Brief Definitive Report

Development of Eosinophilic Airway Inflammation and Airway Hyperresponsiveness in Mast Cell–deficient Mice

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Summary

Mast cells are the main effector cells of immediate hypersensitivity and anaphylaxis. Their role in the development of allergen-induced airway hyperresponsiveness (AHR) is controversial and based on indirect evidence. To address these issues, mast cell–deficient mice (W/W^v) and their congenic littermates were sensitized to ovalbumin (OVA) by intraperitoneal injection and subsequently challenged with OVA via the airways. Comparison of OVA-specific immunoglobulin E (IgE) levels in the serum and numbers of eosinophils in bronchoalveolar lavage fluid or lung digests showed no differences between the two groups of mice. Further, measurements of airway resistance and dynamic compliance at baseline and after inhalation of methacholine were similar. These data indicate that mast cells or IgE–mast cell activation is not required for the development of eosinophilic inflammation and AHR in mice sensitized to allergen via the intraperitoneal route and challenged via the airways.

Mast cells play a central role in immediate allergic reactions (1) and in the early phase of the asthmatic response, but their role in the late phase response or sustained airway hyperresponsiveness is not clearly defined. Arming and activation of mast cells is through the binding of IgE to high affinity IgE receptors (FcεRI) on the cell surface (2, 3). After antigen cross-linking, the cells discharge a group of mediators including histamine and leukotrienes (4, 5), which trigger immediate responses. The fact that mast cells may also synthesize and secrete several cytokines on activation, including IL-4 and TNF-α, indicates their potential role in the sustained airway abnormalities. It is largely based on circumstantial evidence that mast cells are implicated in asthma pathogenesis. Because many other cell types express high or low affinity receptors for IgE and can release biologically active mediators on activation (6), a number of other cell types may be important in the IgE-dependent responses in the airways.

Mast cell–deficient mice can be used to directly assess the role of mast cells in allergen-driven airway hyperresponsiveness (AHR) (7). However, there have been limited investigations of these mice in terms of airway inflammation and the development of AHR. In the present study, we assessed the physiological response of the airways after sensitization and challenge to OVA in W/W^v, genetically mast cell–deficient mice to investigate more directly the role of the mast cell.

Materials and Methods

Animals. Female mast cell–deficient ([WB/Rej-kit^W/+ × C57BL6J-kit^{W-v}/+]F1 − [W/W^v]mice) (W/W^v) and congenic WBB6F1 normal mice (+/+) from 8 to 12 wk of age were obtained from Jackson Labs. (Bar Harbor, ME). The animals were maintained on an OVA-free diet. Experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Sensitization and Airway Challenge. Groups of mice (6–10 mice/group/experiment) receiving the following treatment were studied: (*a*) airway challenge to nebulized OVA alone (N); (*b*) sensitization to OVA with alum plus aerosolized airway challenge with nebulized OVA (ipN). Mice were immunized by intraperitoneal injection of 20 μg of OVA (Grade V; Sigma Chemical Co., St. Louis, MO) emulsified in 2.25 mg alum (AlumImuject; Pierce, Rockford, IL) in a total volume of 100 μl on days 1 and 14. Mice were challenged via the airways with OVA (1% in saline) for 20 min on days 28, 29, and 30 by ultrasonic nebulization, and assessed 48 h after the last OVA exposure for AHR.

Determination of Airway Responsiveness. Airway responsiveness was assessed as a change in airway function after challenge with aerosolized methacholine (MCh) via the airways. Anesthetized, tracheostomized mice were mechanically ventilated and lung function was assessed using methods similar to those described by Martin et al. (8). A four-way connector was attached to the tracheostomy tube, with two ports connected to the inspiratory and expiratory sides of a ventilator (model 683; Harvard Apparatus, South Natick, MA). Ventilation was achieved at 160 breaths/min

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and a tidal volume of 0.15 ml with a positive end–expiratory pressure of 2–4 cm H_2O.

