

# Differences in the Expression of the Cardiopulmonary Alterations Associated with Anti-Immunoglobulin E-induced or Active Anaphylaxis in Mast Cell-deficient and Normal Mice

## Mast Cells Are Not Required for the Cardiopulmonary Changes Associated with Certain Fatal Anaphylactic Responses

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### Abstract

We compared the changes in heart rate (HR), pulmonary dynamic compliance ( $C_{dyn}$ ), and pulmonary conductance ( $G_L$ ) associated with three different models of anaphylaxis in genetically mast cell-deficient WBB6F<sub>1</sub>-*W/W'* and congenic normal (+/+) mice. Intravenous infusion of a monoclonal rat anti-mouse IgE produced a marked tachycardia, diminutions in  $C_{dyn}$  and  $G_L$ , and death in +/+ but not *W/W'* mice, and +/+ mice sensitized to develop high circulating levels of IgE exhibited HR,  $C_{dyn}$ , and  $G_L$  responses to rat anti-IgE challenge which were significantly less intense than those in nonimmunized +/+ mice. By contrast, virtually identical cardiopulmonary responses were observed in either +/+ or *W/W'* mice challenged to elicit pure active anaphylactic responses or simultaneous active and anti-IgE-dependent anaphylaxis. These findings show that anaphylactic responses associated with significant tachycardia, reductions in  $C_{dyn}$  and  $G_L$ , and death can occur in the virtual absence of tissue mast cells. This is true even though, in normal mice, such responses are associated with extensive degranulation of tissue mast cells. By contrast, certain models of anaphylaxis, such as that induced in nonsensitized mice by anti-mouse IgE, can not be elicited in the absence of mast cells. (*J. Clin. Invest.* 1991. 88:598-608.) Key words: asthma • basophils • immunoglobulin E receptors • pulmonary compliance • pulmonary conductance

### Introduction

Several lines of evidence indicate that mast cells represent a potentially critical source of the mediators responsible for the cardiopulmonary alterations associated with IgE-dependent anaphylactic responses (1-4). IgE has been strongly implicated in the pathogenesis of anaphylactic bronchoconstriction (5);

mast cells express large numbers of surface receptors (Fc<sub>RI</sub>)<sup>1</sup> that bind IgE with high affinity (6-8); mast cells can be triggered via Fc<sub>RI</sub> to release potent bronchoactive mediators (1-4, 6-11); and agents that interfere either with mast cell degranulation or the effects of mast cell-associated mediators can diminish the expression of anaphylactic responses (12-16). Finally, we reported that genetically mast cell-deficient mice challenged intravenously (i.v.) with goat anti-mouse IgE (goat anti-IgE) antibodies survived and failed to develop abnormalities of cardiopulmonary function (17). By contrast, identically treated congenic normal mice rapidly developed peribronchial mast cell degranulation, tachycardia, diminished pulmonary dynamic compliance ( $C_{dyn}$ ) and conductance ( $G_L$ ), and, in 8 of 13 cases, death within 60 min of injection of anti-IgE (17).

However, it has been suggested that mast cells may not importantly contribute to certain types of anaphylactic responses. Morphological studies showed that sensitized mice expressing fatal active anaphylaxis upon antigen challenge exhibited no detectable activation of mast cell populations in the skin or mesentery (18, 19). More recently, Jacoby et al. (20) and Ha et al. (21, 22) found that antigen challenge of sensitized genetically mast cell-deficient mice was as likely to result in their death as was identical challenge of sensitized congenic normal mice. Ha et al. (22) also reported that when serum from actively sensitized mast cell-deficient mice was heated to destroy the ability of IgE to bind to Fc receptors, the serum lost its ability to transfer anaphylactic responsiveness to nonsensitized mice. This suggested that IgE antibodies had an essential role in this model of anaphylaxis, whereas mast cells did not. The expression of fatal, IgE-dependent anaphylaxis by mice virtually devoid of mast cells is in accord with evidence that many cell types in addition to the mast cell can release potent biologically active mediators by IgE-dependent mechanisms. Such cells include basophils stimulated via their Fc<sub>RI</sub> (1-5, 7, 8), as well as macrophages, platelets, lymphocytes, and perhaps eosinophils activated via receptors (Fc<sub>R2</sub>/CD23) that can bind IgE with low affinity (23-25).

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance;  $C_{dyn}$ , pulmonary dynamic compliance; Fc<sub>RI</sub>, surface receptors that bind IgE antibodies with high affinity; Fc<sub>R2</sub>/CD23, surface receptors that bind IgE antibodies with low affinity; goat anti-IgE, affinity-purified goat anti-mouse IgE; goat anti-M $\delta$ , affinity-purified goat anti-mouse IgD;  $G_L$ , pulmonary conductance; HR, heart rate; PEEP, positive end expiratory pressure; rat anti-DNP, affinity-purified rat monoclonal anti-DNP; rat anti-IgE, affinity-purified rat monoclonal anti-mouse IgE.

Even though previous work clearly demonstrated that fatal active anaphylaxis can occur in the virtual absence of mast cells, these studies relied on observations of prostration or death as the indicators of a clinical response (20–22). As a result, it was not possible to compare specific cardiopulmonary responses associated with active anaphylaxis in mast cell-deficient mice to those in normal mice. Moreover, the previous studies did not include histological analysis to determine whether the anaphylactic responses were associated with mast cell activation. In fact, Jacoby et al. (20) reported that normal mice expressing active anaphylaxis did not exhibit detectable changes in lung histamine content, suggesting that the model of anaphylaxis studied was not associated with significant activation of mast cell populations. In the present study, we analyzed the changes in heart rate (HR),  $C_{dyn}$ , and  $G_L$ , as well as the occurrence of death, in genetically mast cell-deficient WBB6F<sub>1</sub>- $W/W^v$  and congenic normal (WBB6F<sub>1</sub>- $+/+$ ) mice expressing active anaphylaxis to i.v. goat IgG, anaphylaxis induced by i.v. injection of a rat monoclonal anti-mouse IgE (rat anti-IgE), or a combination of active and anti-IgE-dependent anaphylaxis induced by i.v. injection of a goat anti-mouse IgE. We also examined the tissues of these mice to quantify the extent of mast cell activation associated with the various models of anaphylaxis.

## Methods

**Animals.** We studied male genetically mast cell-deficient WBB6F<sub>1</sub>- $W/W^v$  ( $W/W^v$ ) mice and the congenic normal (WBB6F<sub>1</sub>- $+/+$ ) mice ([WB/ReJ- $W/+ \times C57BL/6J-W^v/+$ ] $F_1$ - $[W/W^v, +/+]$  mice; Jackson Biological Laboratory, Bar Harbor, ME), which were 12–16 wk old (25–35 g of body wt) at the beginning of the experiment. The  $W/W^v$  mice have mutations at the  $W$  locus on chromosome 5, which encodes the *c-kit* tyrosine kinase growth factor receptor (26).  $W/W^v$  mice are anemic, lack melanocytes in the skin, are sterile, and virtually lack tissue mast cells (27, 28). The skin of adult  $W/W^v$  mice contains < 0.5% the number of mast cells present in the skin of the congenic  $+/+$  mice, and no mast cells whatsoever are identifiable in the trachea, lungs, or multiple other organs or anatomical sites (17, 27, 28). However, apart from defects in responses significantly influenced by mast cells, the expression of immunological and inflammatory reactions in  $W/W^v$  mice is similar or identical to that in the congenic normal animals (21, 28–30).

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).

**Materials.** Pentobarbital sodium was obtained from Anthony Products Co., Arcadia, CA. Affinity purified goat anti-mouse IgE (goat anti-IgE) was prepared as described for rabbit anti-mouse IgE (31). In brief, goats were immunized with monoclonal mouse IgE, their immune serum was absorbed with mouse serum-Sepharose, IgD<sub>k</sub>-Sepharose, and IgM<sub>k</sub>-Sepharose, then mixed with normal mouse serum overnight and centrifuged to remove soluble complexes. The supernatant was bound to and then eluted from IgE-Sepharose, and then dialyzed against 0.85 M NaCl. The generation and affinity purification of a rat monoclonal (mc) anti-mouse IgE antibody has been described in detail (32). As a control reagent, we used affinity-purified rat mc anti-DNP (a gift from Dr. Herve Bazin, Brussels, Belgium) of the same isotype (IgG2a) as the rat mc anti-mouse serum IgE. Affinity-purified goat anti-mouse IgD antibodies (goat anti-M $\delta$ ) were prepared as previously described in detail (33). Normal goat IgG was purified from goat serum by ammonium sulfate precipitation and DE-52 ion exchange chromatography.

