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Research Article

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Mast Cell Activation Enhances Airway Responsiveness to Methacholine in the Mouse

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Abstract

Mast cell-deficient mutant mice and their normal littermates were used to determine whether activation of mast cells by anti-IgE enhances airway responsiveness to bronchoactive agonists in vivo. Pulmonary conductance was used as an index of airway response as the mice were challenged with increasing intravenous doses of methacholine (Mch) or 5-hydroxytryptamine (5-HT). Mast cell activation with anti-IgE enhanced pulmonary responsiveness to Mch in both types of normal mice ($P < 0.0001$ by analysis of variance) but not in either genotype of mast cell-deficient mouse. Additionally, anti-IgE pretreatment of genetically mast cell-deficient W/W^v mice whose mast cell deficiency had been repaired by infusion of freshly obtained bone marrow cells or bone marrow-derived cultured mast cells from congenic normal mice led to significant ($P < 0.0001$) enhancement of Mch responsiveness. 5-HT responsiveness was not significantly influenced by anti-IgE pretreatment in any of the mice studied. The data support the hypothesis that IgE-mediated activation of mast cells enhances pulmonary responsiveness to cholinergic stimulation. (*J. Clin. Invest.* 1993. 91:1176-1182.) Key words: allergy • anti-immunoglobulin E • asthma • bronchoconstriction • lung

Introduction

An airway response to bronchoconstrictor agonists at doses that would have little or no effect in normal human subjects is a characteristic feature of people with asthma (1-4). Further, it has been demonstrated, in a variety of experimental systems, that autocoids and proteases produced by mast cells (5, 6) have the capacity to increase bronchial reactivity; these include platelet activating factor (PAF)¹ (7), leukotriene E₄ (LTE₄) (8, 9), thromboxane A₂ (TxA₂) (10, 11), prostaglandin F_{2α} (PGF_{2α}) (12), and tryptase (13). Because the administration of mast cell products can transiently influence airway responsiveness, it has been proposed that mast cell activation may importantly

contribute to the bronchial hyperreactivity observed in asthmatic subjects (14-16). On the other hand, the molecules that have been implicated as potential inducers of airway hyperreactivity can also be produced by other cell types (15-19). As a result, the importance of the mast cell, as a specific source of mediators capable of promoting airway hyperresponsiveness, has been difficult to assess.

To evaluate directly whether mast cell activation influences airway responsiveness in vivo, we studied genetically mast cell-deficient mice, their congenic normal mice, and mast cell-deficient mutant mice whose deficiency had been repaired by adoptively transferring populations of mast cells. We reasoned that, if mast cells represent a crucial source of mediators that are released by IgE-dependent mechanisms and enhance airway responsiveness, infusion of low doses of anti-IgE before agonist administration would enhance responsiveness only in mice that possess mast cells. Our results clearly indicate that mast cell activation enhances airway responsiveness to methacholine in the mouse.

Methods

Animals

We studied WBB6F₁ +/+ normal and WBB6F₁ W/W^v mast cell-deficient mice (Dr. Warren Frost, Bozeman, Montana) and WCB6F₁ +/+ normal and WCB6F₁ Sl/Sl^d mast cell-deficient mice (Jackson Laboratories, Bar Harbor, ME), 3-6 mo of age. Both the WBB6F₁ W/W^v and the WCB6F₁ Sl/Sl^d mutant mice are profoundly deficient in mast cells in all anatomical tissues (20-22). They possess < 0.5% of the normal numbers of cutaneous mast cells and no mast cells are detectable by histology in any other tissues, including the tracheobronchial system and pulmonary parenchyma. All mice were maintained in a facility for viral antibody free mice and fed standard diets until time of study. The study protocols were approved by the Beth Israel and Children's Hospitals' Institutional Animal Care and Use Committees.

We also studied WBB6F₁ W/W^v mice which had received infusion of either freshly obtained bone marrow cells or cultured immature mast cells to repair their mast cell deficiency. Because the W and W^v mutations affect the c-kit receptor on the mast cell membrane (23, 24), tissue mast cell populations can be established in W/W^v mice by adoptive transfer of genetically normal mast cell progenitors or precursors derived from the congenic normal mice. In the first method, bone marrow cells were obtained from WBB6F₁ +/+ mice; recipient W/W^v mice each received 2×10^7 of the bone marrow cells i.v. 12-16 wk before study (20); these mice were designated +/+BM \rightarrow W/W^v mice. This reconstitution method has been shown to provide normal numbers of mast cells in tracheobronchial and other tissues of W/W^v recipients, and also repairs the anemia of these mice (20-22). Because this method does not selectively repair the mast cell deficiency of W/W^v mice, we injected a second group of W/W^v mice with nearly pure populations of bone marrow-derived cultured mast cells. For this, suspended bone marrow cells from WBB6F₁ +/+ mice were cultured in WEHI-3-conditioned medium (containing interleukin 3) for 4-5 wk, at which time the cell populations were composed of > 95% immature

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1. Abbreviations used in this paper: BMCMC, bone marrow-derived cultured mast cells; G_L, lung conductance; 5-HT, 5-hydroxytryptamine (serotonin); Mch, methacholine; PAF, platelet-activating factor; Tx, thromboxane.

