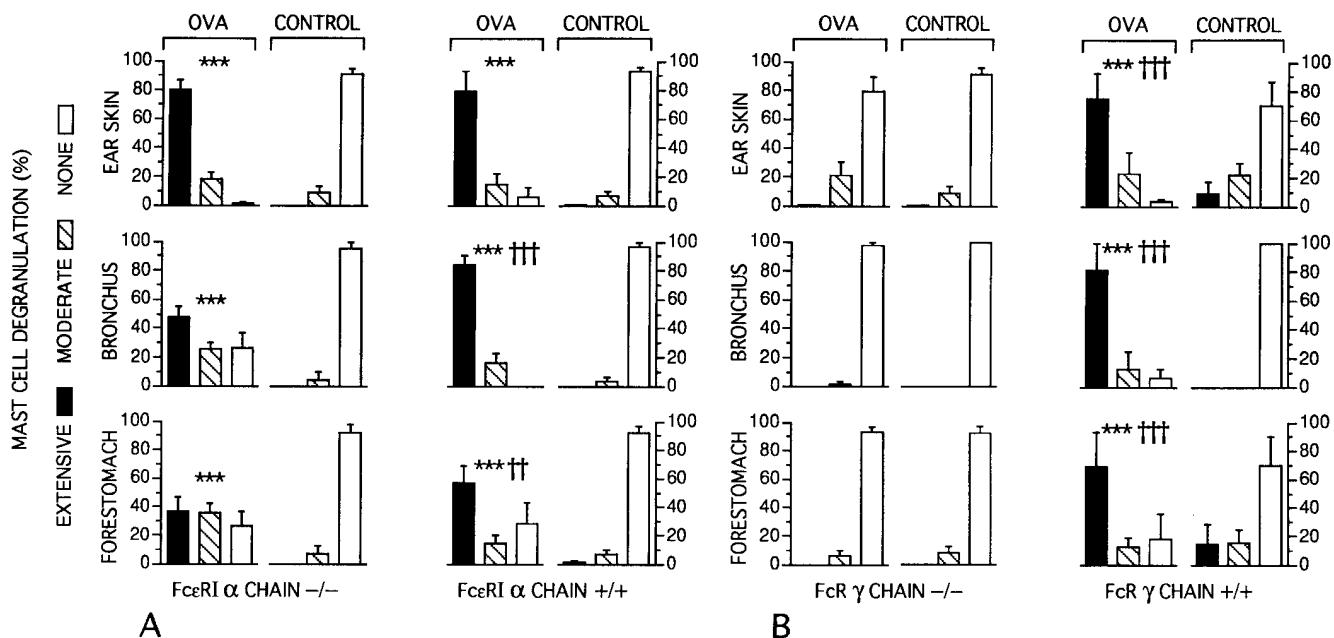


Figure 2. Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in OVA-sensitized (OVA) or sham-immunized (CONTROL) $\text{Fc}_\epsilon\text{RI} \alpha$ chain $-/-$ or $\text{FcR} \gamma$ chain $-/-$ mice and the corresponding $+/+$ mice which had been challenged with 500 μg of OVA intravenously. (A) $\text{Fc}_\epsilon\text{RI} \alpha$ chain $-/-$ and corresponding $+/+$ mice. (B) $\text{FcR} \gamma$ 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 \pm SEM, are from the same mice shown in Fig. 1, A and B. *** $P < 0.001$ by the χ^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 χ^2 test versus data from the same anatomical site in corresponding OVA-sensitized $-/-$ mice.

OVA-sensitized $\text{FcR} \gamma$ 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 $\text{Fc}_\epsilon\text{RI} \alpha$ chain $+/+$ mice (Fig. 1 A). The OVA-sensitized $\text{FcR} \gamma$ 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 $\text{Fc}_\epsilon\text{RI} \alpha$ chain $+/+$ (or $\text{Fc}_\epsilon\text{RI} \alpha$ chain $-/-$) mice (compare Fig. 1, A and B), perhaps reflecting differences in the genetic backgrounds of the mice (BALB/c for the $\text{Fc}_\epsilon\text{RI} \alpha$ chain $+/+$ mice, C57BL/6 \times 129 for the $\text{FcR} \gamma$ chain $+/+$ mice). However, by morphological analysis, the extent of mast cell degranulation in the ear skin, bronchial tissues, and forestomach of the OVA-sensitized $\text{FcR} \gamma$ chain $+/+$ mice (Fig. 2 B) was very similar to that in the corresponding tissues of the OVA-sensitized $\text{Fc}_\epsilon\text{RI} \alpha$ chain $+/+$ mice (Fig. 2 A).

All of the OVA-sensitized $\text{FcR} \gamma$ chain $+/+$ mice died within 20 min of intravenous antigen challenge (Table II). By contrast, none of the antigen-challenged OVA-sensitized $\text{FcR} \gamma$ chain $-/-$ mice died (Table II). The OVA-sensitized $\text{FcR} \gamma$ 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, $\text{FcR} \gamma$ chain $-/-$ mice were unable to express active anaphylaxis.

The lack of anaphylactic responsiveness in $\text{FcR} \gamma$ 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 $\text{FcR} \gamma$ chain $-/-$

mice ($31.9 \pm 14.9 \mu\text{g/ml}$, 10.4 times the baseline level, $P = 0.008$) were even greater than those in the OVA-immunized $\text{FcR} \gamma$ chain $+/+$ mice ($2.96 \pm 0.91 \mu\text{g/ml}$, 4.8 times the baseline level, $P = 0.016$). However, $\text{FcR} \gamma$ chain $+/+$ mice exhibited a larger increase in serum concentrations of total IgG (to $474 \pm 313 \mu\text{g/ml}$, a 260% increase over baseline, $P = 0.016$) than did the $\text{FcR} \gamma$ chain $-/-$ mice (to $520 \pm 236 \mu\text{g/ml}$, 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