**Immunization of mice with goat anti-M $\delta$ .** To sensitize mice for the development of an IgE and IgG<sub>1</sub> antibody response to a defined antigen, some  $W/W^v$  and  $+/+$  mice received a single i.v. injection of 800  $\mu$ g of goat anti-M $\delta$  in 0.2 ml of sterile 0.9% NaCl. Control (nonsensitized)  $W/W^v$  and  $+/+$  mice received a single i.v. injection of 0.2 ml of sterile 0.9% NaCl alone. Immunization with goat anti-M $\delta$  induces significant, T cell-dependent elevations in both goat IgG-specific and nonspecific IgE and IgG<sub>1</sub> antibodies, with the peak of plasma levels of IgE detectable at days 7–9 after immunization (34–36). Levels of total serum IgE and total IgG<sub>1</sub> were measured 7 d after injection of goat anti-M $\delta$  or saline. Mice were placed under light ether anesthesia, 50–80  $\mu$ l of blood was obtained by retro-orbital puncture, and the serum was stored at  $-80^\circ\text{C}$  before assay for IgE and IgG<sub>1</sub> using radial immunodiffusion and ELISA assays (36, 37).

**Physiological measurements.** HR and the pulmonary mechanical parameters,  $C_{dyn}$  and  $G_L$  were measured in mice anesthetized with 70–90 mg/kg i.p. of sodium pentobarbital using a modification (17, 38) of a standard plethysmographic method for rats (39) and guinea pigs (40).  $C_{dyn}$  and pulmonary resistance were calculated from the recordings of volume, flow, and pressure using standard techniques (41). Baseline values of HR,  $C_{dyn}$ , and  $G_L$  were determined 20–30 min after preparation of the animals for recording pulmonary parameters.

**Histologic studies.** The presence of tissue mast cells and their state of activation were assessed in 1  $\mu$ m, Epon-embedded, Giemsa-stained sections taken through the ear pinna or bronchi (17, 42, 43). Tissues were removed and fixed as previously described (42, 43) either immediately after death induced by anti-IgE or goat anti-M $\delta$  challenge, or after injection of a fatal overdose of pentobarbital sodium 60 min after challenge. Sections were coded so that the observer was not aware of the identity of individual specimens, and examined at  $\times 400$  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” (43, 44).

**Protocols.** All mice were studied 8 d after injection of either goat anti-M $\delta$  or saline. When stable values of HR,  $C_{dyn}$ , and  $G_L$  had been obtained, mice received a rapid i.v. infusion of 50  $\mu$ l of 0.9% NaCl containing one of the following: 15, 30, or 100  $\mu$ g of rat anti-mouse IgE; 100  $\mu$ g of normal goat IgG; 100  $\mu$ g of goat anti-mouse IgE; or, as a control reagent, 100  $\mu$ g of rat anti-DNP. HR,  $C_{dyn}$ , and  $G_L$  were measured at multiple intervals until death due to challenge or, after a period of 60 min, overdose of pentobarbital sodium.

**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 analysis of variance (ANOVA). Differences in the maximum diminution in  $C_{dyn}$  or  $G_L$ , or the maximum HR responses, were evaluated for statistical significance by the Mann-Whitney U test (two-tailed). 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 (two-tailed).  $P < 0.05$  was regarded as significant. Unless otherwise specified, results are expressed as the mean  $\pm$  SEM.

## Results

**Serum levels of IgE and IgG<sub>1</sub> antibodies associated with sensitization of  $W/W^v$  or  $+/+$  mice with goat anti-mouse IgD.** In accord with findings in other strains of mice (34–36), WBB6F<sub>1</sub>- $+/+$  or  $-W/W^v$  mice sensitized with goat anti-M $\delta$  7 d previously had levels of IgE and IgG<sub>1</sub> that were markedly elevated compared to those in saline-injected control mice (Table I). Levels of IgE were not significantly different in sensitized WBB6F<sub>1</sub>- $+/+$  or  $-W/W^v$  mice. However, levels of IgE in non-sensitized  $W/W^v$  mice were significantly higher than those in

Table 1. Serum Levels of IgE and IgG<sub>1</sub> in *W/W<sup>o</sup>* and *+/+* Mice 7 d after Sensitization with Goat Anti-Mouse IgD

Mice	IgE			IgG <sub>1</sub>		
	A. Not sensitized	B. Sensitized	<i>P</i> value, A vs. B	A. Not sensitized	B. Sensitized	<i>P</i> value, A vs. B
	<i>μg/ml</i>			<i>mg/ml</i>		
<i>+/+</i>	0.10±0.02*	3.19±0.44	<0.0001	0.73±0.07	2.15±0.19	<0.001
<i>W/W<sup>o</sup></i>	0.16±0.02	3.71±0.41	<0.0001	0.92±0.08	3.60±0.34	<0.001

Mean±SEM for 14–30 mice per group. \* *P* < 0.011 vs. value for *W/W<sup>o</sup>* mice by Mann-Whitney U test (two-tailed). For calculation of mean±SEM of levels of IgE in nonsensitized mice, specimens containing <0.05 μg/ml of IgE were assigned a value of 0.05. Thus, the difference between IgE levels in nonsensitized *+/+* or *W/W<sup>o</sup>* mice may have been even greater than is indicated here (see text).

control *+/+* mice. Indeed, 13 of the 30 values from nonsensitized *+/+* mice were below the lower limit of detection (< 0.05 μg/ml), compared to only 3 of the 20 values from nonsensitized *W/W<sup>o</sup>* mice (*P* < 0.05 by the  $\chi^2$  test).

*Cardiopulmonary responses induced by various doses of rat anti-mouse IgE in WBB6F<sub>1</sub>+/+ mice.* We performed a dose-response experiment to identify an amount of rat anti-mouse IgE that would induce significant alterations in heart rate, *C<sub>dyn</sub>*, and/or *G<sub>L</sub>* upon i.v. injection into nonsensitized *+/+* mice, and to assess whether *+/+* mice sensitized to express high levels of IgE responded to rat anti-IgE challenge differently than did nonsensitized *+/+* mice. In Fig. 1, data are presented as the mean values for the responses of all mice in that group at each time interval after injection. Table II presents the lowest values of *C<sub>dyn</sub>* and *G<sub>L</sub>*, and the highest HR, recorded for each mouse during the first 20 min after injection of rat anti-IgE or rat anti-DNP. Because not all mice developed maximal changes in *C<sub>dyn</sub>*, *G<sub>L</sub>*, or HR at precisely the same interval, the mean values presented in Table II in some instances are slightly greater than those shown in Fig. 1.

A dose-response effect for the cardiopulmonary changes induced by rat anti-IgE was observed both in nonsensitized and sensitized mice. However, cardiopulmonary responses in sensitized mice were generally less marked than those in nonsensitized mice, especially for *G<sub>L</sub>* and HR at a dose of 100 μg of rat anti-IgE and for *C<sub>dyn</sub>* and *G<sub>L</sub>* at 30 μg of rat anti-IgE. By ANOVA, responses of sensitized and nonsensitized mice were different at the *P* < 0.001 level for *C<sub>dyn</sub>*, *G<sub>L</sub>*, and HR at 100 μg of rat anti-IgE. None of the maximal changes in *G<sub>L</sub>* in sensitized mice were significant by the two-tailed Mann-Whitney U test, but the *G<sub>L</sub>* responses to 30 or 100 μg of rat anti-IgE were significant when compared to those to rat anti-DNP by the one-tailed test (*P* < 0.05 for either comparison). Even though sensitization rendered *+/+* mice somewhat less responsive to the cardiopulmonary effects associated with i.v. administration of rat anti-IgE, deaths occurred at lower doses of rat anti-IgE challenge in sensitized than in nonsensitized mice. Thus, the numbers of sensitized mice that died within 60 min of challenge with 15, 30, or 100 μg of rat anti-IgE were two of five, three of five, and two of five, respectively, whereas the corresponding values for the nonsensitized mice were zero of five, zero of five, and three of five. There were no deaths among mice treated with the control reagent, rat anti-DNP.