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mast cells as assessed by staining with toluidine blue (6). 1×10^5 of these bone marrow-derived, cultured mast cells (BMCMC) were infused via tail vein into each W/W^v mouse and the recipients, designated as BMCMC $\rightarrow W/W^v$ mice, were studied at least 26 wk later. W/W^v mice that have received i.v., i.p., or i.d. injection of BMCMC of congenic $+/+$ origin exhibit adoptively transferred mast cell populations without repair of their anemia (25). Thus, this method represents a more selective approach for mast cell reconstitution than transfer of freshly obtained bone marrow cells. The hematocrit of each of the recipient W/W^v mice was measured shortly before the date of study to determine whether their anemia had also been corrected by the reconstitution procedure.

Reagents

Acetyl- β -methacholine chloride (methacholine, Mch) and 5-hydroxytryptamine (serotonin, 5-HT) were purchased from Sigma Chemical Co. (St. Louis, MO). Rat monoclonal anti-mouse IgE antibody (a generous gift from Dr. F. Finkelman, Uniformed Services University of the Health Sciences, Bethesda, MD), was produced and affinity-purified as previously described (26).

Pulmonary function measurements

Lung conductance (G_L) was monitored in anesthetized, tracheotomized, ventilated mice as previously described (27). Agonists were administered via a silastic catheter tied into a jugular vein. Plethysmograph pressure (from which changes in lung volume were derived) and transpulmonary pressure were transduced by differential pressure transducers (Celesco, Canoga Park, CA). The outputs were amplified and digitized, then analyzed using a computerized cross-correlation method (28) for which signals from 8–10 consecutive breaths were stored and averaged. The G_L peak response to each agonist dose was expressed as percentage of the baseline value obtained immediately before that dose of agonist.

Histology

Tracheobronchial and cutaneous tissues were fixed in 2.0% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% CaCl_2 in 0.1 M sodium cacodylate buffer, pH 7.3, and stored overnight at 4°C. 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 (29). The sections were examined at $\times 400$ for assessment of mast cell numbers in tissues of all mice and for quantification of extent of mast cell degranulation in tissues of $+/+$, $+/+$ BM $\rightarrow W/W^v$, and BMCMC $\rightarrow W/W^v$ mice (30, 31). Mast cells were scored as normal ($< 10\%$ of cytoplasmic granules exhibiting fusion, staining alterations, or extrusion from the cell), moderately degranulated (10–50% of granules altered as above) or extensively degranulated ($> 50\%$ of granules altered as above).

Protocols

Normal vs. mast cell-deficient mice not treated with anti-IgE. We first compared bronchial responsiveness of mice with mast cells (WBB6F₁ or WCB6F₁ $+/+$ mice) with those of the congenic mutant mice which lacked mast cells (W/W^v or Sl/Sl^d mice, respectively). After stable lung function measurements had been established, geometrically increasing doses of Mch or 5-HT were infused intravenously in a volume of 1.0 $\mu\text{l/g}$ of mouse per dose, starting with 3.3 or 5.0 $\mu\text{g/kg}$, respectively, and increasing the concentration threefold for each subsequent dose. 5–15 min was allowed to elapse between agonist doses to permit the pulmonary parameters to return to within ten percent of the baseline value obtained prior to the preceding agonist dose.

Anti-IgE vs. saline pretreated normal and mast cell-deficient mice. Additional groups of mice were studied to determine the effect of anti-IgE pretreatment on mediator responses in normal and mast cell-deficient mice. For each of the above four types of mice, agonist dose-response relationships of mice pretreated with normal (0.9% wt/vol) saline were compared with those of mice pretreated with rat monoclonal anti-mouse IgE. A dose of 20 μg per mouse of anti-IgE was chosen

based on previous studies (32) as being below the lethal level for the majority of mice yet large enough to cause substantial mast cell activation. Mch or 5-HT doses were administered starting 20 min after the anti-IgE was administered; dose response measurements were carried out thereafter as described above. Baseline G_L measurements were made once before pretreatment with anti-IgE or saline, again 20 min after pretreatment, at which time Mch or 5-HT administrations were started, and immediately before each subsequent dose of Mch or 5-HT.

Anti-IgE vs. saline pretreated mast cell-reconstituted mice. The effect of anti-IgE on Mch responsiveness was also examined in W/W^v mice containing adoptively transferred normal mast cells ($+/+$ BM $\rightarrow W/W^v$ and BMCMC $\rightarrow W/W^v$ mice). Both types of mast cell-reconstituted mice were pretreated with normal saline or anti-IgE and subsequently administered increasing doses of i.v. Mch as described above.

