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T R Martin, … , I M Katona, J M Drazen

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Research Article

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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

Thomas R. Martin,*‡ Stephen J. Galli,‡ Ildy M. Katona,§ and Jeffrey M. Drazen*‖

*Department of *Pediatrics, Children's Hospital (Ina Sue Perlmutter Laboratory), and Departments of ‡Pathology and ‖Medicine, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Massachusetts 02215; and §Department of Pediatrics and Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814*

Abstract

We used genetically mast cell-deficient WBB6F$_1$-*W/W^v* and WCB6F$_1$-*S1/S1^d* mice and the congenic normal (+/+) mice to investigate the effects of intravenous infusion of goat anti-mouse IgE on heart rate (HR), pulmonary dynamic compliance (C_{dyn}), pulmonary conductance (G_L), and survival. In WBB6F$_1$-+/+ and WCB6F$_1$-+/+ mice, anti-IgE induced extensive degranulation of tracheobronchial mast cells, as well as significant elevation of HR, significant reductions in C_{dyn} and G_L and, in some cases, death. In contrast, *W/W^v* and *S1/S1^d* mice exhibited little or no pathophysiological responses and no mortality after challenge with anti-IgE. In *W/W^v* mice reconstituted with mast cells by intravenous administration of bone marrow cells derived from congenic +/+ mice (+/+ BM → *W/W^v* mice), anti-IgE induced extensive mast cell degranulation, as well as pathophysiological responses and mortality similar to those observed in WBB6F$_1$-+/+ mice. These findings suggest a critical role for mast cells in the development of the cardiopulmonary changes and mortality associated with anti-IgE-induced anaphylaxis.

Introduction

The mast cell has long been considered a critical effector cell in mediating the pulmonary changes associated with anaphylaxis (1–4). Four major lines of evidence support such a role. First, IgE is clearly essential to the pathogenesis of many allergic reactions, including certain anaphylactic responses (5). Mast cells express surface receptors (FcεR),[1] which bind IgE with high affinity (6, 7), priming the cells to release a variety of mediators in response to challenge with specific antigen (1–7). Second, many of the mediators released from appropriately stimulated mast cells are bronchoactive (1–4, 8–10). Third, it is well established that pharmacologic agents capable of interfering with mast cell degranulation, or with the effects of "mast cell-derived" mediators, can inhibit anaphylactic responses (11–15). Fourth, histological studies document the presence of mast cells in the tracheobronchial tissues of man and a number of experimental animals (13–18). Thus, the reasons to believe that mast cells might have an important role in respiratory anaphylaxis seem compelling.

Yet the mere presence of mast cells, even in increased numbers, at sites of IgE-dependent pulmonary responses does not indicate that mast cells represent the source of mediators critical for these responses. A variety of cell types which can elaborate mediators similar or identical to those of mast cells (1–4, 19, 20), including basophils, monocytes/macrophages, eosinophils and platelets, also express receptors capable of binding IgE or aggregates of IgE (21). Moreover, "anaphylactic" syndromes leading to death from apparent cardiorespiratory collapse can be induced in genetically mast cell-deficient mice by appropriate sensitization procedures followed by challenge with specific antigen (22–24). On the other hand, anaphylactic reactions in both mast cell-deficient and normal mice have traditionally been assessed by clinical observations (prostration, death) rather than by actual measurements of pathophysiological responses (22–24). As a result, the extent of the similarities between the pulmonary component of the responses elicited in mice and the anaphylactic reactions of other experimental animals is unknown.

In the present study, we characterized the changes in pulmonary mechanics and heart rate associated with anti-IgE-induced anaphylactic reactions in mice, using an adaptation (25) of a standard technique for measuring pulmonary conductance (G_L) and dynamic compliance (C_{dyn}) in other small animals. In order to assess the contribution of mast cells in this model of anaphylaxis, we compared the effects of anti-IgE administered intravenously to two different types of genetically mast cell-deficient mice and the congenic normal (+/+) mice (26).

WBB6F$_1$-*W/W^v* (*W/W^v*) and WCB6F$_1$-*S1/S1^d* (*S1/S1^d*) mice exhibit a profound mast cell deficiency (26). In each mutant, the adult skin contains < 0.3% the number of mast cells of the congenic normal (+/+) mice, and no mast cells whatsoever are observed in any other organs including the gastrointestinal tract, heart, lungs, or peritoneal cavity (26). Although *W/W^v* and *S1/S1^d* mice share other phenotypic abnormalities (anemia, a lack of cutaneous melanocytes, sterility), the mast cell deficiencies of *W/W^v* and *S1/S1^d* mice are due to different mutations which produce mast cell deficiency

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Address reprint requests to Dr. Galli, Department of Pathology, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215.

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1. *Abbreviations used in this paper:* Anti-IgE, affinity purified goat anti-mouse IgE; anti-IgG, affinity purified goat anti-human IgG; C_{dyn}, pulmonary dynamic compliance; FcεR, surface receptors that bind IgE antibodies; G_L, pulmonary conductance; HR, heart rate; PEEP, positive end expiratory pressure.

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by distinct mechanisms (26). In W/W^v mice, intravenous transplantation of congenic +/+ bone marrow cells repairs both the mast cell deficiency and the anemia of the recipients (27), whereas bone marrow transplantation has no effect on the anemia or mast cell deficiency of Sl/Sl^d mice (28). To assess further the role of mast cells in anti-IgE-induced anaphylaxis, we therefore also studied W/W^v mice containing mast cell populations that were reconstituted by i.v. infusion of congenic +/+ bone marrow cells (+/+ BM → W/W^v mice).