*Cardiopulmonary responses to challenge with rat anti-DNP (control antibody) or during various forms of anaphylactic challenge in mast cell-deficient W/W<sup>o</sup> and congenic +/+ mice.* Groups of nonsensitized and goat anti-Mδ-sensitized mast cell-deficient *W/W<sup>o</sup>* and congenic normal (*+/+*) mice were

challenged i.v. with 100 μg of rat anti-DNP (as a control, Fig. 2 A), rat anti-mouse IgE (Fig. 2 B), goat IgG (Fig. 2 C), or goat anti-mouse IgE (Fig. 2 D), and the *C<sub>dyn</sub>*, *G<sub>L</sub>*, and HR responses were recorded until death or 60 min after challenge, which ever occurred first. Note that the groups of *+/+* mice challenged with 100 μg of rat anti-IgE or rat anti-DNP presented in Fig. 2, A and B, are the same as those shown in Fig. 1. Table III reports for each of the four treatment groups the mean ± SEM of the lowest recordings of *C<sub>dyn</sub>* or *G<sub>L</sub>*, and the highest recording of HR, for each mouse during the first 20 min after challenge. Table IV summarizes the statistical significance of comparisons within the four treatment groups, determined by using ANOVA to compare responses recorded over the first 20 min after challenge. Table V summarizes the statistical significance of comparisons between values recorded in the first 20 min after challenge in the negative control (anti-DNP treated) as opposed to each of the three experimental groups. Table VI reports the death rates and times until death in the four treatment groups. Fig. 3 shows the numbers of dermal and peribronchial mast cells, and the extent of dermal and peribronchial mast cell activation, in sensitized and nonsensitized *+/+* mice in the four treatment groups.

*Mice challenged with rat anti-DNP.* Neither the genotype of the mice nor their sensitization status significantly influenced responses to rat anti-DNP (Table IV). Mice injected with rat anti-DNP (Fig. 2 A) exhibited a slight gradual diminution of *C<sub>dyn</sub>*, with mean maximal decreases to 94–96% of baseline by 20 min after injection (Table III), and also exhibited a gradual diminution in HR, to mean levels 60 min after injection which ranged from 65±1% to 70±4% of baseline values (Fig. 2 A). The values of *C<sub>dyn</sub>* or HR recorded in the four groups of mice tested were quite consistent, with most SEM at individual time points ≤ 10% or 15% of the mean, respectively. As in our previous study (17), values for *G<sub>L</sub>* exhibited more variability than those for *C<sub>dyn</sub>* or HR, with SEM of ≤ 20% of the mean at all time points. Sensitization status had no effect on either numbers of dermal or peribronchial mast cells or on morphological evidence of mast cell activation in *+/+* mice (Fig. 3). Thus > 90% of dermal or peribronchial mast cell populations in either the sensitized or nonsensitized group appeared normal. None of the mice challenged with rat anti-DNP died within 60 min of injection (Table VI).

*Mice challenged with rat anti-IgE.* Injection of rat anti-IgE produced a dramatic anaphylactic response in both sensitized and nonsensitized *+/+* mice but not in either group of *W/W<sup>o</sup>* mice (Fig. 2 B). Moreover, responses were significantly greater in nonsensitized as opposed to sensitized *+/+* mice.

Within the first 20 min after challenge, nonsensitized or

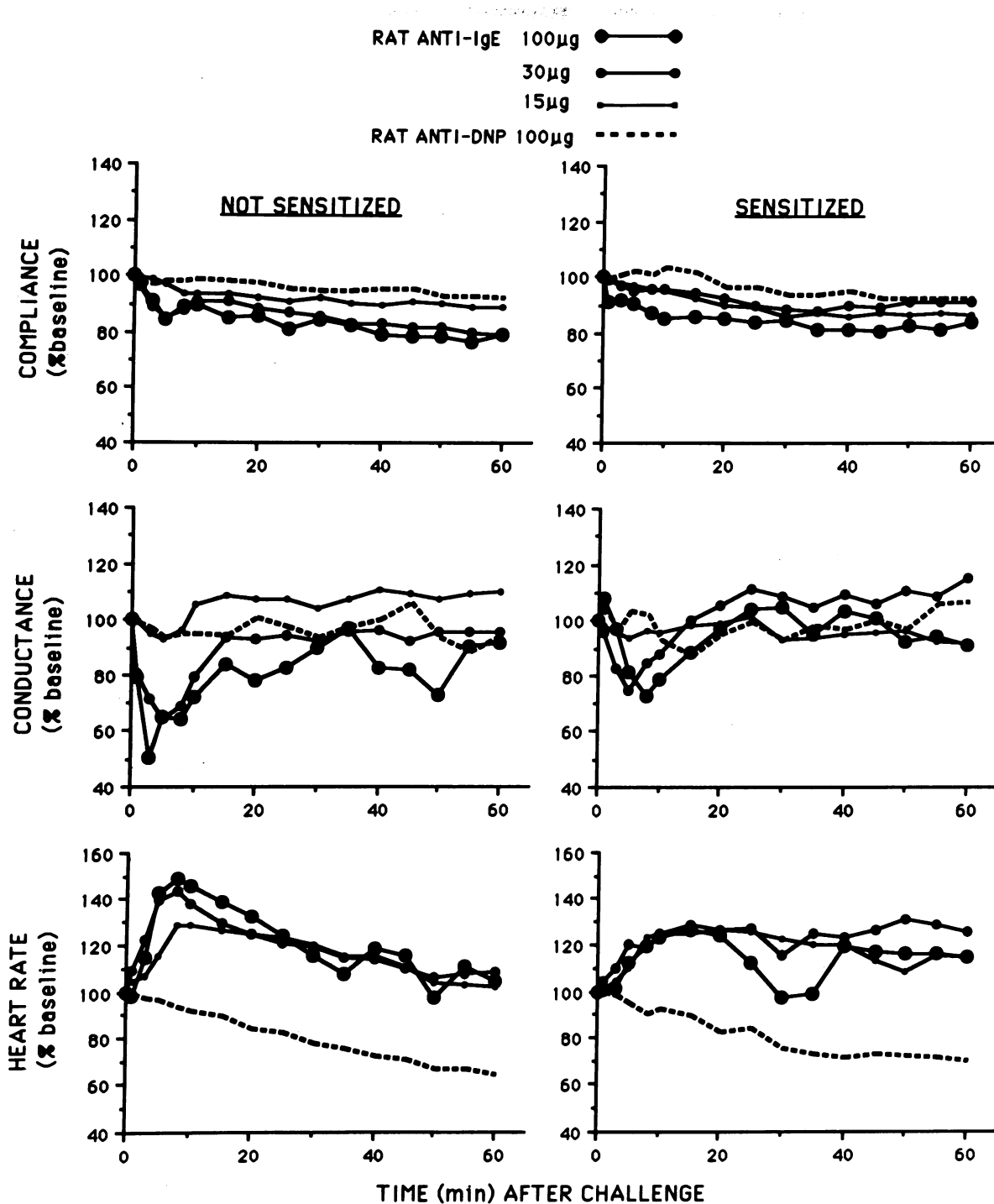


Figure 1. Effects of intravenous infusion of 15, 30, or 100 µg of rat anti-IgE, or, as a control, 100 µg of rat anti-DNP, on  $C_{dyn}$ ,  $G_L$ , and HR in WBB6F<sub>1</sub>+/- mice sensitized with goat anti-Mδ 8 d previously or not sensitized. After recording baseline values for a period of 20–30 min, anti-IgE or anti-DNP was administered at time 0. For each mouse, results obtained at various intervals after challenge were normalized by expressing them as the percentage of the mean baseline measurement. For clarity, only mean values are shown. For most intervals, the SEM for measurements of  $C_{dyn}$ ,  $G_L$ , or HR were < 10% of the mean ( $n = 5$  mice per group).

sensitized +/- mice developed maximal changes in  $C_{dyn}$  to  $80 \pm 2\%$  vs.  $81 \pm 4\%$  of baseline (NS), in  $G_L$  to  $49 \pm 5\%$  vs.  $67 \pm 7\%$  of baseline ( $P < 0.001$ ) and of HR to  $150 \pm 6\%$  vs.  $132 \pm 5\%$  of baseline ( $P < 0.001$ ) (Tables III and IV). Histological analysis demonstrated significant degranulation of dermal and peribronchial mast cell populations in both sensitized and nonsensitized +/- mice, with the response of peribronchial mast cells in nonsensitized mice being significantly greater than that in sensitized animals ( $81 \pm 5\%$  vs.  $43 \pm 15\%$  extensively degranulated mast cells,  $P < 0.001$ ) (Fig. 3). Three of five nonsensitized mice and two of five sensitized mice died within 60 min of challenge with rat anti-IgE (Table VI).