Statistical analysis

To help assess whether responses to methacholine might have been influenced by differences in airway caliber between anti-IgE- or saline-pretreated mice, we used a Student's t test (two-tailed) to compare G_L values ($\text{ml} \cdot \text{s}^{-1} \cdot \text{cm} \text{H}_2\text{O}^{-1}$) obtained 20 min after pretreatment with anti-IgE or saline. We also used ANOVA to compare, in anti-IgE- vs. saline-pretreated mice, the entire series of baseline values beginning with the value obtained 20 min after pretreatment and including all the baseline values obtained just before each subsequent dose of methacholine.

We primarily used ANOVA to compare agonist dose response (expressed as percent of baseline) curves in the various groups of mice. Further, for each mouse dose-response curve, we also calculated the $\text{ED}_{50}G_L$ ($\mu\text{g/kg}$) by linear interpolation as the dose of agonist required to obtain a 50% decrease in G_L . Geometric means of these values and the arithmetic means of the maximal reductions in G_L were compared by unpaired two-tailed Student's t tests. Extent of mast cell degranulation was compared by χ^2 analysis. Differences with a P value of < 0.05 were considered significant. The data in the figures are presented as mean \pm SEM.

Results

Mch

Normal vs. mast cell-deficient mice not treated with anti-IgE. When their responses were compared by analysis of variance, we found that the WBB6F₁ $-/+$ mice were significantly ($P < 0.0001$), albeit slightly, more responsive to methacholine than were the congenic mast cell-deficient W/W^v littermates (Fig. 1). However, the difference in $\text{ED}_{50}G_L$ values between the $+/+$ and W/W^v mice (geometric means, 187.6 vs. 329.7 $\mu\text{g/kg}$; 95% confidence intervals (95% CI) 173.8–251.2 vs. 204.2–536.5) did not quite achieve statistical significance ($P = 0.06$). The mean Mch dose response curve of the WCB6F₁ $-/+$ normal mice did not differ significantly from that of the Sl/Sl^d mast cell-deficient group (Fig. 1). Decreases in G_L with the highest dose of Mch did not differ significantly between $+/+$ and mutant mice of either genotype.

Anti-IgE vs. saline-pretreated normal and mast cell-deficient mice. For mice of each genotype, there were no significant differences in the baseline G_L values obtained 20 min after pretreatment with anti-IgE or saline (see legends to Figs. 2–4). We also used ANOVA to compare the full series of baseline G_L values which were obtained before each of the Mch doses throughout the dose response studies; there was no significant difference in the series of baseline G_L values between the anti-IgE- and saline-treated WBB6F₁ $+/+$ mice and anti-IgE-treated WCB6F₁ $+/+$ mice exhibited $\sim 20\%$ higher G_L values than the saline pretreated group ($P < 0.02$, ANOVA).

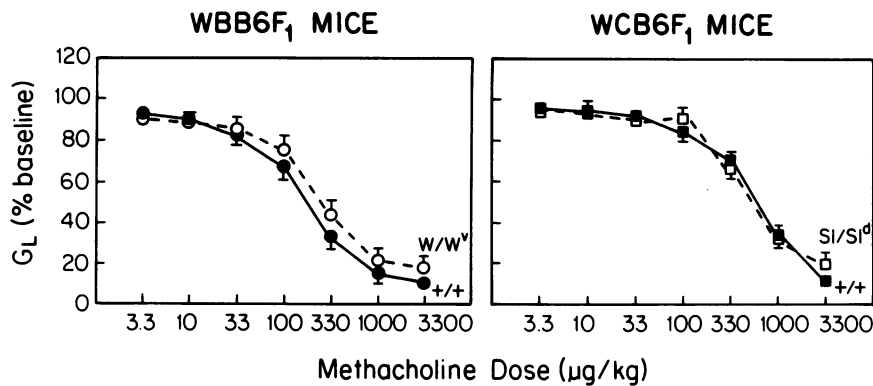


Figure 1. Mch-induced G_L dose-response relationships for normal vs. mast cell-deficient mice. Responses of mast cell-deficient WBB6F₁ W/W^v mice ($n = 14$) are slightly but significantly ($P < 0.0001$, ANOVA) attenuated compared with those of congenic normal WBB6F₁ $+/+$ mice ($n = 14$). No such difference existed between WCB6F₁ Sl/Sl^d ($n = 11$) and $+/+$ ($n = 13$) mice.

Both anti-IgE-pretreated WBB6F₁ $+/+$ and WCB6F₁ $+/+$ mice exhibited greater responsiveness to Mch ($P < 0.0001$, ANOVA) than did the respective matched saline-pretreated group (Fig. 2). In contrast, there was no detectable effect of anti-IgE treatment compared to saline treatment on Mch responsiveness in the W/W^v or Sl/Sl^d mast cell-deficient mice (Fig. 2). $ED_{50}G_L$ and G_L response to the highest Mch dose were not significantly different in any of the four anti-IgE-treated groups compared with the respective saline-treated group.