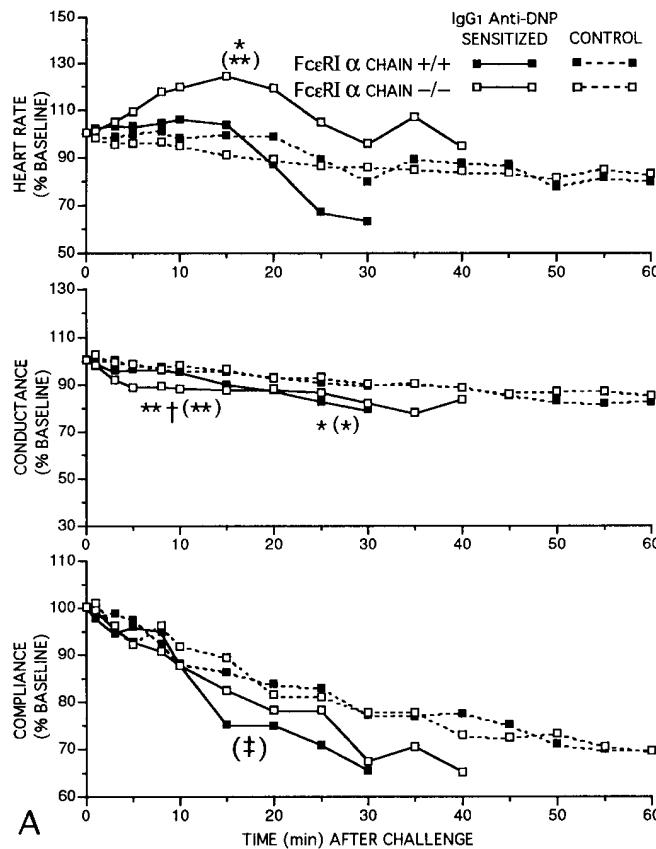
Mice	OVA-immunized	Death rates	Times until death	
			min	
$\text{Fc}_\epsilon\text{RI} \alpha$ chain $-/-$	+	8/9*	20, 20, 20, 25, 25, 25, 25, 50	
	-	0/7	DNA	
$\text{Fc}_\epsilon\text{RI} \alpha$ chain $+/+$	+	5/6*	25, 25, 25, 30, 30	
	-	0/8	DNA	
$\text{FcR} \gamma$ chain $-/-$	+	0/5‡	DNA	
	-	0/5	DNA	
$\text{FcR} \gamma$ 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.

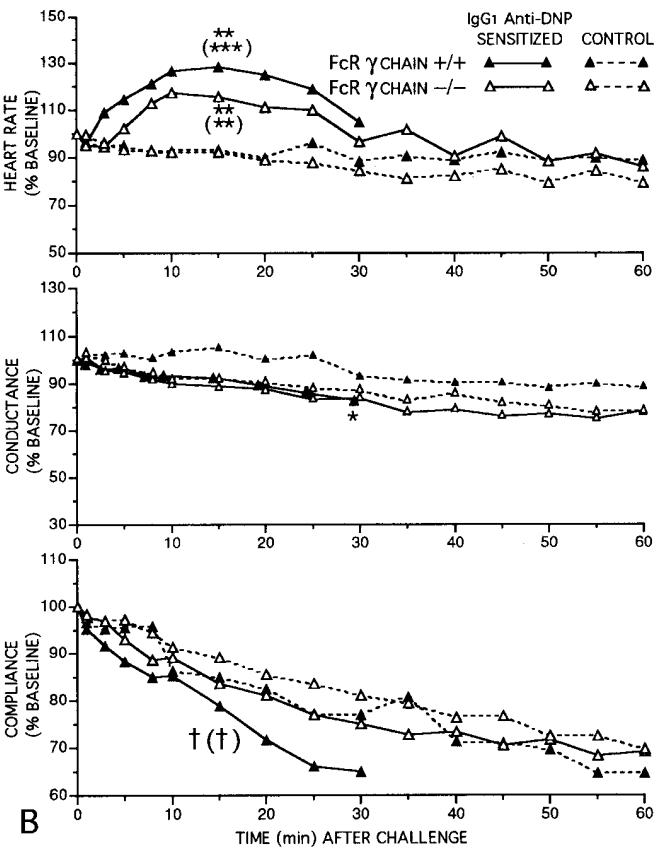
Table III. Death Rates and Times until Death in Mice Passively Sensitized with 400 μ g of Mouse Monoclonal IgG₁ Anti-DNP Antibodies or Injected with 400 μ g of Control Mouse IgG, 1 d before Intravenous Challenge with 1 mg of DNP-HSA

Mice	IgG ₁ anti-DNP	Death rates	Times until death
min			
Fc _ε RI α chain $-/-$	+	6/6*	25, 30, 30, 30, 30, 40
	-	1/6	50
Fc _ε 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.



A



B

Figure 3. Pulmonary C_{dyn} , pulmonary G_L , and HR in Fc_εRI 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 μ g of mouse monoclonal IgG₁ anti-DNP antibodies (IgG₁ Anti-DNP SENSITIZED, solid lines) or injected intravenously with 400 μ g 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_εRI 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 IgG₁-sensitized mice of the other genotype. The exact numbers of mice in the various groups ($n = 5-6$ /group) are given in Table III.

To assess the anaphylactic responsiveness of Fc_εRI 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 IgG₁ antibodies.

IgG₁-dependent passive systemic anaphylaxis is expressed in Fc_εRI α chain $-/-$ mice, but is largely ablated in FcR γ chain $-/-$ mice. When mice were passively sensitized with a monoclonal anti-DNP IgG₁ antibody and then challenged 1 d later with specific antigen, an anaphylactic reaction associated with significant mortality occurred in Fc_εRI 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 IgG₁-sensitized mice occurred within 30–40 min of antigen challenge.