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Table II. Maximum Percent Diminutions of  $C_{dyn}$  and  $G_L$ , and Highest Elevations of HR, for Each Mouse during the First 20 min after Challenge of Sensitized or Nonsensitized  $+/+$  Mice with Rat Anti-IgE or Rat Anti-DNP

Maximum reduction in $C_{dyn}$	Not sensitized	Sensitized
	%	
Rat anti-IgE ( $\mu\text{g per mouse}$ )		
15	10 $\pm$ 1	12 $\pm$ 2
30	17 $\pm$ 3*	11 $\pm$ 2
100	20 $\pm$ 2 <sup>‡</sup>	19 $\pm$ 4 <sup>‡</sup>
Rat anti-DNP (100 $\mu\text{g}/\text{mouse}$ )	4 $\pm$ 2	4 $\pm$ 3
Maximum reduction in $G_L$		
	%	
Rat anti-IgE ( $\mu\text{g per mouse}$ )		
15	10 $\pm$ 4	8 $\pm$ 2
30	39 $\pm$ 9*	18 $\pm$ 11
100	52 $\pm$ 5 <sup>‡</sup>	33 $\pm$ 7
Rat anti-DNP (100 $\mu\text{g}/\text{mouse}$ )	10 $\pm$ 3	17 $\pm$ 4
Maximum elevation in HR		
	%	
Rat anti-IgE ( $\mu\text{g per mouse}$ )		
15	30 $\pm$ 5 <sup>‡</sup>	28 $\pm$ 7 <sup>‡</sup>
30	44 $\pm$ 12 <sup>‡</sup>	27 $\pm$ 7 <sup>‡</sup>
100	49 $\pm$ 6 <sup>‡</sup>	32 $\pm$ 5 <sup>‡</sup>
Rat anti-DNP (100 $\mu\text{g}/\text{mouse}$ )	-1 $\pm$ 1	-1 $\pm$ 2

Data are mean $\pm$ SEM for the maximum difference between baseline (100%) and postchallenge values (as a percentage of baseline) during the first 20 min after challenge.

\*  $P < 0.05$  and <sup>‡</sup> $P < 0.01$  when compared to values in the control (rat anti-DNP challenged) group by the Mann-Whitney U test (two-tailed).

In contrast to our findings in  $+/+$  mice, administration of rat anti-IgE to  $W/W^v$  mice was virtually without effect (Fig. 2 B). Sensitized  $W/W^v$  mice exhibited a slight tachycardia (Table III), which was significant when compared to the responses in either nonsensitized  $W/W^v$  mice challenged with rat anti-IgE (Table IV) or the responses in sensitized  $W/W^v$  mice challenged with rat anti-DNP (Table V). However, neither sensitized nor nonsensitized  $W/W^v$  mice exhibited significant changes in  $C_{dyn}$  or  $G_L$  (Tables III, IV, and V). None of the  $W/W^v$  mice challenged with rat anti-IgE died within 60 min of injection (Table VI).

*Mice challenged with goat IgG.* As would be expected for active anaphylaxis responses, sensitized  $+/+$  mice challenged

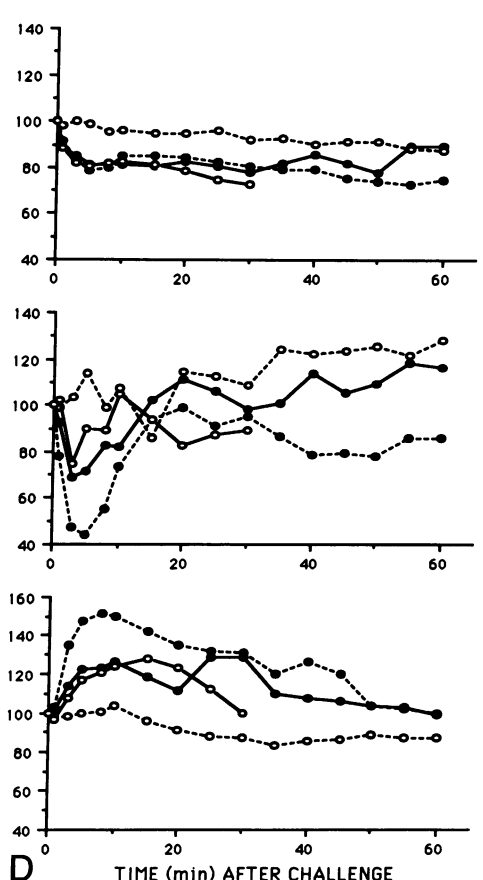
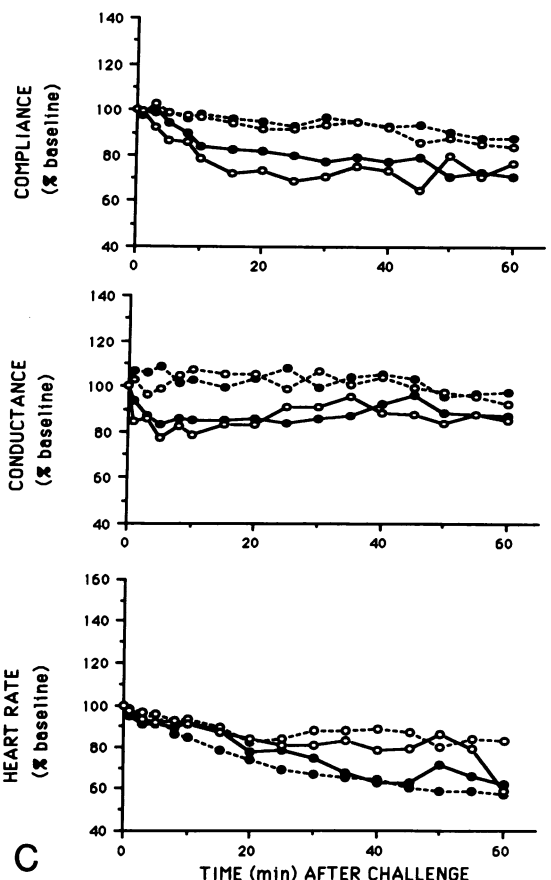
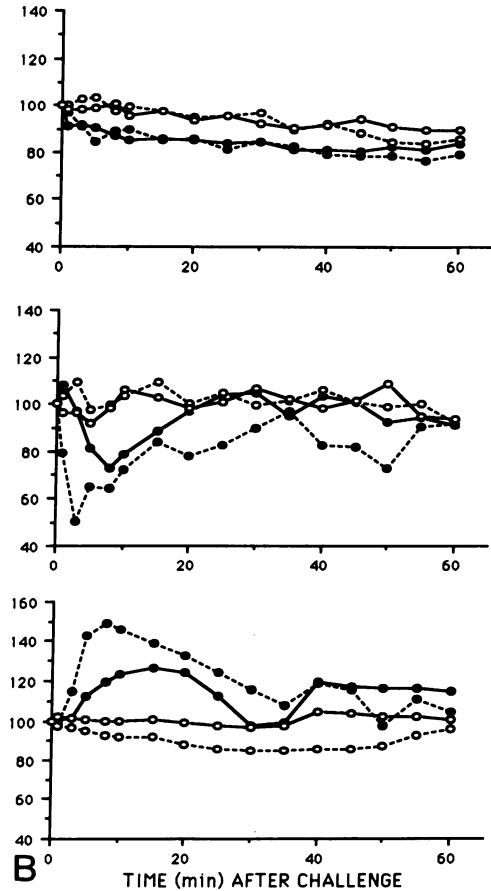
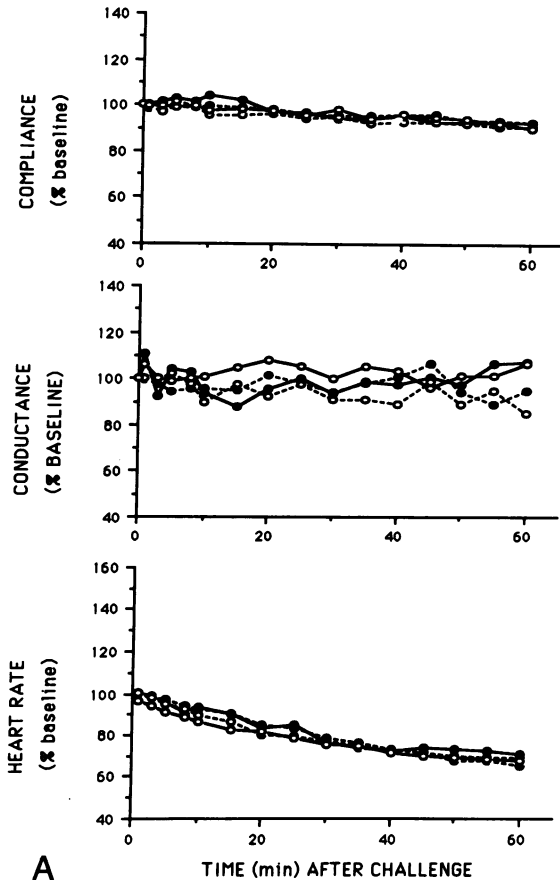
with goat IgG exhibited dramatic responses, whereas nonsensitized control  $+/+$  mice were unresponsive (Fig. 2 C). However, sensitized mast cell-deficient  $W/W^v$  mice also exhibited striking, and in some cases fatal, active anaphylactic responses (Fig. 2 C). Death occurred within 60 min of goat IgG challenge in three of five sensitized  $+/+$  mice and in two of five sensitized  $W/W^v$  mice, but in none of the nonsensitized mice (Table VI).