Anti-IgE- vs. saline-pretreated mast cell-reconstituted mice. In $+/+$ BM \rightarrow W/W^v mice, there was no significant difference in the baseline G_L values obtained 20 min after treatment with anti-IgE vs. saline (Fig. 3, legend). The series of baseline G_L values for the $+/+$ BM \rightarrow W/W^v mice treated with anti-IgE was slightly, but significantly ($P < 0.05$, ANOVA) lower than that of the saline treated group.

Anti-IgE treatment enhanced G_L responses to Mch in

$+/+$ BM \rightarrow W/W^v mice ($P < 0.0001$, ANOVA, Fig. 3). $ED_{50}G_L$ was $87.6 \mu\text{g/kg}$ (geometric mean; 95% CI 60.3–125.9) in the anti-IgE-treated $+/+$ BM \rightarrow W/W^v mice compared with $320.3 \mu\text{g/kg}$ (95% CI, 209.7–489.3) in the matched saline-treated mice ($P < 0.001$). The decrease in G_L with the highest dose of Mch was also significantly ($P < 0.001$) enhanced by anti-IgE in these $+/+$ BM \rightarrow W/W^v mice ($10.5 \pm 2.1\%$ of baseline vs. $34.7 \pm 5.6\%$ of baseline). The hematocrits of the $+/+$ BM \rightarrow W/W^v mice were all $> 40\%$.

In the BMCMC \rightarrow W/W^v mice, there was also no significant difference in the baseline G_L values obtained 20 min after treatment with anti-IgE or saline (Fig. 4, legend). The series of baseline G_L values obtained before each dose of Mch was significantly higher for the anti-IgE-treated BMCMC \rightarrow W/W^v mice than for the matched saline-treated group ($P < 0.02$, ANOVA).

Anti-IgE also significantly enhanced the G_L response to Mch ($P < 0.0001$, ANOVA, Fig. 4) in BMCMC \rightarrow W/W^v mice. The $ED_{50}G_L$ values were 78.5 vs. $343.6 \mu\text{g/kg}$ (geometric means, 95% CI 42.2–149.4 vs. 248.9–483.1) for the BMCMC \rightarrow W/W^v mice which had been pretreated with anti-IgE vs. saline, respectively ($P < 0.001$). The decrease in G_L with the highest dose of Mch was not significantly affected by anti-IgE

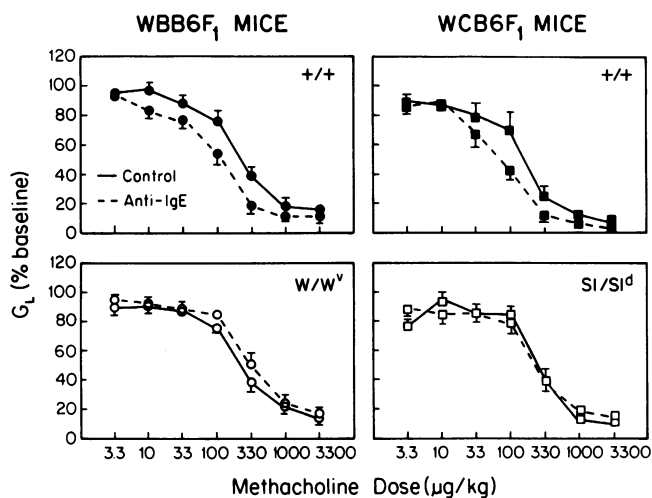


Figure 2. Effect of anti-IgE pretreatment on Mch-induced G_L responses, expressed as percent changes from baseline values, in normal and mast cell-deficient WBB6F₁ and WCB6F₁ mice. Baseline G_L values obtained 20 min after infusion of saline or anti-IgE, just before beginning Mch challenges, were 1.79 for WBB6F₁ $+/+$ control mice vs. 1.58 for anti-IgE-pretreated WBB6F₁ $+/+$ mice (NS), and 1.81 for WCB6F₁ $+/+$ mice pretreated with saline vs. 2.12 for WCB6F₁ $+/+$ mice pretreated with anti-IgE (NS). The G_L responses of both types of $+/+$ (normal) mice ($n = 5-6$) were significantly ($P < 0.001$, ANOVA) enhanced by anti-IgE. For mast cell-deficient W/W^v ($n = 4$ anti-IgE pretreated, 7 saline control) or Sl/Sl^d ($n = 4$ per group) mice, anti-IgE exerted no significant effect on Mch responsiveness.