We found that the physiological changes associated with IgG₁-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-

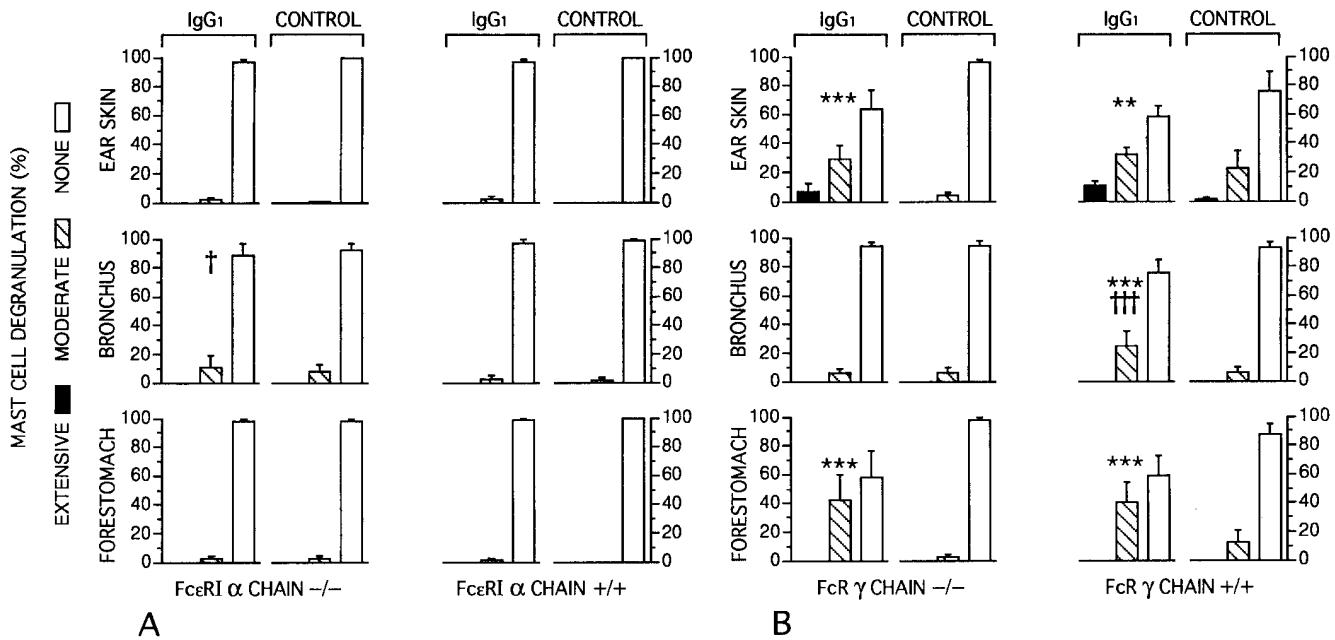


Figure 4. Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ or $\text{FcR } \gamma \text{ chain } -/-$ mice, and the respective $+/+$ mice, which had been passively sensitized by an intravenous injection of 400 μg of mouse monoclonal IgG₁ 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) $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ and corresponding $+/+$ mice. (B) $\text{FcR } \gamma \text{ chain } -/-$ 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 \pm SEM, are from the same mice shown in Fig. 3, A and B. ** $P < 0.01$ and *** $P < 0.001$ by the χ^2 test versus data from the same anatomical site in normal mouse IgG-injected (control) mice of the same genotype. † $P < 0.05$ and ††† $P < 0.001$ by the χ^2 test versus data from the same anatomical site in corresponding IgG₁ anti-DNP sensitized mice of the other genotype.

temic anaphylaxis, the responses to IgG₁ and antigen were associated with little or no morphological evidence of mast cell degranulation (Fig. 4, A and B).

In those IgG₁-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 IgG₁ 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 IgG₁-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 $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ mice and $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } +/+$ mice, the $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ 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 IgG₁-sensitized $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ mice, while minimal and not significantly different than that in control $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ mice which had not been sensitized with IgG₁, was significantly greater than that observed in the bronchial tissues of the identically sensitized and antigen-challenged $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } +/+$ mice (Fig. 4 A). As in the case of the active anaphylaxis responses in $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ 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 IgG₁-dependent passive systemic anaphylaxis in five female $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } +/+$ mice, of 14.8 ± 0.5 wk of age, versus six female $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain } -/-$ mice, all of ~ 14 wk of age.

By contrast, IgG₁-sensitized $\text{FcR } \gamma \text{ chain } -/-$ 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 IgG₁-sensitized $\text{FcR } \gamma \text{ chain } +/+$ mice (Fig. 3 B). It may be of some interest, in this regard, that of the three anatomical sites analyzed in mice challenged with IgG₁ and antigen, the bronchial tissues were the only location where $\text{FcR } \gamma \text{ chain } +/+$ mice exhibited significantly more mast cell degranulation than did the $\text{FcR } \gamma \text{ chain } -/-$ mice (Fig. 4 B). Note, however, that both $\text{FcR } \gamma \text{ chain } +/+$ mice and $\text{FcR } \gamma \text{ chain } -/-$ mice developed a tachycardia response in response to IgG₁ sensitization and antigen challenge. Furthermore, even though all of the $\text{FcR } \gamma \text{ chain } -/-$ mice which had been sensitized with IgG₁ 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 $\text{FcR } \gamma \text{ chain } +/+$ mice (Fig. 4 B).

IgE-dependent passive systemic anaphylaxis requires the $\text{Fc}_\epsilon\text{RI } \alpha \text{ chain}$ and $\text{FcR } \gamma \text{ chain}$ and is associated with cardio-pulmonary responses that mimic those in active anaphylaxis. While tachycardia and declines in G_L and/or C_{dyn} occurred during IgG₁-dependent passive systemic anaphylaxis (Fig. 3, A and B), these changes developed more slowly than in active