The Plexiglas chamber containing the mouse was continuous with a 1.0-liter glass bottle filled with copper gauze to stabilize the volume signal for thermal drift. Transpulmonary pressure was detected by a pressure transducer with one side connected to the fourth port of the four-way connector and the other side connected to a second port on the plethysmograph. Changes in lung volume were measured by detecting pressure changes in the plethysmographic chamber through a port in the connecting tube with a pressure transducer and then referenced to a second copper gauze–filled 1.0-liter glass bottle. Flow was measured by digital differentiation of the volume signal. Lung resistance (R_L) and dynamic compliance (Cdyn) were continuously computed (Labview, National Instruments, TX) by fitting flow, volume, and pressure to an equation of motion.

Aerosolized agents were administered for 10 s with a tidal volume of 0.5 ml (9). From 20 s up to 3 min after each aerosol challenge, the data of R_L and Cdyn were continuously collected. Maximum values of R_L and minimum values of Cdyn were taken to express changes in murine airway function.

Bronchoalveolar Lavage and Lung Cell Isolation. Immediately after assessment of AHR, lungs were lavaged via the tracheal tube with HBSS (1× 1 ml 37°C). The volume of collected bronchoalveolar lavage (BAL) fluid was measured in each sample and numbers of leukocytes were counted (Coulter Counter; Coulter Corporation, Hialeah, FL). Cells in lung tissue were isolated and counted as previously described (10).

Histologic and Immunohistochemistry Studies. After perfusion via the right ventricle, lungs were inflated through the trachea with 2 ml air and then fixed in 10% formalin by immersion. Blocks of the left lung tissue were cut from around the main bronchus and embedded in paraffin blocks, 5-μm tissue sections were affixed to microscope slides and deparaffinized. The slides were then stained with Astra Blue/Vital New Red and mast cells and eosinophils were examined under light microscopy (11).

Cells containing eosinophilic major basic protein (MBP) were identified by immunohistochemical staining as previously described using a rabbit anti–mouse MBP (provided by Dr. G. Gleich and Dr. J. Lee, Mayo Clinic, Rochester, MN and Scottsdale, AZ, respectively) (12). Numbers of eosinophils in the submucosal tissue around central airways were analyzed using the IPLab2 software (Signal Analytics, Vienna, VA) for the Macintosh counting four different sections per animal (12).

Measurement of Anti-OVA Antibody. Serum levels of anti-OVA IgG1 and IgE were measured by ELISA as previously described (10).

Statistical Analysis. All results are expressed as the mean and SEM. Analysis of variance was used to determine the levels of difference between all groups. Pairs of groups were compared by unpaired two-tailed Student's t test. ANOVA was used to compare percent changes of R_L and Cdyn between different strains with the same treatment. The p values for significance were set to 0.05.

Results

Antibody Responses to OVA Sensitization and Challenge. As shown in Fig. 1, serum levels of anti-OVA IgE and IgG1 were comparable in the mast cell–deficient mice and

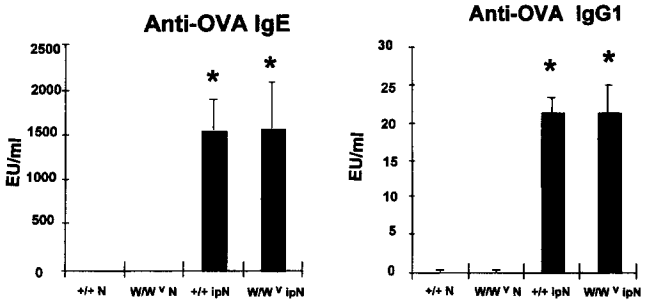

Figure 1. OVA-specific antibody in the serum. Serum titers for OVA-specific antibodies in +/+ and *W/W*^{v} mice were determined after sensitization and challenge (n = 7) compared with mice receiving challenge alone (n = 6). The results for each of the groups are expressed as means ± SEM. *Significant differences (P <0.05) between the groups (N versus IpN). EU, ELISA units; N, challenge (nebulization alone); ipN, sensitization and challenged.