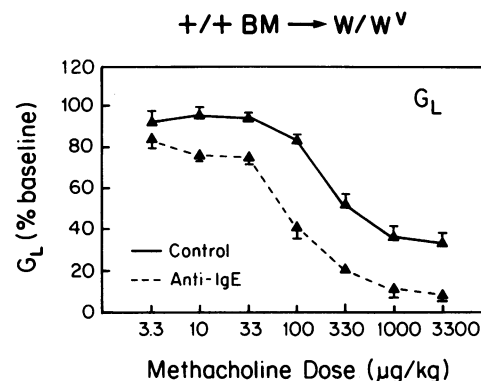


Figure 3. Effect of anti-IgE pretreatment on methacholine-induced G_L dose-response relationship in W/W^v mice whose mast cell deficiency had been repaired by i.v. infusion, at least 12 wk before study, of freshly obtained bone marrow cells from congenic normal mice ($+/+$ BM \rightarrow W/W^v mice). Baseline G_L values measured 20 min after saline pretreatment (1.73 ± 0.24) were not significantly different than those measured 20 min after anti-IgE pretreatment (1.57 ± 0.14). Subsequent G_L responses to Mch were significantly enhanced ($P < 0.0001$, ANOVA) by anti-IgE pretreatment ($n = 4$ per group).

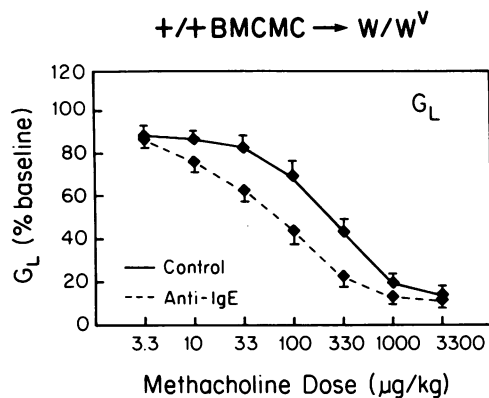


Figure 4. Effect of anti-IgE pretreatment on Mch-induced G_L dose response relationship in W/W^v mice whose mast cell deficiency had been repaired by i.v. infusion, at least 26 wk before study, of cultured mast cells derived in vitro from bone marrow cells of congenic normal $+/+$ mouse origin (BMCMC $\rightarrow W/W^v$ mice). Baseline G_L values measured 20 min after saline pretreatment (1.61 ± 0.15) were not significantly different than those measured 20 min after anti-IgE pretreatment (1.67 ± 0.28). Subsequent G_L responses to Mch were significantly enhanced ($P < 0.0001$, ANOVA) by anti-IgE pretreatment ($n = 6$ anti-IgE pretreated, 11 saline control).

treatment in this group. The hematocrits of the BMCMC $\rightarrow W/W^v$ mice ranged from 32% to 41%; i.e., the values remained within the anemic range found in W/W^v mice (37.9 ± 2.8 , reference 33). There was a significant ($P < 0.001$) negative correlation in the BMCMC $\rightarrow W/W^v$ mice between airway responsiveness and hematocrit at date of study, i.e., the greatest responsiveness was found in the mice with the lowest hematocrits (data not shown).

5-HT

Normal vs. mast cell-deficient mice not treated with anti-IgE. Serotonin dose-response curves were essentially the same in normal mice as in mutant mice of either the WBB6F₁ or the WCB6F₁ type (Fig. 5). There was a modest ($P > 0.05$) increase in the responsiveness of the W/W^v mice compared to their congenic normal $+/+$ group.

Effect of anti-IgE pretreatment. There were no significant differences between the 5-HT dose-response curves of anti-IgE-pretreated vs. saline-pretreated normal or mast cell-deficient mice (Fig. 6). Because anti-IgE pretreatment did not significantly influence 5-HT responsiveness of normal mice, we did not study the effect of anti-IgE on 5-HT responsiveness of mast cell-reconstituted mice.

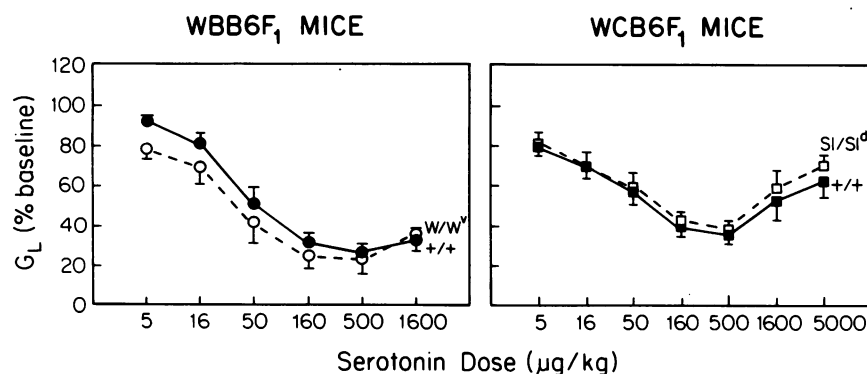


Figure 5. Serotonin (5-HT) G_L dose-response relationships. The WBB6F₁ $+/+$ mice ($n = 10$) exhibited slightly decreased G_L responsiveness when compared with the WBB6F₁ W/W^v mice ($n = 6$), but this difference was not significant.