Methods

Animals. We studied WBB6F$_1$-W/W^v (W/W^v) mast cell-deficient and congenic normal (+/+) mice [(WB/ReJ-W/+ × C57BL/6J-W^v/+)F$_1$-(W/W^v, +/+) mice] and WCB6F$_1$-Sl/Sl^d (Sl/Sl^d) mast cell-deficient and congenic normal (+/+) mice [(WC/ReJ-Sl/+ × C57BL/6J-Sl^d/+)F$_1$-(Sl/Sl^d, +/+) mice] (23–38 g body wt, 12–16 wk old) (Jackson Biological Laboratories, Bar Harbor, ME). We also examined W/W^v mice containing mast cell populations of +/+ origin. The reconstitution procedure has been previously published (27). In brief, femoral bone marrow cells were harvested from 12–16 wk old WBB6F$_1$-+/+ mice, filtered through gauze, washed twice, and resuspended in Dulbecco's minimal essential medium (Gibco Laboratories, Grand Island, NY) at 1.0×10^8 cells/ml and injected (2×10^7 cells in 0.2 ml/mouse i.v.) into WBB6F$_1$-W/W^v mice. These mice were used for physiological studies 12–15 wk later, an interval sufficient to permit population of the recipients' tissues with mast cells of donor origin (26, 27).

Materials. Propranolol (Sigma Chemical Co., St. Louis, MO) and pentobarbital sodium (Anthony Products Co., Arcadia, CA) were obtained commercially as noted. Affinity purified goat anti-mouse IgE (anti-IgE) was prepared as described for rabbit anti-mouse IgE (29). In brief, goats were immunized with monoclonal mouse IgE, their immune serum was absorbed with mouse serum-Sepharose, IgD$_k$-Sepharose, and IgM$_k$-Sepharose, then mixed with normal mouse serum overnight and centrifuged to remove soluble complexes. The supernatant was affinity-purified with IgE-Sepharose, eluted and dialyzed against 0.85 M NaCl. Affinity purified goat anti-human IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) was used as a control reagent.

Physiological measurements. Heart rate (HR) and the pulmonary mechanical parameters, dynamic compliance (C_{dyn}), and lung conductance (G_L) were measured using a modification (25) of a standard plethysmographic method for rats (30) and guinea pigs (31). Mice were anesthetized with 70–90 mg/kg i.p. of sodium pentobarbital, a 19-gauge tubing adapter was inserted as a tracheostomy tube, and a jugular vein was cannulated with a silastic catheter (0.06 cm OD) 6–8 cm in total length. Propranolol (4 mg/kg i.p.) was injected (25). The tracheostomy tube was attached to a fixed volume displacement ventilator (Harvard Apparatus model 651; Ealing Scientific, Natick, MA) set to deliver a tidal volume of 5–6 ml/kg at 150 breaths/min with 3–4 cm H_2O PEEP (positive end expiratory pressure). A 3 mm × 5 mm opening was made in the anterior chest wall to equalize pleural pressure and body surface pressure and the mouse was sealed in the plethysmograph chamber.

Plethysmograph pressure relative to a similar-sized copper gauze-filled glass bottle was measured with a variable reluctance pressure transducer (MP45; Validyne, Northridge, CA). Changes in lung volume were derived from recordings of plethysmograph pressure, flow was obtained by electronic differentiation of the volume signal, and transpulmonary pressure was recorded using a second pressure transducer (268B; Sanborn, Waltham, MA) connected between the proximal end of the tracheostomy tube and the plethysmograph. C_{dyn} and pulmonary resistance were calculated from the recordings of volume, flow, and pressure using standard techniques (32). The resistance of the tracheostomy tube was subtracted from the calculated resistance and the inverse of that difference was taken as G_L. HR was detected by monitoring the cardiac artifact in the transpulmonary pressure signal during brief periods of apnea. Baseline values of HR, C_{dyn}, and G_L were determined 20–30 min after preparation of the animals for recording pulmonary mechanics.

Histologic studies. The presence of tissue mast cells and their state of activation were assessed in 1 μm, Epon-embedded, Giemsa-stained sections (33, 34). Tissues removed either immediately after death induced by anti-IgE or after death of the mice, 60 min after anti-IgE challenge were fixed in 2.0% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% $CaCl_2$ in 0.1 M sodium cacodylate buffer, pH 7.3, at room temperature for 4–5 h. They were then washed in 0.1 M sodium cacodylate buffer, pH 7.3, and stored in the same buffer at 4°C until processing into 1 μm Epon-embedded, Giemsa-stained sections. Sections taken through the trachea or individual bronchi were coded so that the observer was not aware of the identity of individual specimens, and examined at ×400 by light microscopy. The number of mast cells/mm^2 of tissue in the area between the luminal surface and the peritracheal or peribronchial connective tissue was calculated as previously described (33). Mast cells were classified as "extensively degranulated" (> 50% of the cytoplasmic granules exhibiting fusion, staining alterations, and extrusion from the cell), "moderately degranulated" (10–50% of the granules exhibiting fusion or discharge), or "normal" (34).

Protocols. Mast cell-deficient and age-matched congenic normal (+/+) mice were studied during a 3-wk period. When stable values of HR, C_{dyn}, and G_L had been obtained, anti-IgE or anti-IgG were administered rapidly intravenously. The initial dose of anti-IgE was 500 μg/kg given in a volume of 1 ml/kg of normal saline; all mast cell-deficient mice were given a second dose of 1,650 μg/kg, also in a volume of 1 ml/kg, 60 min after the initial dose. Total fluid volume injected was always < 5 ml/kg, including catheter flushes. A similar protocol was used for the bone marrow-reconstituted W/W^v mice; the second dose of anti-IgE was administered to those mice that survived for 60 min after the first dose.