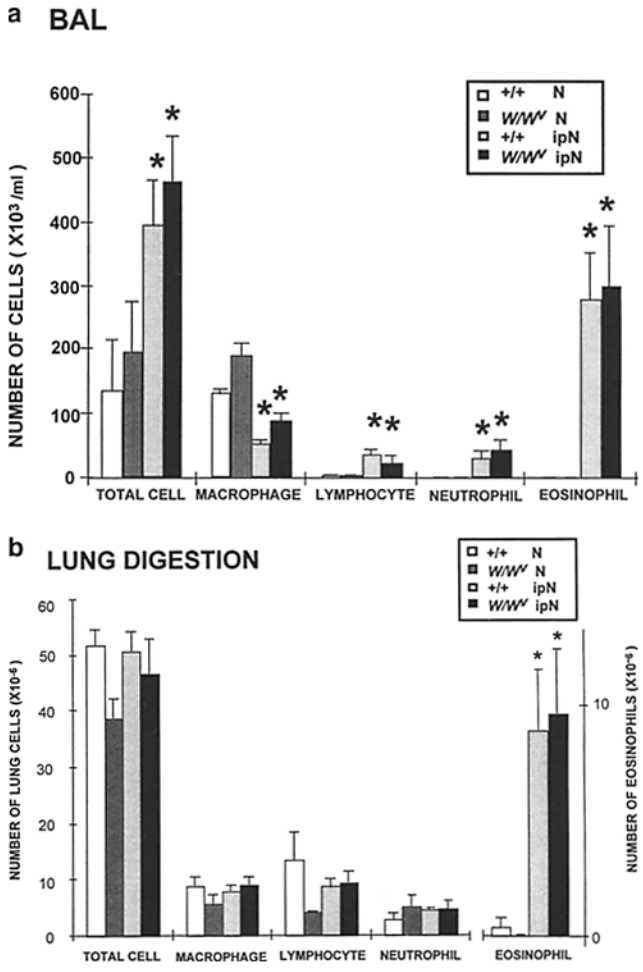

Figure 2. (*a*) Cellular composition of BAL fluid. Mice were sensitized and challenged as described in Materials and Methods. BAL fluid was obtained from the same groups described in the legend to Fig. 1. The results for each group are expressed as means ± SEM. *Significant differences (P <0.05) between the groups (N versus IpN). (*b*) Cellular composition of isolated lung cells. Lung cells were prepared from animals sensitized and challenged as described in the legend to Fig. 1. The results for each group are expressed as means ± SEM (n = 4/group). *Significant differences (P <0.05) between the groups (N versus IpN).