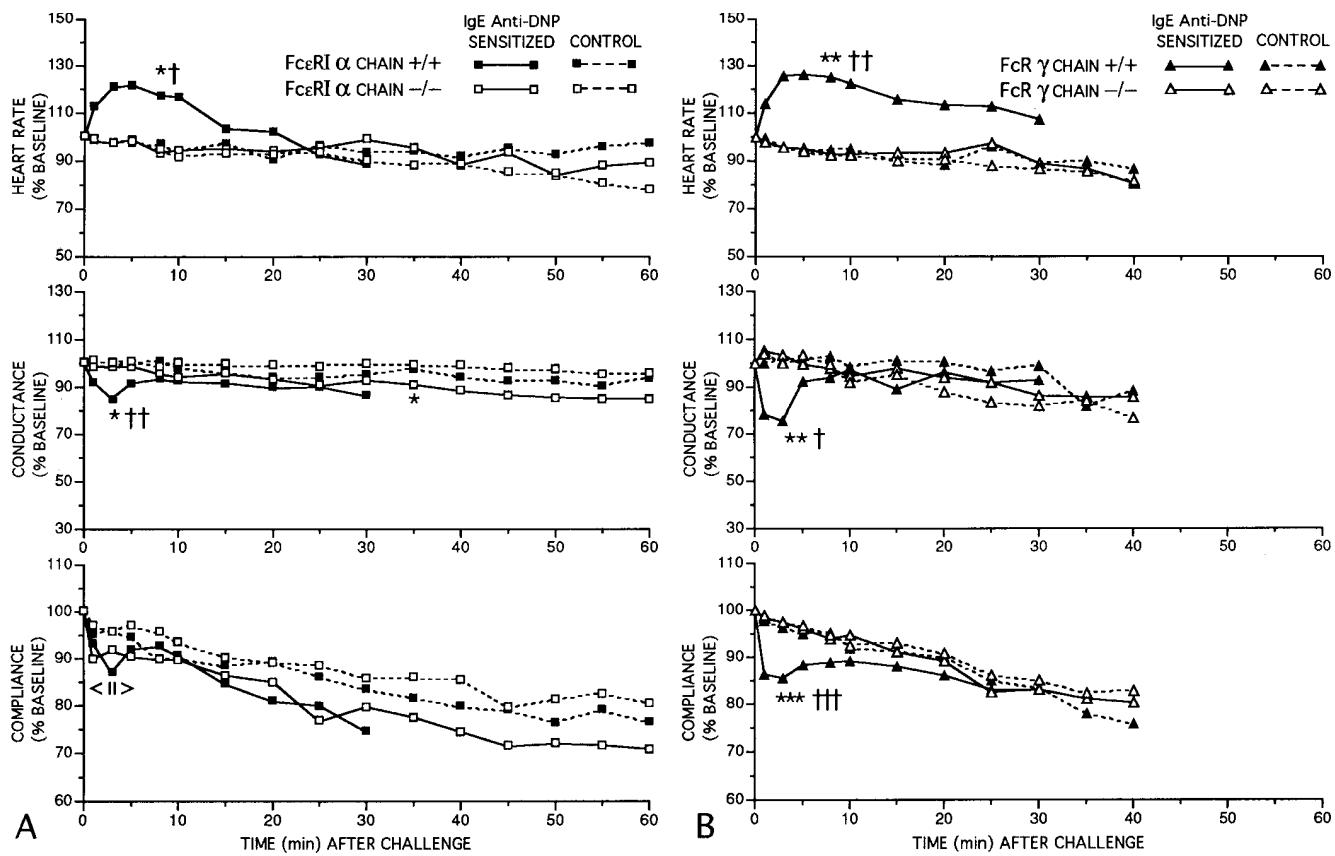


Figure 5. Pulmonary C_{dyn} , pulmonary G_L , and HR in $Fc\epsilon RI\alpha$ chain $-/-$ mice or $FcR\gamma$ chain $-/-$ mice (open symbols, A and B, respectively) or the corresponding normal $(+/+)$ mice (filled symbols) which had been passively sensitized by an intravenous injection of 20 μ g of mouse 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 μ g of DNP-HSA. (A) $Fc\epsilon RI\alpha$ chain $-/-$ and corresponding $+/+$ mice. (B) $FcR\gamma$ 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; $\dagger P \approx 0.06$ by ANOVA over the first 3 min of the response versus data from 0.9% NaCl-injected (control) mice of the same genotype. $\ddagger P < 0.05$, $\ddagger\ddagger P < 0.01$, and $\ddagger\ddagger\ddagger P < 0.001$ 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 IgG₁-dependent reactions.

Both $Fc\epsilon RI\alpha$ chain $+/+$ mice (Fig. 5 A) and, to an even greater extent, $FcR\gamma$ 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 $Fc\epsilon RI\alpha$ chain $+/+$ or $FcR\gamma$ chain $+/+$ mice died after antigen challenge (Table IV). How-

ever, 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 $Fc\epsilon RI\alpha$ chain $-/-$ mice or $FcR\gamma$ 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 IgG₁-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 IgG₁-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-v} mice and the congenic normal