Statistical analysis. Differences among the various types of mice studied in the baseline values of HR, C_{dyn}, and G_L, and in numbers of mast cells/mm^2 of tissue, were examined for statistical significance by the Student's t test (two-tailed). Differences between baseline values of HR, C_{dyn}, and G_L and measurements of peak responses for individual mice measured during specified intervals after anti-IgE challenge within a single group of mice were examined by Student's paired t test (two-tailed). Differences between groups of mice in the time courses of HR, C_{dyn}, and G_L responses were examined for statistical significance by analysis of variance. Differences in the extent of mast cell degranulation in various groups of mice were examined for statistical significance by the X^2 test (two-tailed). $P < 0.05$ was regarded as significant. Unless otherwise specified, results are expressed as the mean±SD.

Results

Cardiopulmonary responses to anti-IgE. For each of the five groups of propranolol pretreated mice studied (WBB6F$_1$-+/+ controls, WBB6F$_1$-W/W^v mutants, +/+ BM → W/W^v mast cell-reconstituted mutants, WCB6F$_1$-+/+ controls, and WCB6F$_1$-Sl/Sl^d mutants), we first performed baseline measurements of HR, C_{dyn}, and G_L. There were no significant differences among the values obtained for the five different groups (Table I).

We then tested the responses of these mice to intravenous challenge with anti-IgE. The time courses of responses of WBB6F$_1$-+/+ and WCB6F$_1$-+/+ normal mice to infusion of 500 μg/kg anti-IgE are shown in Fig. 1–3 for HR, C_{dyn}, and G_L, respectively. WBB6F$_1$-+/+ and WCB6F$_1$-+/+ mice gave similar physiological responses to anti-IgE. Despite propranolol treatment, anti-IgE infusion resulted in an increase in HR that occurred over the first 10 min after infusion and remained

Table I. Baseline Values of HR, C_{dyn}, and G_L in Mice Later Treated with Anti-Mouse IgE*

Mice	HR	C_{dyn}	G_L
	(beats/min)	*(ml·cm H_2O^{-1})*	*(ml·s^{-1}·cm H_2O^{-1})*
$WBB6F_1$-+/+	179±27	0.030±0.006	1.60±0.79
$WBB6F_1$-*W/W^V*	142±22	0.031±0.004	1.50±0.44
+/+ BM → *W/W^V*	180±48	0.035±0.003	1.19±0.32
$WCB6F_1$-+/+	194±72	0.033±0.004	1.34±0.35
$WCB6F_1$-*Sl/Sl^d*	151±19	0.030±0.006	1.47±0.39

* Values are mean±SD (n = 6 to 7/group).

significantly ($P < 0.001$) elevated compared to initial baseline values for the duration of the observation period. Over the period from 10 to 30 min after anti-IgE infusion, mean HR in the $WBB6F_1$-+/+ and $WCB6F_1$-+/+ mice was 284±28 (mean±SD) and 278±32 beats/min, respectively. Dynamic compliance decreased steadily after the infusion of anti-IgE, dropping an average of 12±8% below preinfusion values by 10 min in the $WBB6F_1$-+/+ animals ($P < 0.02$ compared to baseline) and an average of 15±13% below preinfusion values at 8 min after challenge in the $WCB6F_1$-+/+ mice ($P < 0.05$). C_{dyn} continued to diminish during later intervals after anti-IgE challenge, achieving maximal changes compared to baseline in individual mice at 20–40 min (27±4% decreased compared to baseline in $WBB6F_1$-+/+ mice and 28±10% decreased in $WCB6F_1$-+/+ mice, $P < 0.0001$ in both cases).

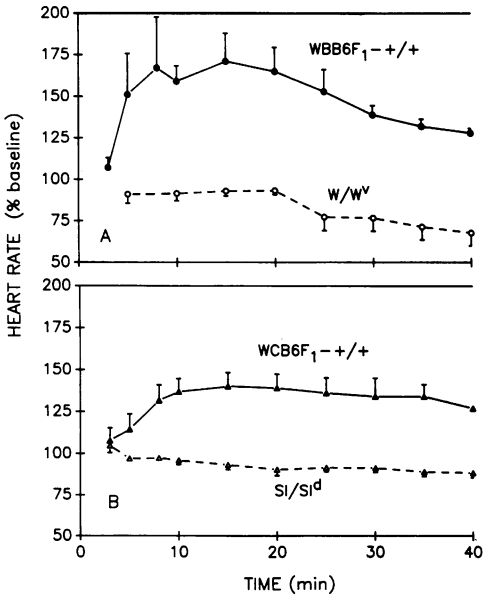

Figure 1. Heart rate measurements in (*A*) mast cell-deficient $WBB6F_1$-*W/W^v* and congenic normal (+/+) mice and (*B*) mast cell-deficient $WCB6F_1$-*Sl/Sl^d* and congenic normal (+/+) mice after infusion of anti-IgE (500 μg/kg i.v.). After recording baseline values for a period of 20–30 min, anti-IgE was administered at time 0. For each mouse, results obtained at various intervals after challenge were normalized by expressing them as percent of the mean baseline measurement. The results for each genotype are expressed as mean ±SEM (n = 6 to 7/group).