In sensitized  $+/+$  or  $W/W^v$  mice, goat IgG challenge produced diminutions in  $C_{dyn}$  and  $G_L$  that were similar to those observed in sensitized  $+/+$  mice challenged with rat anti-IgE (Table III). These responses were significant ( $P < 0.001$ ) when compared either to the corresponding values in mice challenged with rat anti-DNP (Table V) or to the values obtained in nonsensitized mice challenged with goat IgG (Table IV). However, the  $C_{dyn}$  and  $G_L$  responses in sensitized  $+/+$  mice challenged with goat IgG were statistically indistinguishable from those obtained in identically challenged sensitized  $W/W^v$  mice (Table IV).

An unexpected finding was that the HR responses differed dramatically in mice expressing rat anti-IgE-induced anaphylaxis as opposed to active anaphylaxis (compare Fig. 2, B and C). Sensitized WBB6F<sub>1</sub>- $+/+$  mice developed a striking tachycardia upon challenge with rat anti-IgE (Fig. 2 B), whereas the HR response in sensitized  $+/+$  mice challenged with goat IgG (i.e., a gradual diminution in HR, Fig. 2 C) was not significantly different from that observed in mice challenged with the control reagent, rat anti-DNP (Fig. 2 A and Tables III and V). The HR in nonsensitized mice challenged with goat IgG actually was slightly less than that in nonsensitized  $+/+$  mice challenged with rat anti-DNP (Table III). This small difference was significant ( $P < 0.01$ ) over the first 20 min (Table V) but not over the full 60 min after injection. The HR in nonsensitized  $+/+$  mice challenged with goat IgG was also slightly less than that in identically challenged nonsensitized  $W/W^v$  mice (Fig. 2 C and Table IV). The small differences in the HR responses of various groups of nonsensitized mice challenged with goat IgG, and the slightly higher value for  $G_L$  in goat IgG as opposed to rat anti-DNP challenged nonsensitized  $+/+$  mice (Tables III and V), are of uncertain biological significance.

Histological analysis showed that challenge of sensitized  $+/+$  mice with goat IgG resulted in activation of dermal mast cells which was significant when compared to findings in either nonsensitized goat IgG challenged or nonsensitized rat anti-DNP challenged groups ( $P < 0.001$  for either comparison, Fig. 3). However, dermal mast cell degranulation was not as marked as in sensitized or nonsensitized  $+/+$  mice challenged with rat anti-IgE (Fig. 3). When peribronchial mast cells of goat IgG challenged  $+/+$  mice were examined, small numbers of mast cells exhibiting changes consistent with slight activation were observed in both sensitized and nonsensitized groups

Figure 2.  $C_{dyn}$ ,  $G_L$ , and HR in genetically mast cell-deficient  $W/W^v$  mice (open circles) or congenic normal ( $+/+$ ) mice (solid circles) challenged by rapid intravenous infusion of (A) 100  $\mu\text{g}$  of rat anti-DNP (as a negative control), (B) 100  $\mu\text{g}$  of rat anti-mouse IgE, (C) 100  $\mu\text{g}$  of goat IgG, or (D) 100  $\mu\text{g}$  of goat anti-mouse IgE. Both mice sensitized by injection of goat anti-M $\delta$  8 d previously (solid lines) and mice not sensitized to goat anti-M $\delta$  (dashed lines) were tested, and the data are reported as described in the legend of Fig. 1 ( $n = 5$  mice per group at the start of the experiment). 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. The line for sensitized  $W/W^v$  mice challenged with goat anti-IgE (in D) ends because all five mice died within 35 min of challenge. 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  $\leq 25\%$  and usually  $< 10\%$  of the mean. Results of tests of the significance of differences among the various groups of mice are presented in Tables IV and V.



(Fig. 3). Again, these changes were much less striking than those observed in  $+/+$  mice challenged with rat anti-IgE. Examination of the tissues of  $W/W^v$  mice, in this as in all of the other experiments, revealed that all of the animals virtually lacked mast cells (none detected in the peribronchial tissues, < 1% the  $+/+$  value in the skin).

**Mice challenged with goat anti-IgE.** Based on our previous study (17), we expected that challenge of nonsensitized mice with goat anti-IgE would induce responses similar to those observed with rat anti-IgE challenge, i.e., dramatic responses in  $+/+$  mice but little or no responses in  $W/W^v$  mice. This is exactly what we found (Fig. 2, *B* vs. *D*, Tables III through VI).  $C_{dyn}$ ,  $G_L$ , and HR responses, as well as death rates and extent of mast cell degranulation (Fig. 3), were very similar in nonsensitized  $+/+$  mice challenged with rat or goat anti-IgE, whereas nonsensitized  $W/W^v$  mice were essentially unresponsive to either rat or goat anti-IgE.

Mice sensitized to goat anti-M $\delta$  would be expected to exhibit an active anaphylaxis response to i.v. injection of goat anti-IgE. However, in  $+/+$  mice, such active anaphylaxis would occur simultaneously with responses induced by anti-IgE-dependent activation of mast cell populations. Sensitized  $+/+$  mice challenged with goat anti-IgE exhibited significant diminutions in  $C_{dyn}$  or  $G_L$ , which were very similar to those observed in sensitized  $+/+$  mice challenged with either rat anti-IgE or goat IgG (Table III). However, these mice also developed a significant tachycardia which was virtually identical in magnitude to that seen in sensitized mice challenged with rat anti-IgE. By contrast,  $+/+$  mice experiencing active anaphylaxis to goat IgG did not develop tachycardia (Table III).

Even though death rates were identical in sensitized or nonsensitized  $+/+$  mice challenged with goat anti-IgE (Table VI), the nonsensitized mice developed a diminution in  $G_L$  and an elevation of HR which were significantly greater ( $P < 0.001$ ) than those observed in identically challenged sensitized mice (Fig. 2 *D* and Tables III and IV). Nonsensitized  $+/+$  mice also developed significantly more intense degranulation of dermal or peribronchial mast cells upon goat anti-IgE challenge than did the sensitized  $+/+$  animals (Fig. 3).

Sensitized  $W/W^v$  mice challenged with goat anti-IgE developed changes in  $C_{dyn}$  and  $G_L$  that were similar to those observed in sensitized  $W/W^v$  mice challenged with goat IgG (Tables III, IV, and V). However, unlike the  $W/W^v$  mice expressing active anaphylaxis to goat IgG, sensitized  $W/W^v$  mice challenged with goat anti-IgE developed a significant tachycardia (Fig. 2 *D*, Tables III through V). Indeed, whether judged by  $C_{dyn}$ ,  $G_L$ , HR, or death rate, the responses to goat anti-IgE challenge of sensitized  $W/W^v$  mice were statistically indistinguishable from those of sensitized  $+/+$  mice.