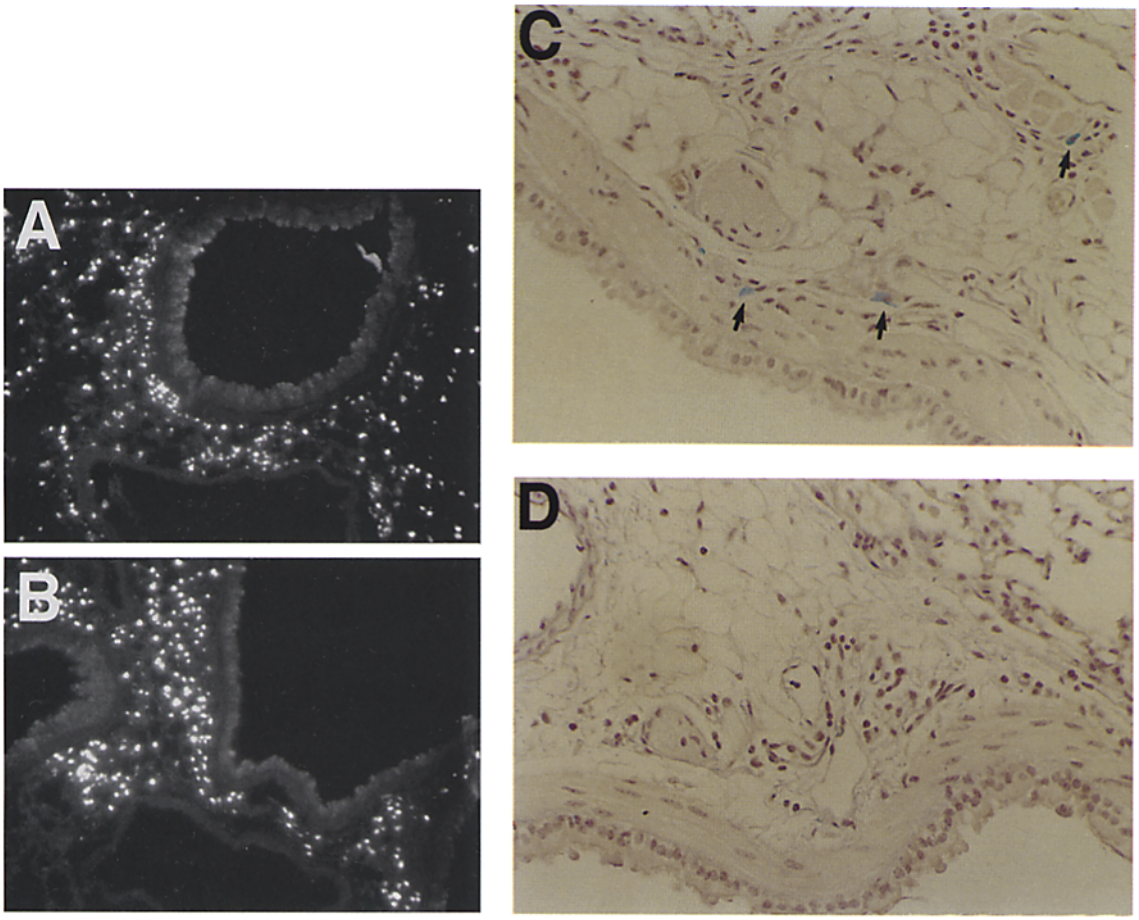

Figure 3. Immunohistochemistry of peribronchial tissue after sensitization and challenge with OVA. Localization of eosinophils and mast cells are shown. +/+ mice are shown in *a* and *c* and *W/W*^{v} mice in *b* and *d*. In *c* and *d*, cells were stained with Astra Blue/Vital New Red. For *a* and *b*, staining was with a rabbit anti–mouse MBP antibody and fluorescein-labeled goat anti–rabbit IgG. Original magnification of 500.

the congenic littermates after sensitization and challenge with OVA. Challenge alone on three occasions was insufficient to trigger antibody responses in either group of mice.

Eosinophilic Accumulation in the BAL and Lung. As shown in Fig. 2 *a*, sensitization and challenge with OVA had a marked effect on the numbers and composition of the cells recovered. In both groups of mice, macrophages were the predominant cell type in the mice receiving three challenges with OVA alone (similar to control mice; data not shown). However, after both sensitization and challenge, cell numbers increased and the predominant cells in the BAL were eosinophils, comprising roughly 60% of the cells in both the mast cell–deficient and congenic littermates.

When lung digests were examined, sensitization and challenge also resulted in a marked increase in eosinophil numbers (Fig. 2 *b*), although total cell numbers were little changed when compared with challenge alone. As in the BAL, there were no differences in the numbers of eosinophils in the lung digests between mice that were mast cell deficient or sufficient.

Localization of Eosinophils and Mast Cells in Lung Tissue. After staining with anti-MBP antibody, sensitization and challenge significantly increased the numbers of eosinophils per area in the peribronchial tissue of both groups of mice (Fig. 3, *a* and *b*) to 187 ± 23/mm^2 in +/+ mice, 168 ± 18/mm^2 in *W/W*^{v} mice (n = 4). In animals challenged alone very few eosinophils were detected in these sites (13 ± 4/mm^2). Staining with Astra Blue/Vital New Red revealed the accumulation of mast cells in the submucosal tissue of the bronchi in sensitized and challenged +/+ mice (Fig. 3 *c*). None could be identified in any of the sections examined from *W/W*^{v} mice (Fig. 3 *d*).