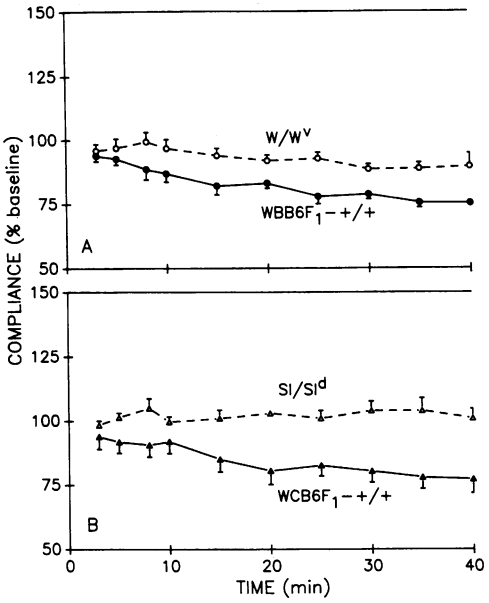

Figure 2. Pulmonary dynamic compliance in (*A*) mast cell-deficient $WBB6F_1$-*W/W^v* and congenic normal (+/+) mice and (*B*) mast cell-deficient $WCB6F_1$-*Sl/Sl^d* and congenic normal (+/+) mice after infusion of anti-IgE (500 μg/kg i.v.). The challenge with anti-IgE was conducted as described in legend to Fig. 1, and the results are expressed as mean±SEM (n = 6 to 7/group).

At the time of peak response (determined for each mouse 3–15 min after challenge), the differences between baseline and postchallenge G_L were significant ($P < 0.05$) for either group of normal (+/+) mice. 15 min after anti-IgE infusion, G_L had returned nearly to pre-challenge values in both $WBB6F_1$-+/+ and $WCB6F_1$-+/+ mice. In both the $WBB6F_1$-

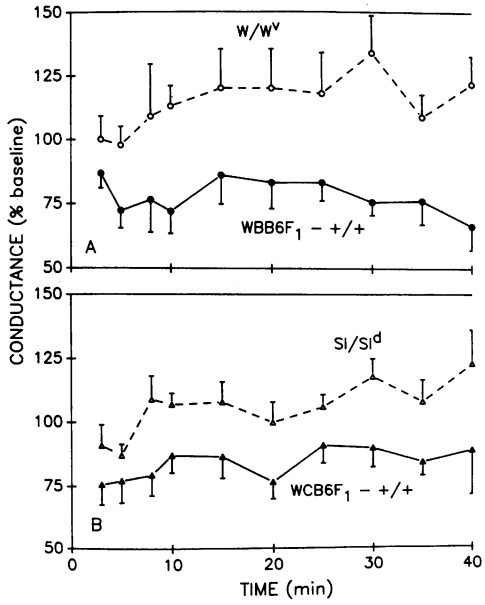

Figure 3. Pulmonary conductance in (*A*) mast cell-deficient $WBB6F_1$-*W/W^v* and congenic normal (+/+) mice and (*B*) mast cell-deficient $WCB6F_1$-*Sl/Sl^d* and congenic normal (+/+) mice after infusion of anti-IgE (500 μg/kg i.v.). The challenge with anti-IgE was conducted as described in legend to Fig. 1, and the results are expressed as mean±SEM (n = 6 to 7/group).

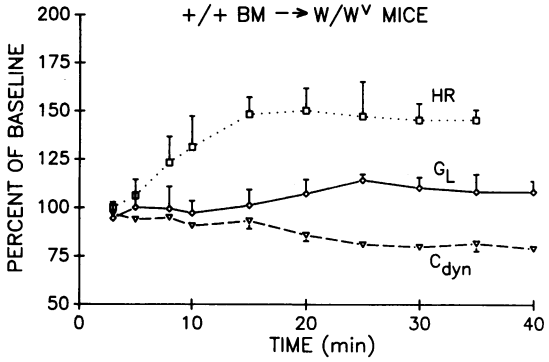

Figure 4. Heart rate (HR), pulmonary dynamic compliance (C_{dyn}) and pulmonary conductance (G_L) after infusion of anti-IgE (500 μg/kg i.v.) in +/+ BM → *W/W^v* mice. 12–15 wk before anti-IgE challenge, the WBB6F$_1$-*W/W^v* mice were given intravenously 2 × 10^7 bone marrow cells derived from the congenic +/+ mice, in order to reconstitute mast cell populations in the *W/W^v* recipients (see Methods for details). The challenge with anti-IgE was conducted as described in legend to Fig. 1, and the results are expressed as mean ±SEM (n = 6).

+/+ and the WCB6F$_1$-+/+ mice, there was a second significant ($P < 0.05$) decrease in G_L compared with baseline in the period 20–40 min after challenge (Fig. 3).

The time courses of responses of the mast cell-deficient *W/W^v* and *S1/S1^d* mice to infusion of 500 μg/kg of anti-IgE are shown in Fig. 1–3 for HR, C_{dyn}, and G_L, respectively. In both *W/W^v* and *S1/S1^d* mice, anti-IgE infusion was followed by small decreases in HR compared to preinfusion values. Anti-IgE infusion was followed by a small but statistically significant decline in C_{dyn} during the period 20–40 min after challenge in *W/W^v* mice, with maximal declines in C_{dyn} in individual mice averaging 15±4% ($P < 0.05$ compared to baseline values). However, these mice exhibited no significant change in G_L. In contrast, the *S1/S1^d* mice challenged with anti-IgE exhibited no significant change in C_{dyn}, but expressed a small transient decrease in G_L which achieved statistical significance 3–8 min after challenge (average maximal decline = 18±14%, $P < 0.05$ compared to baseline values). When an additional higher dose of anti-IgE (1,650 μg/kg) was administered to *W/W^v* or *S1/S1^d* mice, no further significant responses in any of the physiological indices monitored were noted.

In addition to comparing postchallenge values with baseline measurements for each group of mice, we also compared responses of mast cell-deficient mutants with those of the congenic normal (+/+) mice. Analysis of variance revealed significant differences ($P < 0.0001$) between the responses of mast cell-deficient mice and the congenic +/+ mice for each parameter measured.