## Discussion

**Differences in the expression of different models of anaphylaxis in  $WBB6F_1-+/+$  mice.** We found that nonsensitized  $+/+$  mice challenged by i.v. infusion of rat monoclonal or goat polyclonal anti-mouse IgE antibodies rapidly developed tachycardia, as well as diminished  $C_{dyn}$  and  $G_L$ . The pattern of changes in HR,  $C_{dyn}$ , and  $G_L$  were similar to those reported previously (17). In nonsensitized  $+/+$  mice, injection of rat or goat anti-IgE produced nearly identical changes in dermal or peribronchial mast cell populations, with > 70% of dermal and > 80% of peribron-

chial mast cells exhibiting extensive degranulation. Sensitization of  $+/+$  mice with goat anti-M $\delta$  was associated with the development of serum levels of IgE and IgG $_1$ , which were ~ 32- and ~ 3-fold those in control mice injected with saline instead of goat anti-M $\delta$ . Even though the death rates of sensitized or nonsensitized  $+/+$  mice challenged with 100  $\mu$ g of rat or goat anti-IgE were similar, the extent of peribronchial mast cell degranulation was significantly less in sensitized  $+/+$  mice than in nonsensitized animals. Also, the  $G_L$  response in sensitized mice lacked the second phase of diminution observed in the nonsensitized animals.

The predominant mechanism by which rat anti-IgE challenge produces anaphylactic responses is probably by cross-linking Fc $\epsilon$ RI on the surface of mast cells and basophils, thereby inducing the release of bronchoactive and other mediators. It is possible that high plasma levels of IgE in sensitized  $+/+$  mice, by reacting with rat anti-IgE in the circulation, reduced the amount of anti-IgE available to enter the interstitial compartment and to react with IgE bound to mast cell Fc $\epsilon$ RI. This mechanism could explain both the reduced mast cell degranulation and the attenuated cardiopulmonary responses in sensitized as opposed to nonsensitized  $+/+$  mice challenged with rat anti-IgE. However, some  $WBB6F_1-+/+$  mice sensitized with goat anti-M $\delta$  died when challenged with 15 or 30  $\mu$ g of rat anti-IgE, whereas no fatalities occurred in similarly challenged control mice. Thus, sensitization resulted in a change in  $WBB6F_1-+/+$  mice which rendered them more susceptible than control mice to anti-IgE-induced fatal anaphylactic responses, even though they were less susceptible than controls to the effects of anti-IgE on mast cell activation and cardiopulmonary responses. We recently found that sensitization of mice with goat anti-M $\delta$  strikingly increases basophil levels (45), suggesting that anti-IgE-induced basophil activation may contribute to fatal anaphylactic responses in sensitized mice.

In contrast to responses induced by rat anti-IgE, active anaphylaxis to goat IgG challenge was not associated with tachycardia. In fact, HR responses in  $+/+$  mice exhibiting active anaphylaxis were statistically indistinguishable from those in  $+/+$  mice challenged with rat anti-DNP. In either instance, HR gradually and progressively declined throughout the period of observation. By contrast,  $+/+$  mice expressing active anaphylaxis did develop a significant reduction in  $C_{dyn}$ , which was similar in magnitude to that observed in nonsensitized  $+/+$  mice challenged with rat anti-IgE (to  $79 \pm 2\%$  vs.  $80 \pm 2\%$  of baseline in the first 20 min after challenge, respectively). They also developed a significant drop in  $G_L$ , although not as marked as that in nonsensitized  $+/+$  mice challenged with rat anti-IgE ( $75 \pm 5\%$  vs.  $48 \pm 5\%$  of baseline in the first 20 min after challenge, respectively,  $P < 0.008$ ). Active anaphylaxis was also associated with significantly less mast cell degranulation than was observed in either sensitized or nonsensitized  $+/+$  mice challenged with rat anti-IgE. Thus, in mice expressing active anaphylaxis to IgG,  $59 \pm 12\%$  of dermal mast cells and  $80 \pm 3\%$  of peribronchial mast cells exhibited no morphological evidence of degranulation, whereas these values were  $\leq 21 \pm 11\%$  in  $+/+$  mice challenged with rat anti-IgE (Fig. 3).

Even though active anaphylaxis to goat IgG was associated with less dramatic mast cell degranulation and less impressive cardiopulmonary changes than occurred in mice challenged with rat anti-IgE, the death rates in these two models of anaphylaxis were identical (three of five) and the times until death

Table III. Lowest Recordings of  $C_{dyn}$  and  $G_L$ , and Highest Recordings of HR, for Individual Sensitized or Nonsensitized Control Mice in the First 20 min after Challenge with 100  $\mu$ g of Rat or Goat Anti-IgE, Goat IgG, or Rat Anti-DNP

		Rat anti-DNP	Rat anti-IgE	Goat IgG	Goat anti-IgE
Lowest $C_{dyn}$					
+/+	C	96 $\pm$ 2	<u>80<math>\pm</math>2</u>	90 $\pm$ 3	<u>75<math>\pm</math>2</u>
+/+	S	96 $\pm$ 3	<u>81<math>\pm</math>4</u>	<u>79<math>\pm</math>2</u>	<u>75<math>\pm</math>4</u>
$W/W^o$	C	94 $\pm$ 2	93 $\pm$ 2	91 $\pm$ 3	92 $\pm$ 1
$W/W^o$	S	95 $\pm$ 2	92 $\pm$ 2	<u>68<math>\pm</math>10</u>	<u>75<math>\pm</math>2</u>
Lowest $G_L$					
+/+	C	90 $\pm$ 3	<u>48<math>\pm</math>5</u>	94 $\pm$ 6	<u>43<math>\pm</math>10</u>
+/+	S	83 $\pm$ 6	67 $\pm$ 7*	75 $\pm$ 5	66 $\pm$ 15
$W/W^o$	C	82 $\pm$ 4	92 $\pm$ 4	88 $\pm$ 7	96 $\pm$ 7
$W/W^o$	S	96 $\pm$ 2	85 $\pm$ 5	<u>69<math>\pm</math>7</u>	<u>75<math>\pm</math>4</u>
Highest HR					
+/+	C	99 $\pm$ 1	<u>149<math>\pm</math>6</u>	97 $\pm$ 5	<u>154<math>\pm</math>8</u>
+/+	S	99 $\pm$ 2	<u>132<math>\pm</math>5</u>	96 $\pm$ 4	<u>130<math>\pm</math>12</u>
$W/W^o$	C	98 $\pm$ 2	97 $\pm$ 2	98 $\pm$ 3	108 $\pm$ 4*
$W/W^o$	S	94 $\pm$ 2	<u>103<math>\pm</math>4</u>	95 $\pm$ 1	<u>131<math>\pm</math>10</u>

Data are mean $\pm$ SEM of lowest ( $C_{dyn}$  or  $G_L$ ) or highest (HR) values recorded for each mouse during the 20-min period immediately after challenge. Underlined values are significantly different than those for the corresponding group of rat anti-DNP challenged mice of the same genotype and sensitization status ( $P < 0.05$  to  $0.01$ , two-tailed Mann-Whitney U test). See Table V for statistical significance of comparisons between results for experimental groups and the control (rat anti-DNP treated) group over the entire 20-min period after challenge. Abbreviations: C, control; S, sensitized.

\*  $P < 0.05$  vs. the corresponding group of rat anti-DNP challenged mice of the same genotype and sensitization status by one-tailed Mann-Whitney U test.

in mice which succumbed before the end of the 60-min period of observation were very similar (Table VI). We do not know why rat anti-IgE-induced anaphylaxis was associated with a striking tachycardia whereas active anaphylaxis to goat IgG was not. However, we already have reported that in a different model of active anaphylaxis +/+ mice develop tachycardia whereas  $W/W^o$  mice do not (46). Taken together, our previous study (46) and the present one indicate that distinct models of active anaphylaxis may be associated with strikingly different cardiopulmonary responses.

WBB6F<sub>1</sub>-+/+ mice sensitized to goat anti-M $\delta$  and challenged with goat anti-mouse IgE expressed simultaneously an active anaphylactic response to the goat antibodies and an anti-IgE-dependent response. The changes in HR,  $C_{dyn}$ , and  $G_L$  in these mice were very similar to those recorded in sensitized +/+ mice challenged with rat anti-IgE (Table III) whereas the extent of mast cell degranulation was somewhat less (Fig. 3). Mice exhibiting a combined anti-IgE dependent and active anaphylaxis reaction differed from those experiencing a pure active anaphylactic response in that they expressed a significant tachycardia. The magnitude of this tachycardia was statistically indistinguishable from that observed in sensitized +/+ mice challenged with rat anti-mouse IgE to express a pure anti-IgE-dependent anaphylactic response.