Airway Responsiveness. We examined baseline lung function and assessed the airway response to inhaled methacholine. Baseline (before MCh challenge) measures of lung function as assessed with R_L and Cdyn are presented in Table 1. The values in all four groups were comparable. The response to aerosolized methacholine in mice that were challenged with antigen alone revealed small, dose-dependent changes in R_L and a 20–30% dose-dependent fall in Cdyn (Fig. 4). After sensitization and challenge, resistance values increased by almost fivefold and dynamic compliance was reduced by 60–70% with comparable doses of methacholine. The responses in the mast cell–deficient mice, if any-

Table 1. *Baseline Values of RL and Cdyn in Mice*

Mice	Group	RL	Cdyn
		$cmH_2O \cdot ml^{-1} \cdot sec$	$ml \cdot cm\ H_2O^{-1}$
+/+	N	0.45 ± 0.057	0.039 ± 0.0012
W/W^v	N	0.42 ± 0.070	0.039 ± 0.0013
+/+	ipNeb	0.40 ± 0.062	0.036 ± 0.0020
W/W^v	ipNeb	0.44 ± 0.063	0.035 ± 0.0018

Lung resistance and dynamic compliance values in sensitized and challenged mice. RL and Cdyn values were obtained in the different groups of animals after sensitization and challenge but before exposure to MCh. The results for each group are expressed as means ± SEM (n = 8).

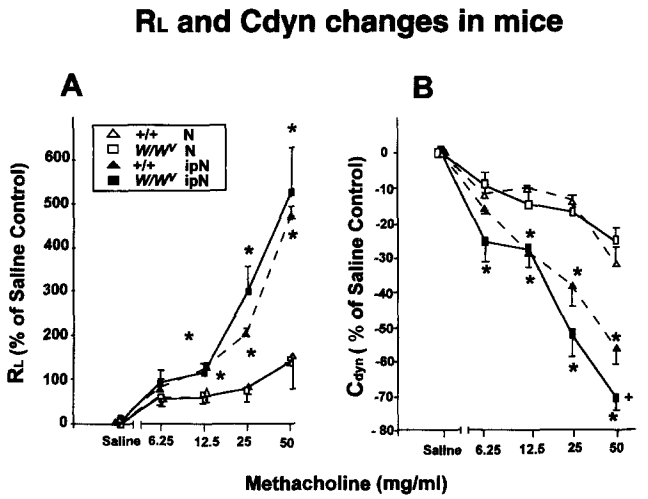

Figure 4. Lung resistance (*A*), and pulmonary dynamic compliance (*B*) in sensitized and challenged mice. RL and Cdyn values were obtained in response to increasing concentrations of methacholine as described in Materials and Methods. The results for each group are expressed as means ± SEM (n = 8). *Significant differences (P <0.05) between the groups. +Significant differences (P <0.05) between W/W^v and +/+ mice.

thing, exceeded the response in the congenic littermates, with a shift to the left in the methacholine dose–response curve for both RL and Cdyn.

Discussion

Mast cells and their released products are widely believed to contribute to the development of allergic respiratory disorders. IgE-dependent activation of mast cells can induce these cells to release a panel of preformed or newly synthesized mediators including histamine, tryptase, prostaglandins, leukotrienes, and platelet activating factor, which can result in acute phase allergic reactions in the lung including airway obstruction, airway microvascular leakage, and mucosal edema, as well as mucus gland hypersecretion (13–15). Although a role for mast cells has been defined in the acute phase of allergic reactions, much less is known about their role in chronic allergic lung inflammatory responses and their contribution to lung dysfunction in this setting. After allergen sensitization and challenge in the mouse, the changes in airway function that have generally been monitored include the response to MCh (8) or electrical field stimulation of tracheal smooth muscle preparations (16) and likely reflect a more chronic, eosinophil-dependent response (12). The current study extends previous investigations by assessing airway responsiveness in vivo and factors, such as cells and antibodies, which may contribute to the development of airway responsiveness.