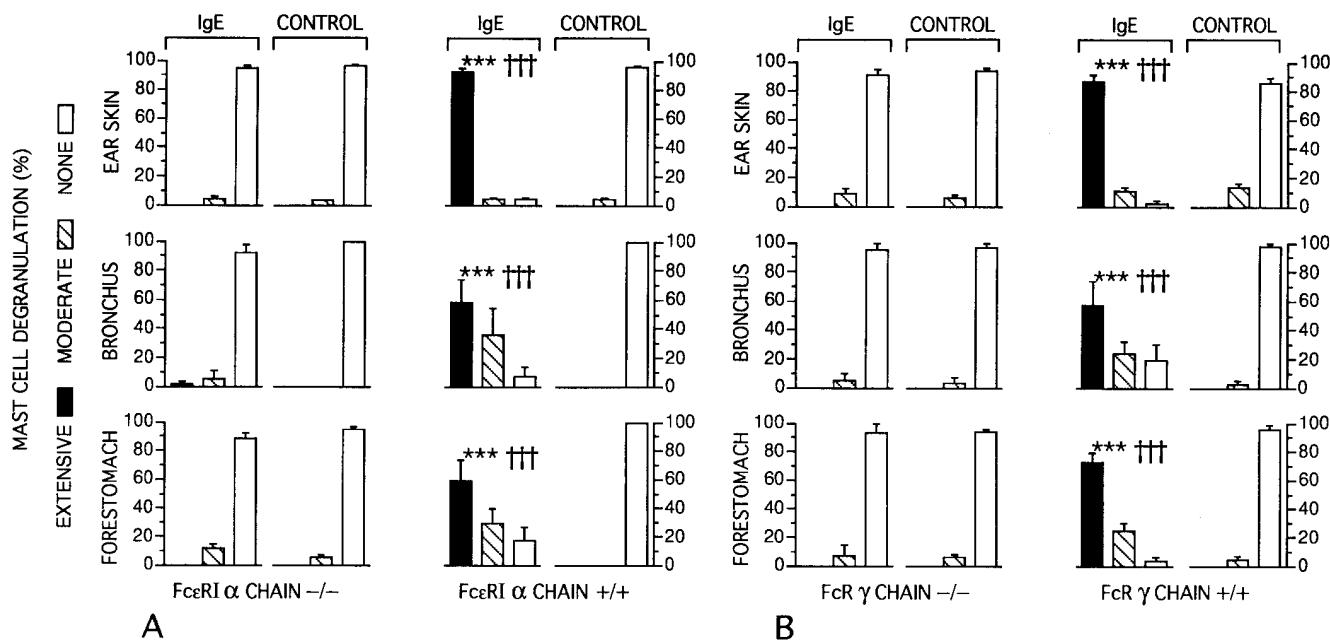


Figure 6. Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in IgE anti-DNP-sensitized (IgE) or 0.9% NaCl-injected (CONTROL) Fc_εRI α chain -/- or FcR γ chain -/- mice, or the respective +/+ mice, which had been challenged with 200 µg of DNP-HSA intravenously. (A) Fc_εRI 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 χ^2 test versus data from the same anatomical site in 0.9% NaCl-injected (control) mice of the same genotype. †††P < 0.001 by the χ^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-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-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-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-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 _ε RI α chain -/-	+	1/6*	60
	-	0/5	DNA
Fc _ε 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 WBB6F₁-*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 IgG₁-dependent Systemic Anaphylaxis to DNP-HSA*

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

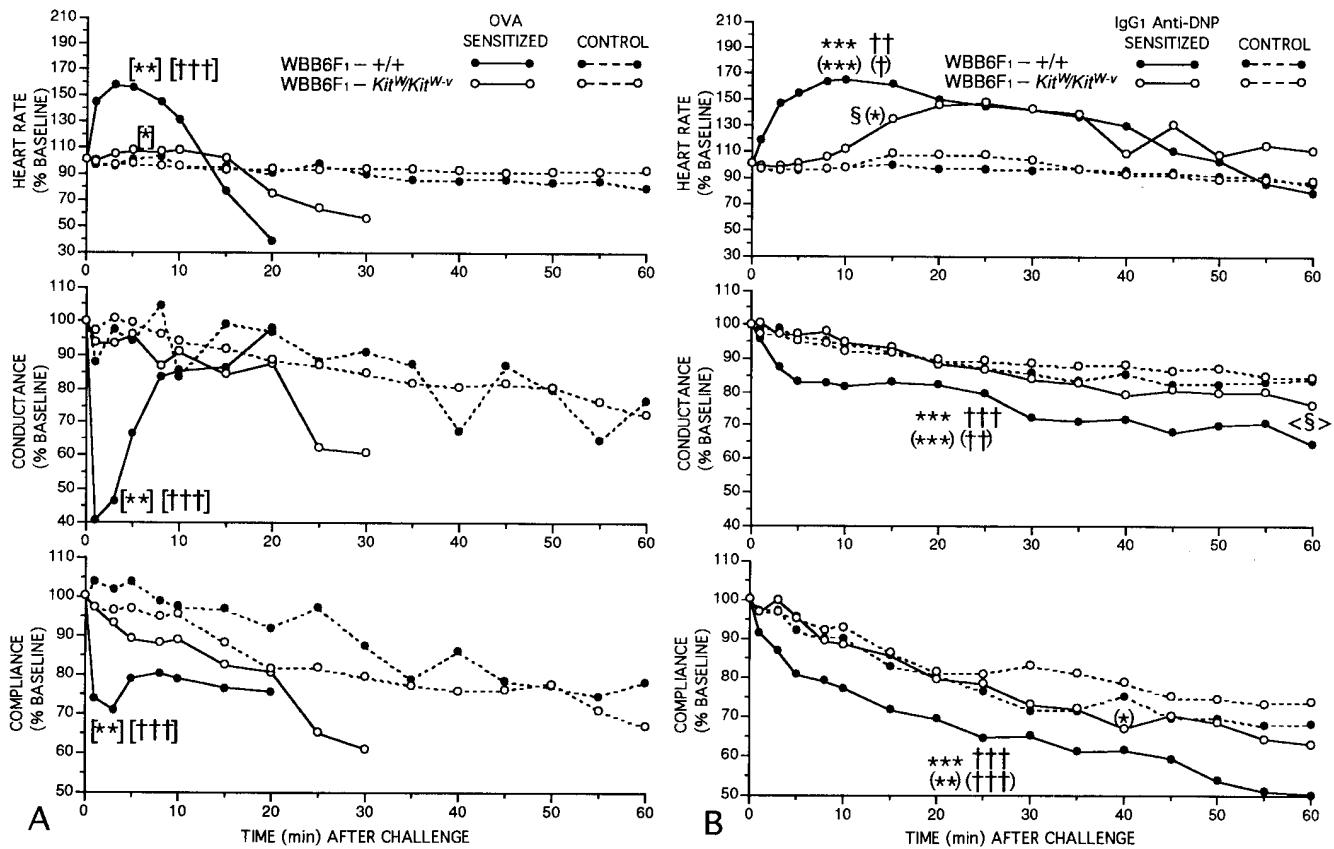


Figure 7. (A) Pulmonary C_{dyn} , pulmonary G_L , and HR in WBB6F1- Kit^W/Kit^{W-v} (Kit^W/Kit^{W-v}) mice (open symbols) or the corresponding normal, WBB6F1- $+/+$ ($+/+$) mice (filled symbols) which had been sensitized with OVA (OVA SENSITIZED, solid lines) or sham-immunized (CONTROL, dotted lines), and then 18–21 d later, challenged by rapid intravenous infusion of 0.9% NaCl containing 500 μ g 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 $\leq 29\%$ 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 WBB6F1- Kit^W/Kit^{W-v} (Kit^W/Kit^{W-v}) mice (open symbols) or the corresponding normal, WBB6F1- $+/+$ ($+/+$) mice (filled symbols), which had been passively sensitized by an intravenous injection of 400 μ g of mouse monoclonal IgG₁ anti-DNP antibodies (IgG₁ Anti-DNP SENSITIZED, solid lines) or injected intravenously with 400 μ g 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 IgG₁-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-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-v} mice) (Fig. 7 A). WBB6F1- $+/+$ 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 IgG₁-induced passive systemic ana-

phylaxis, WBB6F1- $+/+$ mice exhibited responses which in in some respects were even stronger than those of FcR γ chain $+/+$ mice (Figs. 3 B and 4 B). The WBB6F1- $+/+$ 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 IgG₁-dependent passive systemic anaphylaxis (Fig. 4 B), but was less striking than that in WBB6F1- $+/+$ mice which had been challenged for active anaphylaxis to OVA (compare Fig. 8 A).