WBB6F<sub>1</sub>- $W/W^o$  mice express active anaphylaxis but little or no response to challenge with anti-IgE. Genetically mast cell-deficient  $W/W^o$  mice expressed active anaphylaxis responses to goat IgG that were statistically indistinguishable from those in +/+ mice in terms of HR,  $C_{dyn}$ ,  $G_L$ , or death rate. Careful histological analysis confirmed that the tissues of the  $W/W^o$  mice remained virtually devoid of mast cells at the end of the experiment, ruling out the possibility that the sensitization protocol somehow resulted in the development of mast cell populations in these animals (42). This experiment thus confirmed, using the goat anti-M $\delta$  model of active anaphylaxis, results obtained by Jacoby et al. (20) and Ha et al. (21, 22), indicating that fatal active anaphylaxis responses can be expressed in the virtual absence of tissue mast cells. However, our findings extend the previous work by demonstrating that the mast cell-deficient mice exhibit pulmonary function changes associated with active anaphylaxis which are not distinguishable from those in normal mice containing mast cells. Thus, mast cells are not required for the development of the changes in pulmonary mechanics associated with at least some models of active anaphylaxis.

One explanation for the expression of active anaphylaxis in mast cell-deficient mice would be that the model of anaphylaxis tested occurred by mechanisms which failed to activate tissue mast cells in normal mice. However, this explanation was ruled out by our histological analysis, which indicated that +/+ mice expressing active anaphylaxis exhibited significant activation of dermal mast cell populations (Fig. 3). On the other hand, these +/+ mice exhibited only modest activation of peribronchial mast cell populations, a response which was significant when compared to that in sensitized +/+ mice challenged with the control reagent, rat anti-DNP, but which was not significant when compared to the findings in nonsensitized +/+ mice challenged with goat IgG (Fig. 3). If these results were to be considered in the absence of studies in mast cell-deficient mice, one might be tempted to attribute the pulmonary changes associated with active anaphylaxis in +/+ mice to the effects of mediators released into the circulation by dermal and perhaps other nonbronchial mast cell populations. However, the findings in  $W/W^o$  mice clearly show that diminished pulmonary compliance and conductance can occur during active anaphylaxis by mechanisms completely independent of contributions by mature mast cells.

Infusion of rat anti-mouse IgE into nonsensitized  $W/W^o$  mice had no discernable effect on HR,  $C_{dyn}$ , or  $G_L$ , findings consistent with those of our previous study (17). However, in  $W/W^o$  mice with high levels of IgE as a result of immunization with goat anti-M $\delta$ , injection of rat anti-mouse IgE produced a modest but highly significant tachycardia ( $P < 0.001$  vs. values in sensitized  $W/W^o$  mice challenge with rat anti-DNP). The tachycardia response in sensitized  $W/W^o$  mice challenged with goat anti-mouse IgE was even more striking. Indeed, it was statistically indistinguishable from the response in the corresponding group of +/+ mice (Table III). These findings, in contrast to those obtained in a different model of active anaphylaxis (46), indicate that mast cell-deficient  $W/W^o$  mice are fully capable of exhibiting significant tachycardia in response to appropriate immunological stimulation.

Differences in serum levels of IgE in WBB6F<sub>1</sub>- $W/W^o$  and congenic +/+ mice. In accord with previous reports (21), we found that serum IgE levels in sensitized  $W/W^o$  mice were



Table IV. Summary of Statistical Significance of Differences in Responses of +/+ vs. *W/W<sup>v</sup>* Mice, or Sensitized vs. Nonsensitized Control Mice, within Each of the Four Challenge Groups

	Rat anti-DNP	Rat anti-IgE	Goat IgG	Goat anti-IgE
<b>C<sub>dyn</sub></b>				
+/+ C vs. S	NS	NS	S ↓ vs. C <sup>§</sup>	NS
<i>W/W<sup>v</sup></i> C vs. S	NS	NS	S ↓ vs. C <sup>§</sup>	S ↓ vs. C <sup>§</sup>
C +/+ vs. <i>W/W<sup>v</sup></i>	NS	+/+ ↓ vs. <i>W/W<sup>v</sup></i> §	NS	+/+ ↓ vs. <i>W/W<sup>v</sup></i> §
S +/+ vs. <i>W/W<sup>v</sup></i>	NS	+/+ ↓ vs. <i>W/W<sup>v</sup></i> §	NS	NS
<b>G<sub>L</sub></b>				
+/+ C vs. S	NS	C ↓ vs. S <sup>§</sup>	S ↓ vs. C <sup>§</sup>	C ↓ vs. S <sup>§</sup>
<i>W/W<sup>v</sup></i> C vs. S	NS	NS	S ↓ vs. C <sup>§</sup>	S ↓ vs. C <sup>§</sup>
C +/+ vs. <i>W/W<sup>v</sup></i>	NS	+/+ ↓ vs. <i>W/W<sup>v</sup></i> §	NS	+/+ ↓ vs. <i>W/W<sup>v</sup></i> §
S +/+ vs. <i>W/W<sup>v</sup></i>	NS	+/+ ↓ vs. <i>W/W<sup>v</sup></i> *	NS	NS
<b>HR</b>				
+/+ C vs. S	NS	C ↑ vs. S <sup>§</sup>	NS	C ↑ vs. S <sup>§</sup>
<i>W/W<sup>v</sup></i> C vs. S	NS	S ↑ vs. C <sup>§</sup>	NS	S ↑ vs. C <sup>§</sup>
C +/+ vs. <i>W/W<sup>v</sup></i>	NS	+/+ ↑ vs. <i>W/W<sup>v</sup></i> §	+/+ ↓ vs. <i>W/W<sup>v</sup></i> §	+/+ ↑ vs. <i>W/W<sup>v</sup></i> §
S +/+ vs. <i>W/W<sup>v</sup></i>	NS	+/+ ↑ vs. <i>W/W<sup>v</sup></i> ‡	NS	NS

Results obtained during the first 20 min after challenge with 100 μg of each agent were compared by ANOVA (see text). Abbreviations: C, control; S, sensitized. \*  $P < 0.05$ , ‡  $P < 0.01$ , and §  $P < 0.001$ , respectively.

slightly higher than those in sensitized +/+ mice. However, we also found that IgE levels in nonsensitized *W/W<sup>v</sup>* mice were at least 60% higher than those in the congenic +/+ mice ( $0.16 \pm 0.02$  vs.  $0.10 \pm 0.02$  μg/ml,  $P < 0.011$ ). Sensitized or nonsensitized *W/W<sup>v</sup>* mice also had levels of IgG<sub>1</sub> that were somewhat higher than those of the congenic +/+ mice (Table I). However, these differences were not statistically significant. The high circulating levels of IgE in sensitized *W/W<sup>v</sup>* or +/+ mice may favor the participation of effector cells bearing Fc<sub>γ</sub>RII/CD23 in the expression of active anaphylaxis responses. However, the reason that *W/W<sup>v</sup>* mice develop higher levels of plasma IgE than do the congenic +/+ mice is not known. By binding IgE to surface Fc<sub>γ</sub>RI, populations of tissue mast cells may serve to limit the amount of IgE that gains access to or remains in the circulation. This effect would be most apparent at levels of IgE that are too low to saturate fully mast cell Fc<sub>γ</sub>RI. In accord with this idea, differences in the IgE levels in *W/W<sup>v</sup>* and +/+ mice in our experiments achieved statistical significance only in nonsensitized mice.