The time courses of responses of mast cell-reconstituted *W/W^v* mice challenged with anti-IgE are shown in Fig. 4. Bone marrow reconstitution restored the HR response to anti-IgE; by 15 min there was a 48% increase in HR compared to preinfusion values ($P < 0.001$). By analysis of variance, the HR response of the mast cell-reconstituted mice was not significantly different from that of the WBB6F$_1$-+/+ mice. Bone marrow reconstitution also resulted in a restoration of the C_{dyn} response, such that there was no significant difference between the responses of +/+ and bone marrow-reconstituted *W/W^v* mice ($P > 0.1$). By contrast, anti-IgE treated +/+ BM → *W/W^v* mice exhibited a G_L response that appeared intermediate between that of *W/W^v* and congenic +/+ mice. Analysis of variance confirmed that the G_L responses of *W/W^v*, +/+, and +/+ BM → *W/W^v* mice were all different (*W/W^v* vs +/+ BM → *W/W^v*, $P = 0.017$, +/+ vs *W/W^v* or +/+ BM → *W/W^v*, $P < 0.0001$).

Mortality following anti-IgE challenge. Of the normal mice, three of six WBB6F$_1$-+/+ mice and five of seven WCB6F$_1$-+/+ mice died within 60 min after infusion of 500 μg/kg of anti-IgE. In contrast, none of the six *W/W^v* or seven *S1/S1^d* mast cell-deficient mice died within 60 min after the initial 500-μg/kg dose of anti-IgE, or even within 60 min of a subsequent 1,650-μg/kg dose of anti-IgE. Of the seven +/+ → BM *W/W^v* mice tested, two died within 60 min after the initial dose of anti-IgE and one additional mouse died 10 min after the administration of the subsequent 1,650-μg/kg dose.

Histologic studies of anti-IgE-challenged mice. Histologic examination of tracheobronchial tissues of the +/+ mice challenged with anti-IgE (Fig. 5, *A* and *B*) exhibited marked degranulation of most of the mast cells present. Many of the mast cells in the tracheobronchial tissues of the +/+ bone marrow-reconstituted *W/W^v* mice also exhibited degranulation (Fig. 5, *C* and *D*). There were no significant differences between WBB6F$_1$-+/+, +/+ BM → *W/W^v*, and WCB6F$_1$-+/+ mice in the numbers of tracheal and peribronchial mast cells (22±12, 23±11, and 14±12 mast cells/mm^2 of tissue, respectively), or in the extent of degranulation of these cells (% extensively degranulated, moderately degranulated, and normal mast cells were 95, 3, and 2%, respectively, for WBB6F$_1$-+/+ mice; 85, 12, and 3%, respectively, for +/+ BM → *W/W^v* mice; and 94, 6, and 0%, respectively, for WCB6F$_1$-+/+ mice). Representative sections of the tracheobronchial tissues of mast cell-deficient mice confirmed the many previous studies indicating that these tissues are devoid of mast cells.

Findings in control mice challenged with anti-human IgG. Control groups of WBB6F$_1$-+/+ (n = 4), WBB6F$_1$-*W/W^v* (n = 5), and +/+ BM → *W/W^v* (n = 3) mice were pretreated with propranolol (4 mg/kg i.p., as above) and then challenged with goat anti-human IgG (500 μg/kg i.v.). HR, C_{dyn}, and G_L were recorded during the 40 min after challenge (Table II), survival was assessed, and then the mice were killed for histological determination of the numbers and extent of degranulation of tracheobronchial mast cells.

All of the mice survived anti-IgG challenge. Compared to baseline prechallenge values, each of the three groups exhibited a modest bradycardia throughout the period of observation, as well as a modest decline in C_{dyn} during the period from 20 to 40 minutes after challenge (Table II). G_L was stable in the *W/W^v* and +/+ mice, but declined slightly in the +/+ BM → *W/W^v* mice. The HR, C_{dyn}, or G_L responses of anti-IgE treated +/+ mice (Fig. 1–3) were significantly different than the corresponding values in anti-IgG treated +/+ mice (Table II) at $P < 0.0001$, $P < 0.0001$, or $P < 0.03$, respectively. By contrast, anti-IgE treated *W/W^v* mice (Fig. 1–3) exhibited patterns of HR or C_{dyn} not significantly different than those of anti-IgG treated *W/W^v* mice, and exhibited values of G_L which were slightly greater than those of the anti-IgG treated animals. Anti-IgE treated +/+ BM → *W/W^v* mice (Fig. 4) exhibited significant tachycardia ($P < 0.0001$) and significantly diminished C_{dyn} ($P < 0.03$) compared to anti-IgG treated +/+ BM → *W/W^v* mice, whereas the G_L of the +/+ BM → *W/W^v* mice