Although IgG₁-dependent passive systemic anaphylaxis was expressed in Kit^W/Kit^{W-v} mice, the responses were substan-

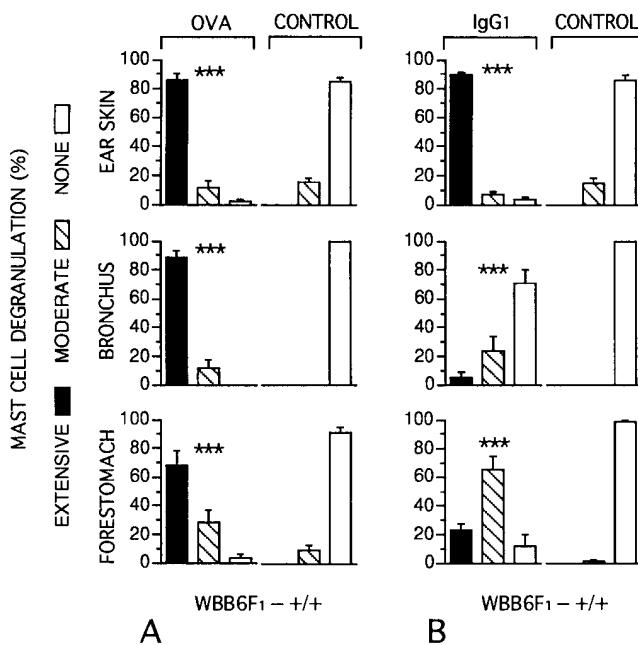


Figure 8. (A and B) Extent of activation of mast cell populations in the ear skin, peribronchial tissues, or forestomach in (A) OVA-sensitized (OVA) or sham-immunized (CONTROL) WBB6F₁-+/+ (+/+) mice which had been challenged with 500 µg of OVA intravenously or (B) in WBB6F₁-+/+ (+/+) mice which had been passively sensitized by an intravenous injection of 400 µg of mouse monoclonal IgG₁ anti-DNP antibodies (IgG₁) 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 \pm SEM, are from the same mice shown in Fig. 7. *** P < 0.001 by the χ^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-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-v}* died as a result of IgG₁-dependent passive anaphylaxis, as opposed to five out of six of the congenic +/+ mice (Table V).

In confirmation of our previous findings (4, 5), WBB6F₁-+/+ 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-v}* mice gave no detectable responses (data not shown). Five out of six WBB6F₁-+/+ 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-v}* mice challenged for IgE-dependent systemic anaphylaxis died versus zero out of four antigen-challenged control *Kit^W/Kit^{W-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 physiologi-

cal responses, mast cell degranulation, and death rates associated with the three types of anaphylactic reactions in the Fc_εRI α chain -/- and corresponding +/+ mice, the FcR γ chain -/- and corresponding +/+ mice, and the mast cell-deficient *Kit^W/Kit^{W-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 WBB6F₁ > FcR γ chain +/+ (C57BL/6 \times 129) > Fc_εRI 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_εRI, or lacking both Fc_εRI and Fc_γRI/III, exhibited very distinct patterns of defects in their ability to express active systemic anaphylaxis or IgE- or IgG₁-dependent passive systemic anaphylaxis. Fc_εRI α chain -/- mice that lacked Fc_εRI 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_εRI 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_εRI 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_εRI α chain, mast cells can exhibit increased expression of Fc_γRIII (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_εRI α chain -/- versus the corresponding +/+ mice is that the Fc_εRI α chain -/- mice exhibited increased levels of Fc_γRIII-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_εRI α chain -/- versus corresponding wild-type mice, at least as could be assessed by histological analysis. Alternatively, the Fc_εRI 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_εRI 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_εRI α chain.

In contrast to their ability to express all of the features of active systemic anaphylaxis reactions that we analyzed, Fc_εRI

Table VI. Characteristics of Active, or IgE- or IgG₁-dependent Passive, Systemic Anaphylactic Reactions in Fc_εRI α Chain *−/−* Mice, FcR γ Chain *−/−* Mice, and Mast Cell-deficient Kit^{W/W} Mice, and the Corresponding Normal (+/+) Mice

Feature	Type of anaphylaxis	Mice					
		Fc _ε RI α +/+	Fc _ε RI α <i>−/−</i>	FcRγ +/+	FcRγ <i>−/−</i>	Kit (+/+)	Kit ^{W/W}
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_εRI 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_εRI-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_εRI, 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 IgG₁ antibodies, which bind much more preferentially to FcγRIII than to FcγRI (23), can sensitize Fc_εRI α chain *−/−* mice, Fc_εRI 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 IgG₁-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 IgG₁-dependent passive systemic anaphylactic responses and active anaphylactic reactions which we studied were not fully identical. Although the IgG₁-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, IgG₁-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 IgG₁ 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, IgG₁-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 IgG₁-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 IgG₁-dependent passive anaphylaxis can also occur independently of any critical contribution of the mast cell. On the other hand, the cardiopulmonary changes associated with IgG₁-dependent passive systemic anaphylaxis, as well as the mortality associated with this response, were greater in WBB6F₁-+/+ (normal) mice than in the congenic mast cell-deficient WBB6F₁- *Kit*^W/*Kit*^{W-v} mice (Fig. 7 B). These findings suggest that IgG₁-dependent mast cell degranulation can, under some circumstances, contribute to the intensity of IgG₁-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 IgG₁ antibodies and specific antigen, IgG₁-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 fore-stomach, responses that were statistically indistinguishable from those in the IgG₁-sensitized and antigen-challenged FcR γ chain +/+ mice (Fig. 4 B).