*Summary: the role of mast cells in the pathogenesis of the cardiopulmonary changes and death associated with various forms of anaphylaxis in mice.* In nonsensitized +/+ mice containing mast cells, challenge with either rat or goat anti-IgE produced extensive degranulation of dermal and peribronchial mast cell populations, tachycardia, diminished C<sub>dyn</sub> and G<sub>L</sub> and, in some cases, death. By contrast, injection of either rat or goat anti-IgE into nonsensitized mast cell-deficient *W/W<sup>v</sup>* mice was virtually without effect. These results support those of our previous study (17) in indicating that mast cells contribute significantly to the pathogenesis of anti-IgE-induced anaphylactic responses. However, in active anaphylactic responses to goat IgG, the pulmonary function changes and death rates associated with the reactions in *W/W<sup>v</sup>* mice were very similar to those in +/+ mice. This was true even though active anaphylaxis was associated with significant mast cell activation in +/+

mice. These findings indicate that even though mast cells are activated in +/+ mice expressing active anaphylaxis to goat IgG, mast cell degranulation is not required for either the diminished C<sub>dyn</sub> and G<sub>L</sub>, or the death, associated with these re-

Table V. Summary of Statistical Significance of Differences in +/+ or *W/W<sup>v</sup>* Sensitized or Nonsensitized Control Mice Challenged with Rat Anti-IgE, Goat IgG, or Goat Anti-IgE Compared to the Corresponding Group of Mice Challenged with the Control Reagent, Rat Anti-DNP

Challenge group	Rat anti-IgE	Goat IgG	Goat anti-IgE
<b>C<sub>dyn</sub></b>			
+/+ C	↓ <sup>§</sup>	NS	↓ <sup>§</sup>
+/+ S	↓ <sup>§</sup>	↓ <sup>§</sup>	↓ <sup>§</sup>
<i>W/W<sup>v</sup></i> C	NS	NS	NS
<i>W/W<sup>v</sup></i> S	NS	↓ <sup>§</sup>	↓ <sup>§</sup>
<b>G<sub>L</sub></b>			
+/+ C	↓ <sup>§</sup>	↑*	↓ <sup>§</sup>
+/+ S	↓ <sup>§</sup>	↓ <sup>§</sup>	NS
<i>W/W<sup>v</sup></i> C	NS	NS	NS
<i>W/W<sup>v</sup></i> S	NS	↓ <sup>§</sup>	↓ <sup>‡</sup>
<b>HR</b>			
+/+ C	↑ <sup>§</sup>	↓ <sup>‡</sup>	↑ <sup>§</sup>
+/+ S	↑ <sup>§</sup>	NS	↑ <sup>§</sup>
<i>W/W<sup>v</sup></i> C	NS	NS	↑ <sup>‡</sup>
<i>W/W<sup>v</sup></i> S	↑ <sup>§</sup>	NS	↑ <sup>§</sup>

Results obtained during the first 20 min after challenge with 100 μg of each agent were compared by ANOVA (see text). Abbreviations: C, control; S, sensitized.

\*  $P < 0.05$ , ‡  $P < 0.01$ , and §  $P < 0.001$ , respectively.

Table VI. Death Rates and Times until Death in +/+ or W/W<sup>o</sup> Mice Challenged with 100 μg of Rat Anti-DNP, Rat Anti-IgE, Goat IgG, or Goat anti-IgE

	Rat anti-DNP		Rat anti-IgE		Normal goat IgG		Goat anti-IgE	
	Not sensitized	Sensitized	Not sensitized	Sensitized	Not sensitized	Sensitized	Not sensitized	Sensitized
Death rate								
+/+	0/5	0/5	3/5	2/5	0/5	3/5	3/5	3/5
W/W <sup>o</sup>	0/5	0/5	0/5	0/5	0/5	2/5	0/5	5/5
Time until death (min)								
+/+	DNA	DNA	35, 40, 55	35, 40	DNA	35, 45, 50	40, 50, 60	25, 25, 35
W/W <sup>o</sup>	DNA	DNA	DNA	DNA	DNA	45, 60	DNA	20, 25, 30, 35, 35

sponses. Taken together, these findings indicate that even in anaphylactic responses associated with significant activation of mast cell populations, the importance of the mast cell in the

pathogenesis of the cardiopulmonary changes associated with specific examples of anaphylactic reactions can vary from critical to inconsequential.

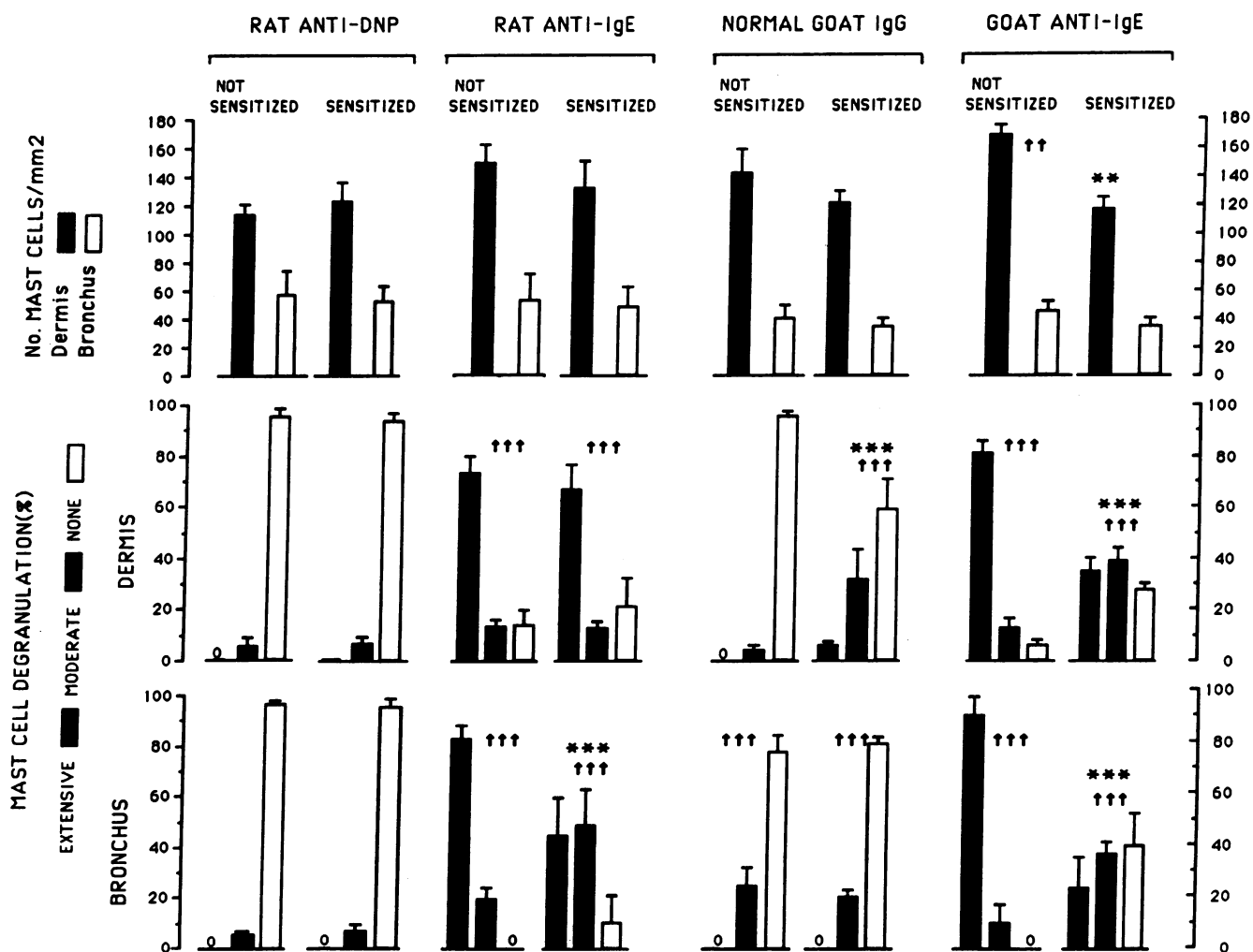


Figure 3. Number and extent of activation of cutaneous and peribronchial mast cells in WBB6F<sub>1</sub>-+/+ mice challenged with rat anti-DNP, rat anti-IgE, goat IgG, or goat anti-IgE. 1-μm-thick, Epon-embedded, Giemsa-stained sections of the ear pinnae or major bronchi were examined as described in the text to quantify the numbers of mast cells per square millimeter of dermis or peribronchial tissues, and 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. 2 and Table III. Results significantly different than those in the corresponding (i.e., sensitized or not sensitized) group of rat anti-DNP challenged mice are indicated as: †† *P* < 0.01; ††† *P* < 0.001. Results significantly different than those obtained in the not sensitized +/+ mice challenged with the same agent are indicated as: \*\**P* < 0.01; \*\*\**P* < 0.001.

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