Histology

Histological assessment confirmed the presence of mast cells in the tracheal tissues (Table I) and/or skin (data not shown) of all $+/+$ and $+/+BM \rightarrow W/W^v$ mice. In the tissues from eleven BMCMC $\rightarrow W/W^v$ mice not treated with anti-IgE, 10 mice displayed small numbers of mast cells in ear skin, sections of back skin, and/or lung parenchyma. By contrast, no mast cells were detected in any of these tissues from W/W^v mice that had not received bone marrow cells or mast cells or in tissues from Sl/Sl^d mice. Examination of the ear skin sections of the WBB6F₁ $+/+$, WCB6F₁ $+/+$, and $+/+BM \rightarrow W/W^v$ mice treated with anti-IgE or saline (Table II) revealed a much greater degree of mast cell degranulation in the anti-IgE-pretreated mice than in the saline-pretreated mice ($P < 0.0001$ for each comparison). Mast cells were not detectable in any tissues from four of six anti-IgE-treated BMCMC $\rightarrow W/W^v$ mice; the rare mast cells detected in the other two mice all exhibited moderate to extensive degranulation. Although the differences for each group were not significant, tracheal mast cell numbers/mm² were lower in anti-IgE-treated mice of all groups compared with respective saline treated control groups (Table I). These latter findings together with the results obtained in previous studies of mice which have undergone extensive mast cell activation (31, 32) are consistent with the possibility that our histological assessment of the Giemsa-stained sections may have underestimated the numbers of mast cells in BMCMC $\rightarrow W/W^v$ mice treated with anti-IgE. However, the numbers of mast cells in the tissues of BMCMC $\rightarrow W/W^v$ mice not treated with anti-IgE also were very low. This result is in accord with previous work indicating that transfer of 1×10^5 BMCMC i.v. produces very low levels of tissue mast cell reconstitution (25).

Discussion

Our data demonstrate that mast cell activation can enhance airway responsiveness to intravenous Mch in the mouse. When anti-IgE was administered to WBB6F₁ or WCB6F₁ $+/+$ mice in amounts adequate to induce degranulation of mast cells without causing persisting effects on airway physiology, the mice exhibited significantly enhanced airway responsiveness to Mch. By contrast, airway responsiveness to Mch in genetically mast cell-deficient WBB6F₁- W/W^v or WCB6F₁- Sl/Sl^d mice injected with anti-IgE was indistinguishable from that observed after injection of saline (Fig. 2).

Several lines of evidence indicate that the increase in Mch responsiveness observed in the anti-IgE-treated animals was not due simply to an anti-IgE-induced bronchoconstriction. In

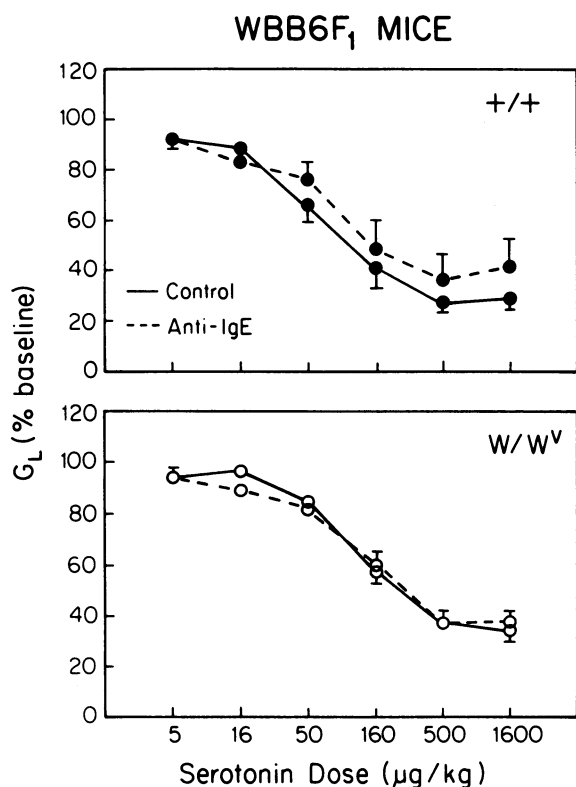


Figure 6. The effect of anti-IgE pretreatment on serotonin G_L dose response relationships in WBB6F₁ +/+ and W/W^v mice ($n = 4$ per group). Anti-IgE pretreatment was associated with a small, but not statistically significant, decrease in G_L responsiveness in the +/+ group.

our previous studies (32, 34), infusion of anti-IgE at 30 or 500 $\mu\text{g}/\text{kg}$ induced significant decreases in G_L but G_L returned to baseline levels within 15 min after antibody infusion. In the current study, values of G_L measured just before commencement of Mch infusions were not significantly different in anti-IgE- vs. saline-pretreated mice in any of the experimental groups. Furthermore, the serial baseline G_L values obtained prior to subsequent Mch doses over the course of the experiments were not significantly lower by ANOVA in three of the four anti-IgE-treated groups of mice possessing mast cells as

Table I. Tracheal Mast Cell Numbers

	Pretreatment	
	Saline	Anti-IgE
	<i>cells/mm² cross-sectional area</i>	
WBB6F ₁ - +/+	30.3±18.1 (6)*	7.5±3.5 (8)
WCB6F ₁ - +/+	13.5 (2)*	8.0 (2)
+/+BM → W/W^v	15.5±4.4 (8)*	6.0±2.9 (5)
BMCMC → W/W^v	1.9 (13) [‡]	0.26 (6)

* Data are mean±SEM. The numbers in parentheses indicate number of mice from which sections were examined.