These results indicate that sensitization with IgG₁ and challenge with specific antigen can induce both tachycardia responses and small amounts of mast cell degranulation independently of Fc γ RI/III- (or Fc ϵ 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 IgG₁-injected FcR γ chain -/- mice reflected complement activation by anti-DNP IgG₁-DNP-HSA immune complexes. Nevertheless, it should be emphasized that FcR γ chain -/- mice exhibited neither pulmonary changes nor increased mortality upon challenge with IgG₁ and specific anti-

gen. Therefore, we can conclude that, no matter what IgG₁-dependent but FcR-independent processes may have occurred in our experiments, the development of the most clinically significant consequences of IgG₁-dependent passive anaphylaxis reactions required adequate signaling through the FcR γ chain.

If virtually all of the pathophysiology of both active and IgG₁-dependent passive systemic anaphylaxis responses reflects the signaling function of the FcR γ chain, and if both active and IgG₁-dependent passive systemic anaphylaxis responses can be expressed in mice that lack the Fc ϵ 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-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 WBB6F₁-+/+ mice), mast cell degranulation may also contribute to the intensity of the cardiopulmonary changes and mortality associated with IgG₁-dependent passive systemic anaphylaxis. Mast cell degranulation during active anaphylaxis may have occurred primarily via IgE and Fc ϵ RI in normal mice, but by IgG₁-dependent mechanisms in Fc ϵ RI α chain -/- mice that express increased levels of mast cell activation via Fc γ RIII (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.

Acknowledgments

We thank Drs. Fu-Tong Liu and David Katz for H 1 DNP- ϵ -26 hybridoma cells, Dr. David M. Segal for the mouse monoclonal IgG₁ (clone U7.6) antibodies, Dr. Zhen-Sheng Wang for assistance with the histology, and Dr. Beverly H. Koller for helpful discussions.

This work was supported in part by United States Public Health Service grants CA/AI-72074 and AI/CA-23990 (to S.J. Galli), K11HL-02240 (to T.R. Martin), GM-53950 (to J.-P. Kinet), by National Institutes of Health grants to J.V. Ravetch, and by the Beth Israel Hospital Pathology Foundation. D. Dombrowicz was supported in part by a fellowship from the FNRS (Belgium).

References

1. Bochner, B.S., and L.M. Lichtenstein. 1991. Anaphylaxis. *N. Engl. J. Med.* 324:1785-1790.
2. Marquardt, D.L., and S.I. Wasserman. 1993. Anaphylaxis. In *Allergy: Principles and Practice*, Vol. II, 4th Ed. E. Middleton, Jr., C.E. Reed, E.F. Ellis, N.F. Adkinson, Jr., J.W. Yunginger, and W.W. Busse, editors. Mosby-Year Book, Inc., St. Louis, MO. 1525-1536.
3. Ando, A., T.R. Martin, and S.J. Galli. 1993. Effects of chronic treatment with *c-kit* ligand, stem cell factor, on immunoglobulin E-dependent anaphylaxis in mice. *J. Clin. Invest.* 92:1639-1649.
4. Martin, T.R., S.J. Galli, I.M. Katona, and J.M. Drazen. 1989. Role of mast cells in anaphylaxis. Evidence for the importance of mast cells in the cardiopulmonary alterations and death induced by anti-IgE in mice. *J. Clin. Invest.* 83:1375-1383.
5. Takeishi, T., T.R. Martin, I.M. Katona, F.D. Finkelman, and S.J. Galli. 1991. Differences in the expression of the cardiopulmonary alterations associated with anti-immunoglobulin E-induced or active anaphylaxis in mast cell-deficient and normal mice. *J. Clin. Invest.* 88:598-608.
6. Martin, T.R., A. Ando, T. Takeishi, I.M. Katona, J.M. Drazen, and S.J.

Galli. 1993. Mast cells contribute to the changes in heart rate, but not hypotension or death, associated with active anaphylaxis in mice. *J. Immunol.* 151:367-376.