Sensitization and challenge of the mast cell–deficient mice resulted in IgE and IgG1-specific antibody responses, increased eosinophils in the BAL and lung digests, and peribronchial infiltration of eosinophils. In all of these aspects, they were indistinguishable from their congenic littermates. The only difference was that mast cells were identified histologically in the submucosa of +/+ mice and not in the W/W^v animals. These findings suggest that the development of an allergic inflammatory reaction is not dependent on the presence of functional mast cells. These results are similar to what has been suggested in other systems. For example, Nogami and coworkers (17) showed no evidence for the involvement of mast cells in the pulmonary eosinophilic response to challenge with an extract from the parasite *Ascaris suum*. Further, Brusselle et al. (18) demonstrated no effect of mast cells on eosinophil influx in BAL fluid after repeated challenge with OVA in sensitized mice. In contrast, Kung et al. (19) reported that OVA challenge of sensitized mast cell–deficient mice produced fewer eosinophils in the BAL fluid and lungs compared with similarly sensitized and challenged congenic littermates. However, in this study both the sensitization and challenge protocol were attenuated and the number of eosinophils was significantly lower than we and others (18) generally observe after sensitization and challenge as described in this study. In their protocol, mice were challenged with antigen on only 1 d; in our studies we have found that 1 or 2 d of antigen challenge were not sufficient to develop airway hyperresponsiveness (our unpublished data). In our protocol, airway inflammation and eosinophil accumulation may have been sufficiently strong so that a role for the mast cells could not be demonstrated.

The presence of a comparable peribronchial eosinophil response in the W/W^v and +/+ mice was associated with a similar response to MCh in this study. Monitoring both resistance and dynamic compliance, aerosolized MCh resulted in a dose-dependent increase in RL as well as a 60–70% decrease in compliance. These changes were only observed in sensitized and challenged animals. At virtually all concentrations of MCh, the findings in the W/W^v mice exceeded those in the +/+ mice. At present, there is no apparent explanation for these differences. One possibility is that in the airways of mast cell–sufficient animals, heparin is released after activation (20), and may limit the response to the cationic protein mediators released by these same or other cells. In this regard, it has been previously shown that heparin sulfate and other polyanionic molecules block the

increase in airway responsiveness caused by highly charged cationic proteins (21, 22).

If sensitization and repeated challenge with antigen triggers both eosinophilic inflammation and AHR, does this eliminate a role for mast cells in the development of these changes? It is possible that the same physiologic response, AHR to MCh challenge, may be mediated by distinct cellular mechanisms in different strains of mice (23). For example, in BALB/c mice AHR could be induced in an IgE and mast cell–dependent fashion. In strains genetically deficient in important mast cell mediators (e.g., mast cell protease 7 deficiency in C57BL/6 mice) AHR may be more dependent on other cell types, such as eosinophils. Mast cell deficient mice of the same background as studied here do demonstrate reduced severity of anaphylaxis induced by anti-IgE treatment as well as reduced airway responsiveness to MCh shortly after systemic administration of anti-IgE (25, 26).

After limited bronchoprovocation mast cells may play a role in the liberation of cytokines such as IL-4, IL-5, and TNF-α (15); because of preformed stores, mast cells could provide the initial source of TNF-α in IgE-dependent reactions (27). However, we have shown in nude mice that were passively sensitized with IgE, that despite adequate mast cell degranulation, insufficient cytokines are liberated to trigger an eosinophilic response (28). A similar inconsistency centers around the role of IgE in triggering allergic inflammation and AHR. Sensitization exclusively via the airways, which results in limited eosinophilic infiltration of the peribronchial regions, results in AHR that appears to be IgE dependent (29). On the other hand, after sensitization and challenge as performed in this study, AHR may be IgE independent but eosinophil dependent (our unpublished data). Similar results have recently been reported by Korsgren et al. (30) demonstrating normal development of eosinophilic airway inflammation in B cell–deficient mice. However, we can not discount that in the mast cell–deficient mice, the presence of a normal IgE response serves to trigger other cells expressing either high affinity IgE receptors, e.g., basophils, macrophages, or other cells expressing low affinity receptors (6) to release important proinflammatory mediators.

In summary, in this study of mast cell–deficient mice we have shown that after sensitization and airway challenge they are capable of developing an allergic antibody response and changes in airway resistance and dynamic compliance that are similar to their congenic littermates. Although this does not exclude contributions of mast cells in other aspects of chronic allergic inflammatory responses, it indicates that mast cells do not have an essential role in development of eosinophilic airway inflammation and airway hyperresponsiveness to MCh in mice sensitized and challenged as described in this report.

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