[‡] Mean values are shown; mast cells were detected in only 2 of 13 sections for saline-pretreated mice and in only 1 of 6 sections in anti-IgE-pretreated mice.

Table II. Mast Cell Activation in Ear Skin

Mouse type	Normal	Moderate	Extensive
After normal saline			
WBB6F ₁ +/+	84.2 (3.8)*	11.8 (1.0)	3.9 (3.0)
WCB6F ₁ +/+	90.2 (4.6)	6.0 (1.9)	1.6 (1.0)
+/+BM → W/W^v	79.5 (9.0)	12.2 (4.6)	8.3 (6.8)
After anti-IgE			
WBB6F ₁ +/+	10.0 (4.3)	10.0 (2.1)	80.0 (4.6)
WCB6F ₁ +/+	14.2 (4.6)	15.7 (3.1)	70.0 (7.0)
+/+BM → W/W^v	17.7 (7.2)	16.3 (2.1)	66.0 (9.2)

* Mean (SEM) of percentages of mast cells with various degrees of degranulation in ear skin from mice studied for comparisons of Mch responses after anti-IgE vs. saline pretreatment. For mice of each genotype the differences in extent of degranulation between anti-IgE- and saline-pretreated groups was highly significant ($P < 0.0001$, χ^2).

compared to their saline treated control groups. Finally, if the increased Mch responsiveness observed after the injection of anti-IgE was simply the result of a mechanical effect of residual constriction, the responsiveness to 5-HT would have been similarly increased, which was not the case (Fig. 5).

The initial finding that anti-IgE treatment enhanced Mch responsiveness in normal mice could have been explained by the activation of Fc_εR-bearing cell types other than the mast cell. However, W/W^v mice are not defective in basophils (35), the only effector cell other than the mast cell which expresses the high-affinity Fc_εR on its surface. Yet we observed no increase in the Mch responsiveness of W/W^v mice pretreated with anti-IgE. In addition, we used two mast cell reconstitution approaches to demonstrate that it was the mast cell deficiency of the W/W^v or Sl/Sl^d mice, not other consequences of their mutations, which was responsible for their inability to exhibit increased airway responsiveness to Mch challenge after injection with anti-IgE.

We first examined W/W^v mice which contained adoptively transferred mast cell populations as a result of i.v. infusion of 2.0×10^7 bone marrow cells freshly obtained from the congenic +/+ mice. Such bone marrow-reconstituted W/W^v mice (+/+BM → W/W^v mice) exhibited markedly enhanced responsiveness to Mch after priming with anti-IgE (Fig. 3). Indeed, the magnitude of the enhancement effect of anti-IgE injection in the +/+ BM → W/W^v mice appeared to be even greater than that observed in the congenic +/+ mice (compare Figs. 2 and 3).

Because transplantation of +/+ bone marrow cells repairs the anemia of W/W^v recipients and perhaps also results in the development of other +/+ hematopoietic lineages besides the mast cell, we sought additional evidence that mast cell reconstitution was responsible for enabling W/W^v recipients to exhibit enhanced airway responsiveness to Mch after anti-IgE infusion. For this purpose W/W^v mice received i.v. infusions of 1.0×10^5 immature mast cells of congenic +/+ origin (BMCMC → W/W^v mice). We administered a relatively small number of cultured mast cells per mouse as this approach may be more likely to achieve a selective mast cell reconstitution. Although some of the BMCMC → W/W^v mice may have exhibited partial repair of their anemia, i.e., the mast cell

reconstitution may not have been completely selective in some of the animals, increased hematocrit was not associated with enhanced Mch responsiveness. Hence the enhanced responsiveness with two approaches to mast cell reconstitution was attributed to the mast cell rather than other cell types.

As a group, BMCMC \rightarrow W/W^v mice injected with anti-IgE, like anti-IgE injected $+/+BM \rightarrow W/W^v$ mice, exhibited significantly enhanced airway responsiveness to Mch compared to control mice pretreated with saline (Fig. 4). The amount of BMCMC transferred to each recipient W/W^v mouse (1×10^5) was chosen because our previous work indicated that this number would result in low levels of tissue mast cells without repairing the recipients' anemia due to the transfer of small numbers of pluripotent hematopoietic precursor cells which may have been present in the BMCMC preparations (25). Indeed, random tissue sections confirmed the presence of only limited numbers of skin or tracheal mast cells in the saline pretreated BMCMC $\rightarrow W/W^v$ mice as compared with normal or $+/+BM \rightarrow W/W^v$ mice. Moreover, random tissue sections revealed no identifiable mast cells in a few of the BMCMC $\rightarrow W/W^v$ mice which had been injected with anti-IgE. This may have been due, in part, to difficulty in identifying very small populations of extensively degranulated mast cells in the histological sections of these mice. Taken together, these observations suggest that restoration of relatively small mast cell populations is sufficient to allow mice to exhibit anti-IgE-induced enhancement of pulmonary responsiveness to Mch.