7. Jacoby, W., P.V. Cammarata, S. Findlay, and S. Pincus. 1984. Anaphylaxis in mast cell-deficient mice. *J. Invest. Dermatol.* 83:302-304.
8. Ha, T.Y., N.D. Reed, and P.K. Crowley. 1986. Immune response potential of mast cell-deficient *W/W^v* mice. *Int. Arch. Allergy Appl. Immunol.* 80:85-94.
9. Ha, T.Y., and N.D. Reed. 1987. Systemic anaphylaxis in mast cell-deficient mice of *W/W^v* and *Sl/Sl^d* genotypes. *Exp. Cell Biol.* 55:63-68.
10. Oettgen, H.C., T.R. Martin, A. Wynshaw-Boris, C. Deng, J.M. Drazen, and P. Leder. 1994. Active anaphylaxis in IgE-deficient mice. *Nature (Lond.)* 370:367-370.
11. Finkelman, F.D., I. Scher, J.J. Mond, S. Kessler, J.T. Kung, and E.S. Metcalf. 1982. Polyclonal activation of the murine immune system by an antibody to IgD. II. Generation of polyclonal antibody production and cells with surface IgG. *J. Immunol.* 129:638-646.
12. Finkelman, F.D., I.M. Katona, J.F. Urban, Jr., and W.E. Paul. 1989. Control of *in vivo* IgE production in the mouse by interleukin 4. In IgE, Mast Cells and the Allergic Response. *Ciba Found. Symp.* 147:3-22.
13. Watanabe, N., and Z. Ovary. 1977. Antigen and antibody detection by *in vivo* methods; a reevaluation of passive cutaneous anaphylactic reactions. *J. Immunol. Methods*. 14:381-390.
14. Ovary, Z., B. Benacerraf, and K. Bloch. 1963. Properties of guinea pig 7S antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis. *J. Exp. Med.* 117:951-964.
15. Ovary, Z., N.M. Vaz, and N.L. Warner. 1970. Passive anaphylaxis in mice with gamma-G antibodies. V. Competitive effects of different immunoglobulins and inhibition of reactions with antigen-globulin sera. *Immunology*. 19:715-727.
16. Harada, M., M. Nagata, M. Takeuchi, T. Ohara, S. Makino, and A. Watanabe. 1991. Age-dependent difference in susceptibility to IgE antibody- and IgG₁ antibody-mediated passive anaphylactic shock in the mouse. *Immunol. Invest.* 20:515-523.
17. Oshiba, A., E. Hamelmann, K. Takeda, K.L. Bradley, J.E. Loader, G.L. Larsen, and E.W. Gelfand. 1996. Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific immunoglobulin (Ig)E and IgG₁ in mice. *J. Clin. Invest.* 97:1398-1408.
18. Dombrowicz, D., V. Flamand, K.K. Brigmann, B.H. Koller, and J.-P. Kinet. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor Fc_εRI α chain gene. *Cell*. 75:969-975.
19. Takai, T., M. Li, D. Sylvestre, R. Clynes, and J.V. Ravetch. 1994. Fc_γR γ chain deletion results in pleiotropic effector cell defects. *Cell*. 76:519-529.
20. Ravetch, J.V., and J.-P. Kinet. 1991. Fc receptors. *Ann. Rev. Immunol.* 9:457-492.
21. Jeselma, C.L., and J.-P. Kinet. 1994. The high affinity receptor for immunoglobulin E. In Molecular and Cellular Biology of the Allergic Response. A.I. Levinson and I. Patterson, editors. Marcel Dekker, New York. 211-244.
22. Lin, S., C. Cicala, A. Scharenberg, and J.-P. Kinet. 1996. The atopy-associated Fc_εRI β chain gene: the encoded subunit functions as an amplifier of Fc_εRI γ -mediated cell activation signals. *Cell*. 85:985-995.
23. Snapper, C.M., and F.D. Finkelman. 1993. Immunoglobulin class switching. In Fundamental Immunology. W.E. Paul, editor. Raven Press, New York. 837-863.
24. Ravetch, J.V. 1994. Fc receptors: rubor redux. *Cell*. 78:553-560.
25. Daéron, M., S. Latour, O. Malbec, E. Espinosa, P. Pina, S. Pasmans, and W.H. Friedman. 1995. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of Fc_γRIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity*. 3:635-646.
26. Bonnerot, C., and M. Daéron. 1994. Biological activities of murine low-affinity Fc receptors for IgG. *Immunomethods*. 4:41-47.
27. Toshiyuki, T., M. Ono, M. Hikida, H. Ohmori, and J.V. Ravetch. 1996. Augmented humoral and anaphylactic responses in Fc_γRII-deficient mice. *Nature (Lond.)*. 379:346-349.
28. Katz, H.R., M.B. Raizman, C.S. Gartner, H.C. Scott, A.C. Benson, and K.F. Austen. 1992. Secretory granule mediator release and generation of oxidative metabolites of arachidonic acid via Fc-IgG receptor bridging in mouse mast cells. *J. Immunol.* 148:868-871.
29. Kitamura, Y., S. Go, and S. Hatanaka. 1978. Decrease of mast cells in *W/W^v* mice and their increase by bone marrow transplantation. *Blood*. 52:447-452.
30. Galli, S.J., K.M. Zsebo, and E.N. Geissler. 1994. The kit ligand, stem cell factor. *Adv. Immunol.* 55:1-96.
31. Witham, B.A. 1995. Nomenclature update: symbols affecting mutant genes. *JAX Notes* No. 461. Jackson Laboratory, Bar Harbor, ME.
32. Liu, F.-T., J.W. Bohn, E.L. Ferry, H. Yamamoto, C.A. Molinaro, L.A. Sherman, N.R. Kliman, and D.H. Katz. 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 124:2728-2737.
33. George, A.J., J.A. Titus, C.R. Jost, I. Kurucz, P. Perez, S.M. Andrew, P.J. Nicholls, J.S. Huston, and D.M. Segal. 1994. Redirection of T cell-mediated cytotoxicity by a recombinant single-chain Fv molecule. *J. Immunol.* 152:1802-1811.
34. Martin, T.R., N.P. Gerard, S.J. Galli, and J.M. Drazen. 1988. Pulmonary responses to bronchoconstrictor agonists in the mouse. *J. Appl. Physiol.* 64: 2318-2323.
35. Von Neergaard, K., and K. Wirz. 1927. Die Messung der Strömungswiderstände in der Atemwegen des Menschen, insbesondere bei Asthma und Emphysem. *Z. Klin. Med.* 105:51-82.
36. Galli, S.J., N. Arizono, T. Murakami, A.M. Dvorak, and J.H. Fox. 1987. Development of large numbers of mast cells at sites of idiopathic chronic dermatitis in genetically mast cell-deficient WBB6F₁-W/W^v mice. *Blood*. 69:1661-1666.
37. Wershil, B.K., T. Murakami, and S.J. Galli. 1988. Mast cell-dependent amplification of an immunologically nonspecific inflammatory response: mast cells are required for the full expression of cutaneous acute inflammation induced by phorbol 12-myristate 13-acetate. *J. Immunol.* 140:2356-2360.
38. Dombrowicz, D., V. Flamand, I. Miyajima, J.V. Ravetch, S.J. Galli, and J.-P. Kinet. 1997. Absence of Fc_εRI α chain results in upregulation of Fc_γRIII-dependent mast cell degranulation and anaphylaxis. Evidence of competition between Fc_εRI and Fc_γRIII for limiting amounts of FcR β and γ chains. *J. Clin. Invest.* 99:915-925.
39. Takizawa, F., M. Adamczewski, and J.-P. Kinet. 1992. Identification of the low affinity receptor for immunoglobulin E on mouse mast cells and macrophages as Fc_γRII and Fc_γRIII. *J. Exp. Med.* 176:469-476.
40. Hazenbos, W.L.W., J.E. Gessner, F.M.A. Hofhuis, H. Kuipers, D. Meyer, I.A.F.M. Heijnen, R.E. Schmidt, M. Sandor, P.J.A. Capel, M. Daéron, et al. 1996. Impaired IgG-dependent anaphylaxis and arthus reaction in Fc_γRIII (CD16) deficient mice. *Immunity*. 5:181-188.
41. Schwartz, L., and T. Huff. 1993. Biology of mast cells and basophils. In Allergy: Principles and Practice. 4th ed. E. Middleton, Jr., C.E. Reed, E.F. Ellis, N.F. Adkinson, Jr., J.W. Yunginger, and W.W. Busse, editors. Mosby-Year Book, Inc., St. Louis, MO. 135-168.