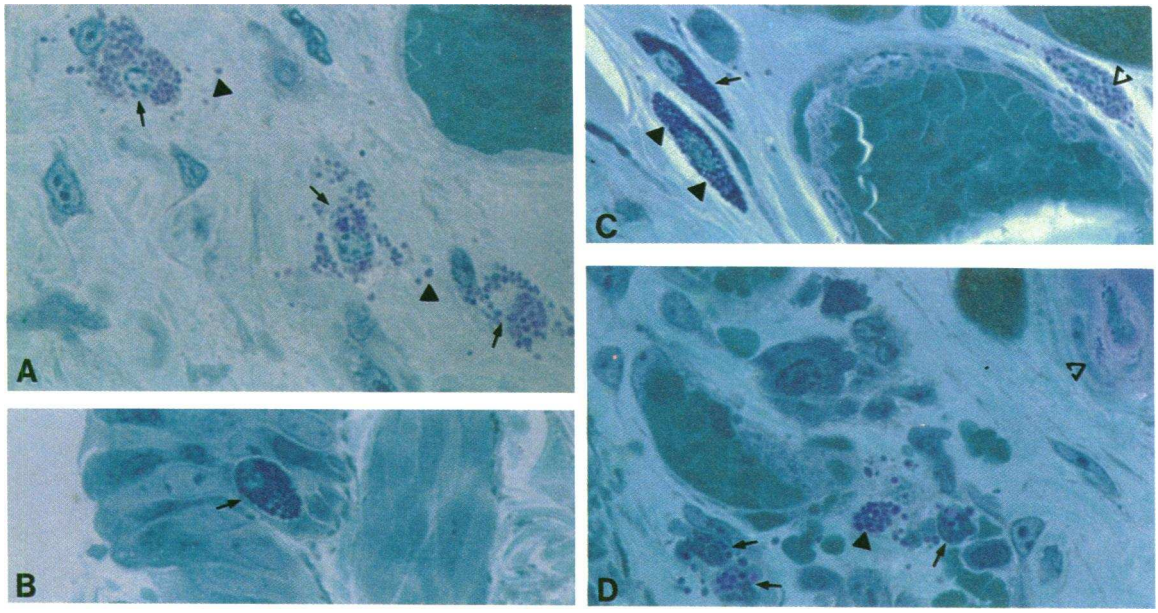

Figure 5. Histology of tracheobronchial tissues from (*A* and *B*) an anti-IgE-challenged $WBB6F_1$-+/+ mouse and (*C* and *D*) an anti-IgE-challenged +/+ BM → W/W^v mouse (a genetically mast cell-deficient $WBB6F_1$-W/W^v mouse whose mast cell deficiency was repaired by intravenous transplantation of bone marrow cells derived from $WBB6F_1$-+/+ mice, see text). (*A*) Three mast cells (*solid arrows*) in the lamina propria of the posterior wall of the trachea. All three cells are extensively degranulated, with > 50% of the granules exhibiting staining changes and/or extrusion (*solid arrowheads*) from the cell. ×900. (*B*) A single mast cell (*solid arrow*) within the mucosa of a bronchus. This cell exhibits no evidence of activation. ×900. (*C*) Three mast cells in peritracheal connective tissue. One "normal" cell exhibits only a few granules with altered staining characteristics (*solid arrow*), one moderately degranulated cell exhibits altered staining of many granules (*solid arrowheads*) and one cell (*open arrowhead*) is extensively degranulated, with staining alterations involving all of the granules. ×900. (*D*) Tracheal lamina propria containing an extensively degranulated mast cell (*solid arrowhead*), and three neutrophils which have phagocytosed mast cell granules (*solid arrows*). The open arrowhead indicates the edge of a cartilage plate. ×900. A-D are 1 μm, Epon-embedded, Giemsa-stained sections.

treated with anti-IgE was not significantly less than that recorded in the anti-IgG treated animals.

Histological analysis of the tissues of anti-IgG challenged mice confirmed that the trachea and lungs of W/W^v mice lacked detectable mast cells, and that bone marrow reconstitution succeeded in establishing tracheal and pulmonary mast cell populations in +/+ BM → W/W^v mice. The number of tracheal and peribronchial mast cells in +/+ or +/+ BM → W/W^v mice treated with anti-IgG was 20±12 or 28 ±16/mm², respectively. However, these mice exhibited little or no degranulation of tracheobronchial mast cells (15% extensively degranulated in +/+ mice and 0% extensively degranulated in +/+ BM → W/W^v mice treated with anti-IgG, vs. 95% and 85%, respectively, for anti-IgE treated mice; $P < 0.0001$ for either comparison).

*Table II. Values of HR, C_{dyn}, and G_L in Control Mice Treated with Anti-human IgG**

	$WBB6F_1$+/+	$WBB6F_1$-W/W^v	+/+ BM → W/W^v
HR			
Early	93.0±5.4	90.6±4.7	86.4±6.8
Late	86.9±8.1	84.9±7.7	81.1±12.7
C_{dyn}			
Early	95.7±9.7	98.3±7.5	98.7±7.6
Late	84.1±14.0	86.8±10.2	90.2±4.3
G_L			
Early	99.0±9.4	104.0±13.0	87.1±15.4
Late	98.7±14.2	102.1±25.4	94.9±13.9

* Values are mean±SD (n = 3 to 4/group) for each parameter, expressed as percent of the baseline values. "Early" refers to the period from 3 to 15 min after injection of anti-IgG; "late" refers to the period from 20 to 40 min after anti-IgG injection.

Discussion

This study demonstrates that the development of specific cardiopulmonary consequences of anti-IgE infusion in mice is closely associated with the presence of mast cells, and with the occurrence of mast cell degranulation. Naive $WBB6F_1$-+/+ and $WCB6F_1$-+/+ mast cell-sufficient mice developed increases in HR and decreases in C_{dyn} and G_L when challenged intravenously with 500 μg/kg of anti-IgE; furthermore, 8 of 13 anti-IgE-treated +/+ mice died within 40 min of challenge. All +/+ mice challenged with anti-IgE exhibited extensive degranulation of tracheobronchial mast cells. In contrast to their normal littermates, W/W^v or Sl/Sl^d mast cell-deficient mice developed little or no pathophysiological responses after infusion of 500 or 1,650 μg/kg i.v. of anti-IgE. Moreover, none of the 13 mast cell-deficient mice challenged with 500 and then 1,650 μg/kg of anti-IgE died.

Before this study, the changes associated with various models of anaphylaxis in mice had been characterized using descriptive terms. However, it was impossible to compare the pulmonary mechanical manifestations of anaphylactic reactions in mice to the much better characterized responses of

larger animals (35–37), since no physiological measurements of the murine responses were available. It is well established that administration of antigen to a wide variety of animals sensitized to express anaphylaxis, including rats, rabbits, ferrets, dogs, sheep, and horses, results in significant decrements in C_{dyn} and in G_L (35–37). Our data clearly demonstrate that anti-IgE-induced murine anaphylaxis is accompanied by pulmonary mechanical abnormalities that are similar in magnitude and kinetics to those observed in other species after anaphylactic challenge.