In contrast to their enhanced responsiveness to Mch (Fig. 1), normal ($+/+$) mice treated with anti-IgE had slightly, but not significantly, diminished responsiveness to 5-HT compared with saline pretreated $+/+$ mice. Tachyphylaxis to 5-HT has been demonstrated in the mouse (27) and it is possible that anti-IgE-induced mast cell degranulation led to release of quantities of 5-HT in the lung which were sufficient to reduce pulmonary responsiveness to exogenous 5-HT. Since mice of this genetic background do not develop bronchoconstrictor responses to other mediators, including histamine, LTC_4 , $PGF_{2\alpha}$, substance P, and PAF (27), we could not determine whether the anti-IgE-treated mice exhibited generalized pulmonary hyperresponsiveness.

It has been shown by others that IgE-mediated antigenic stimulation enhances cholinergic bronchial reactivity (36–39). Although the mechanism of enhanced cholinergic responsiveness due to mast cell activation is not known, it is known that a number of mast cell products (5–13) can enhance airway responses. Of particular interest is the observation that mastocytoma cell culture supernatants or purified mast cell tryptase can induce a dose-related increase in canine bronchial responsiveness in vitro (13). The in vitro enhancement of bronchial responsiveness by mast cell products was observed for agonists such as histamine, 5-HT, and potassium chloride, mediators which contract airway smooth muscle by a mechanism predominantly involving membrane potential-dependent Ca^{2+} transport, but not for acetylcholine, which contracts airway smooth muscle by a mechanism independent of calcium channels. The differences in agonist responses following putative mast cell and IgE dependent priming of canine tissues in vitro and of mice in vivo may be related to species differences, to the difference between a constant tissue bath exposure vs. a bolus injection, or to differences between large airway responses and those of the more peripheral small airways. The differences in

results may also reflect important differences between in vitro studies, in which tissues are examined after separation from their blood supply and connection with the central nervous system, and in vivo studies, in which airway smooth muscle cell function can be influenced by other cell types such as airway epithelial cells, as well as by factors dependent on an intact vasculature and nerve supply.

Mast cell products other than proteases may also contribute to the enhanced bronchial reactivity observed after IgE-dependent mast cell activation. Both in vitro-derived mouse mast cells (40–43) and freshly isolated mouse peritoneal mast cells (41, 42) can produce tumor necrosis factor- α in response to IgE-dependent activation. Several lines of evidence indicate that tumor necrosis factor- α can contribute to airway hyperactivity in the rat in vivo (44). In addition to exerting potentially important direct effects on bronchial reactivity, mast cell-derived cytokines might influence airway reactivity indirectly, by promoting the recruitment and/or modulating the function of other cell types which have effects in the airways. For example, eosinophils represent a prominent feature of the pathology of asthma (45, 46), eosinophils produce mediators which can influence bronchial reactivity (45), and mast cell-derived cytokines may contribute to the recruitment of eosinophils to sites of mast cell activation (47).

However, it is unlikely that any of the results obtained in our experiments were eosinophil dependent. In this study we examined responsiveness to methacholine or 5-HT 20 min after inducing IgE-dependent mast cell activation in immunologically naive mice. This is too short an interval for significant mast cell-dependent leukocyte infiltration to occur (42). Moreover, histological analysis of these mice indicated that neither the anti-IgE-treated nor the saline-treated mice exhibited eosinophils or other leukocytic infiltrates in their respiratory tissues.

T lymphocytes may also contribute to the pathology associated with chronic allergic asthma (46). However, an extensive body of data (reviewed in references 21 and 32) indicates that W/W^v or Sl/Sl^d mice exhibit no T cell deficiencies. These animals also express no impairment in their ability to mount an IgE response (reviewed in reference 32). This evidence, taken together with the fact that we studied acute rather than chronic pulmonary responses, indicates that the differences in bronchial reactivity we observed in mast cell-deficient as opposed to congenic normal mice probably did not reflect abnormalities of T cell function in the mutant animals.

In summary, our data provide strong evidence that mast cell activation can promote airway hyperactivity, and that this effect can be produced by activation of a relatively small number of mast cells. Although the mast cell mediators responsible for this effect in the mouse have not been defined, it is tempting to speculate that ongoing IgE-mediated activation of human mast cells may contribute significantly to the airway hyperactivity that characterizes the asthmatic state.

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