Despite the similarities between the pulmonary changes induced by anti-IgE challenge of naive mice and those observed in other species during antigen-induced anaphylaxis, the pathogenesis of these responses may not be identical. However, there were several reasons why we chose to examine, as our initial model of mouse anaphylaxis, anti-IgE challenged immunologically naive mice rather than actively or passively immunized mice challenged with specific antigen. First, anti-IgE had been demonstrated to cause pulmonary mechanical changes in the rat (38), a species whose pattern of responsiveness to bronchoconstrictor agonists (e.g., serotonin) is similar to that of the mouse (25). Second, anti-IgE challenge of naive mice is experimentally straightforward in comparison to antigen challenge of sensitized mice. Responses elicited in individual mice using the latter approach are susceptible to several potential sources of biological variability, including those related to differences in the primary antibody responses of individual mice immunized with the same dose of antigen (in active anaphylaxis), and individual variation in the extent of sensitization of mast cells and other effectors by adoptively transferred serum (in passive systems). Third, anti-IgE represents a more specific mechanism than antigen challenge for initiating exclusively IgE-dependent processes, at least in actively immunized mice. Finally, two groups already had reported that fatal anaphylaxis could be elicited in mast cell-deficient mice challenged with specific antigen after active (22, 23) or passive (24) sensitization, and we were searching for an approach to reveal potentially mast cell-dependent differences in the anaphylactic responsiveness of mast cell-deficient and normal mice.

Before challenging our mice with anti-IgE, we treated them with propranolol. Such treatment is standard procedure in assessing the effects of bronchoactive agonists because the drug maximizes the induced bronchoconstriction by preventing β-adrenergic bronchodilation due to endogenous epinephrine release. In addition to this effect on airway smooth muscle, it is possible that propranolol also altered the extent of anti-IgE-induced mast cell degranulation. Adrenergic agonists have been demonstrated to decrease anaphylactic histamine release from guinea pig lungs in vitro (39) and from dispersed human lung mast cells challenged with anti-IgE (40). Propranolol has been shown to cause a small amount of histamine release (< 25% of total cell-associated histamine) from rat pleural and peritoneal mast cells in vitro (41), although only at drug concentrations that were more than five times the maximum tissue concentrations which may have developed in our mice.

While the findings described in (39–41) are of interest, they were not obtained using mouse mast cells. As a result, the relevance of these observations to our own study is uncertain. Unfortunately, information about the effects of propranolol on mouse mast cells is quite limited. Guirgis and Townley reported that the direct injection of propranolol, at 0.3 or 1.0 mg/ml, into the skin of mice caused augmented vascular permeability, as assessed by a dye extravasation assay (42). Although these authors did not search for mast cell degranulation at sites of propranolol injection, their findings are consistent with the possibility that high doses of propanolol can induce cutaneous mast cell degranulation in mice.

Taken together, the studies reviewed above (39–42) raise the possibility that treatment with propranolol might have augmented anti-IgE-induced effects on mast cells in our experiments. However, our histological examination of the tissues of control +/+ or +/+ BM → W/W^v mice pretreated with propranolol before anti-IgG challenge did not identify extensive mast cell degranulation: no degranulation of cutaneous mast cells was observed and the small amount of degranulation of tracheopulmonary mast cells documented (0–15%) may have reflected the effects of instrumentation before challenge and/or damage to tissues upon their removal after sacrifice.

In addition to effects on airway smooth muscle and possible effects on mast cells, β-adrenergic blockade with propranolol would be expected to interfere with chronotropic effects of epinephrine released during anaphylaxis. In spite of this, $WBB6F_1$-+/+, +/+ BM → W/W^v, and $WCB6F_1$-+/+ mice all developed tachycardia after anti-IgE challenge. This response presumably reflected either massive epinephrine release sufficient to overcome beta blockade, and/or IgE-dependent chronotropic mechanisms other than epinephrine. A less likely explanation for the tachycardia observed in anti-IgE treated normal mice is that the mast cell-deficient mutants absorb propranolol more rapidly or metabolize it more slowly than the congenic normal mice. Whatever the explanation for the difference in the HR responses of mast cell-deficient and normal mice, the abnormality of the response in W/W^v mice was abolished by reconstitution of the mutant animals with congenic +/+ bone marrow cells.

Our data not only establish that intravenous infusion of anti-IgE results in cardiopulmonary mechanical abnormalities in normal mice, they also provide strong evidence that these responses require the presence of mast cells. We demonstrated in two different types of genetically mast cell-deficient mice that anti-IgE induced significantly smaller pulmonary mechanical responses than those of the congenic +/+ mice and did not cause death. Indeed, the changes in HR, C_{dyn}, or G_L observed in anti-IgE-treated W/W^v mice were not statistically different than those seen in control W/W^v mice treated with anti-human IgG. W/W^v and $S1/S1^d$ mice express profound mast cell deficiencies because of the effects of distinct mutations which produce mast cell deficiency by different mechanisms (26, 43, 44). On the other hand, except as influenced by their mast cell abnormalities, the immune responses of W/W^v and $S1/S1^d$ mice are remarkably similar to those of the congenic normal (+/+) mice (26). Thus, the mutants exhibit a normal ability to synthesize specific IgM and IgG upon exposure to antigen (23), develop serum IgE concentrations after appropriate antigenic stimulation which can be even higher than those of the congenic +/+ mice (23), and express T cell-dependent, delayed-type hypersensitivity reactions (23, 45–47) and immunological tolerance responses (46) which are not significantly different than those of their normal littermates. W/W^v and $S1/S1^d$ mice also have normal numbers of blood granulocytes and platelets (26) and, while mice have very low numbers of circulating granulocytes with the morphology of

basophils (48, 49), Jacoby et al. reported that similar numbers of circulating basophils were present in W/W^v and congenic +/+ mice (22).

Despite these similarities between mast cell-deficient and congenic normal mice, the complete pattern of phenotypic abnormalities associated with the mutations in W/W^v and Sl/Sl^d mice have not yet been defined (26, 43, 44). It is therefore theoretically possible that these mutations might influence pulmonary responsiveness by mechanisms independent of the mast cell and other bone marrow-derived cells. To compare the effects of bone marrow-dependent and -independent consequences of the W/W^v mutations on pulmonary responsiveness to anti-IgE challenge, we analyzed the responses of W/W^v mice which had undergone repair of their mast cell deficiency by intravenous transplantation of bone marrow cells derived from the congenic +/+ mice (+/+ BM → W/W^v mice). In +/+ BM → W/W^v mice, anti-IgE challenge induced extensive degranulation of the adoptively transferred mast cell population, and produced changes in HR and C_{dyn} which were virtually identical to those observed in similarly challenged congenic +/+ mice (compare Fig. 4 with Figs. 1 and 2).

The findings in +/+ BM → W/W^v mice greatly strengthen the evidence that the inability of W/W^v mice to express the changes in HR and C_{dyn} associated with anti-IgE-induced anaphylaxis is due to the mast cell deficiency of these animals, rather than to the consequences of other effects of the W/W^v mutations. It should be noted, however, that this experiment cannot distinguish between effects due to correction of the mutants' mast cell deficiency and other consequences of bone marrow transplantation (26). Thus, it remains possible that the differences between the anti-IgE-induced responses of W/W^v and +/+ BM → W/W^v mice were influenced by the actions of bone marrow-derived elements other than (or in addition to) the mast cell.

We were interested to note that the G_L response in anti-IgE treated +/+ BM → W/W^v mice, although significantly greater than that of W/W^v animals, was also significantly less than that of the congenic +/+ mice. The reason bone marrow reconstitution of W/W^v mice did not fully "normalize" the G_L response to anti-IgE is not clear. Although +/+ BM → W/W^v mice were similar to +/+ mice in numbers and extent of degranulation of tracheobronchial mast cells, it is possible that the adoptively transferred mast cell population in +/+ BM → W/W^v mice differed from that in native +/+ mice in the types or amounts of mediators elaborated in response to anti-IgE, or in precise anatomical relationships with important targets of mediator action, such as bronchial smooth muscle. Another possibility is that the abnormality of the G_L response was influenced by a genetically determined, but mast cell- and bone marrow-independent, defect in the W/W^v animals.

Unlike the W/W^v mutants, the Sl/Sl^d mutants cannot be repaired of their anemia or mast cell deficiency by bone marrow transplantation from the congenic normal mice (26–28). The difference in their response to bone marrow-reconstitution represents a major phenotypic distinction between the W/W^v and Sl/Sl^d mutants. However, some of our findings may point to additional, more subtle differences between these animals. For example, anti-IgE-treated W/W^v mice exhibited a small (~ 15%) but significant decrease in C_{dyn}, an effect not observed in Sl/Sl^d mice (Fig. 2). By contrast, Sl/Sl^d mice, but not W/W^v animals, exhibited a small but significant transient decrease in G_L 3–8 min after challenge with anti-IgE (Fig. 3). We have no data which would permit speculation concerning the reasons for the differences in the responses of W/W^v and Sl/Sl^d mice to anti-IgE.

The results we obtained using anti-IgE to induce anaphylaxis are especially interesting in view of evidence indicating that mast cells may not be critical for the development of anaphylaxis, as defined by clinical criteria, in some other murine model systems. Mota (50) reported that mice or rats dying of anaphylaxis after passive sensitization did not exhibit significant mast cell degranulation; and Lima et al. (51) reported that little mast cell degranulation occurred in mice dying of reverse passive anaphylaxis. Similarly, mice (51) or rats (50) exhibiting severe or fatal anaphylaxis due to injection of soluble antigen-antibody complexes exhibited little or no mast cell degranulation. More recently, two separate groups have established that actively sensitized W/W^v (22–24) or Sl/Sl^d (24) mast cell-deficient mice can exhibit fatal active anaphylaxis upon intravenous challenge with specific antigen. These findings eliminate any doubt that death from anaphylaxis can occur in the absence of histologically identifiable mast cells. Moreover, passive transfer experiments indicate that fatal anaphylaxis in this model system is at least in part IgE-dependent (24). Recently, we also have observed fatal active anaphylaxis in mast cell-deficient mice, and found that W/W^v or Sl/Sl^d mice and the congenic +/+ mice, and +/+ BM → W/W^v mice, exhibited similar, albeit modest, pulmonary responses during active anaphylaxis (52). However, $WBB6F_1$-+/+, +/+ BM → W/W^v and $WCB6F_1$-+/+ mice developed tachycardia in association with anaphylaxis, whereas tachycardia did not occur in W/W^v or Sl/Sl^d mice (52).

There is no readily available explanation for the striking difference in responsiveness of W/W^v and Sl/Sl^d mast cell deficient mice to active anaphylaxis as opposed to challenge with anti-IgE. The active anaphylaxis responses elicited in our +/+ or +/+ BM → W/W^v mice, like the responses to anti-IgE challenge, were associated with extensive mast cell degranulation (52). While mast cell degranulation may have contributed to the tachycardia associated with active anaphylaxis in mice containing mast cells, it obviously was not essential for the pulmonary changes and death seen in this model of anaphylaxis at the doses of antigen tested. Elucidation of the reasons why mast cells may be essential for the pathogenesis of certain forms of anaphylaxis in mice, but do not contribute detectably in others, will represent an important topic of our future work.

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