Do Mucosal Mast Cells Contribute to the Immediate Asthma Response?

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ABSTRACT—In rat trachea, two types of mast cells have been identified, connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs). We previously reported that CTMCs play an important role in tracheal contraction in vitro via 5-hydroxytryptamine (5-HT) release in a rat model. In this study, we investigated whether MMCs also play a role in tracheal contraction by employing mast cell-deficient (Ws/Ws) rats and their congenic (/G2b/G2b) rats. Rats were actively sensitized with ovalbumin and challenged with it 2 weeks later. To exclude the influence of CTMCs, rats were pretreated for 7 days with compound 48/80 injected i.p. in increasing doses. Histological study confirmed that degranulation occurred in CTMCs, but MMCs still remained. Histamine levels in trachea decreased to 9.31% of control levels. Ovalbumin-specific IgE production showed a time-dependent increase in both Ws/Ws and +/+ rats after sensitization with no significantly different values between the two groups. Ovalbumin challenge caused contraction of the trachea in sensitized control (+/+ rats), but not in sensitized Ws/Ws and compound 48/80-pretreated +/+ rats. Ketanserin inhibited the contraction, but leukotriene antagonist ONO-1078 did not, indicating that the contraction was due to 5-HT, whereas leukotriene, a mediator specific derived from MMCs, has no significant effect. The results suggest that MMCs has minimal, if any, contribution to tracheal contraction and might have another function. Furthermore, Ws/Ws and the congenic rats provide a good model for studying the role of mast cells in the immunologic response in airways.

Keywords: 5-Hydroxytryptamine, Ovalbumin, Mast cell heterogeneity, Ws/Ws rat, Tracheal contraction

Mast cells are implicated in the pathogenesis of diseases of the airways, especially in the immediate type hypersensitivity reaction in a number of immunologic and non-immunological disorders. However, much controversy and confusion concerning mast cells has arisen because of their heterogeneity (1).

It has long been known that in rodents, there are two major populations of mast cells, connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs) (2, 3). These two types of mast cells have been observed in rat trachea (4, 5), in the submucosa region and epithelial layer, respectively. Differences in the mediators generated by the two cell types also exist. Both MMCs and CTMCs release histamine and serotonin, but the predominant arachidonic acid metabolite of CTMCs is prostaglandin (PGD₂), whereas the MMCs generate more lipoxygenase products such as leukotrienes (6). However, there is little available information concerning the functional differences between the two distinct types of mast cells in the respiratory region, especially with regard to tracheal contraction, one feature of asthmatic disease. It is therefore of interest to study the role each type of mast cell plays in such a function since drugs may act differently in different type of mast cells.

The discovery of mast cell-deficient rats by Kitamura and colleagues (7) has made it possible to make more direct studies of the role of mast cells in various regions and, furthermore, the role of the different types of mast cells. Our previous work performed in mast cell-deficient rats (Ws/Ws) and their congenic (+/+ rats) demonstrated that CTMCs contributed to compound 48/80-induced tracheal contraction in non-immunological events (5). In that study, the role of MMCs could be excluded, since compound 48/80 does not degranulate this type of mast cells (8). In this present study, we wanted to investigate the potential role of MMCs in tracheal contraction during immunological conditions.

We chose an immunological event that involves IgE since the MMCs are not affected by non-immunological stimuli (8). IgE antibody is the major homocytotropic antibody produced in rats after sensitization (9), as in humans (10). After sensitization, IgE was produced and distributed...
in the tracheal section, bound to IgE receptors on the surface of mast cells, and concentrated in the cytoplasm of subepithelial mast cells and globule leukocyte/mucosal mast cells (11). While compound 48/80 does not activate MMCs, both types of mast cells may be activated by antigen crosslinking with IgE to high affinity IgE receptors (FcεRI) on the cell surfaces (12, 13).

The present study addressed whether or not MMCs play a significant role, as do CTMCs, on tracheal contraction. Recognition of this complexity is crucial for understanding mast cell biology and, potentially, for treating mast cell-associated diseases. The study also addressed whether the specific mast cell-deficient (Ws/Ws) rat provides a good tool for studying the role of airway mast cells in immunological events.

MATERIALS AND METHODS

Experimental animals

Male and female Ws/+ rats, both from the Donryu strain, were crossed to obtain male Ws/Ws, heterozygous Ws/+ , and wild type +/+ rats, using the procedure described by Niwa et al. (7). Male Ws/Ws and +/+ rats, weighing between 250 – 300 g and aged 3 – 4 months, were used. The animals were housed at a constant temperature of 22 ± 2°C with a humidity of 55 ± 10% on an automatically controlled 12:12 h light-dark cycle (lights on at 7.00 A.M.) and had free access to food and water.

The animal experiments performed in the present study were conducted according to the guidelines of the Animal Care Committee of the Ehime University School of Medicine, and all experimental protocols had been approved by this Committee.

Chemicals

Carbamylcholine chloride (carbachol), compound 48/80, indomethacin, naphthol AS-D chloroacetate and pararosanilin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Bordetella pertussis toxin, ovalbumin, p-nitropheryl-2-acetamido-2-deoxy-β-D-glucopyranoside, 5-hydroxytryptamine creatinine sulfate (5-HT) and ketaserin were purchased from Wako Company (Osaka). Eagles’s minimum essential medium and antibiotics were obtained from Gibco (Grand Island, NY, USA), fetal calf serum was purchased from JRH Biosciences (Lenexa, KS, USA), and PIPES was purchased from Dosindo (Kumamoto). ONO-1078 was kindly supplied by Ono Pharmaceutical Co., Ltd. (Osaka). All other chemicals were of the highest grade commercially available.

Active sensitization and blood sampling

The rats were sensitized by subcutaneous injection of 1.0 ml ovalbumin (1.0 mg/ml) mixed with 10% aluminium hydroxide suspension in physiologic saline and intraperitoneal injection of a Bordetella pertussis antigen solution containing 1.0 × 10⁶ organisms/ml. Blood samples were obtained from the tail vein on day 0 (before sensitization) and thereafter on days 3, 7, 10 and 14. After coagulation for 30 min at room temperature, the samples were centrifuged at 2000 × g and 4°C for 20 min to obtain sera. The sera were stored at −20°C until further processing. Rats were studied for contractile response on day 14.

Negative control animals (n = 2, both for +/+ and Ws/Ws rats) were not sensitized with ovalbumin. Sera from negative control animals were pooled and used as negative control samples in the ovalbumin-specific IgE determination.

Determination of functional IgE production

Functional anti ovalbumin-IgE produced in rat serum after sensitization was determined by detecting its capability to induce β-hexosaminidase release from RBL-2H3 cells. RBL-2H3 cells were cultured in Eagle’s minimum essential medium (MEM) containing 15% fetal calf serum in a flask in a humidified atmosphere of 5% CO₂ in air according to Barsumian et al. (14). RBL-2H3 cells were seeded in 96-well culture plates (0.5 × 10⁵ cells/well) in 0.2 ml medium in each well. Cells were incubated overnight and sensitized with sera samples in several dilution times.

After sensitizing cells with IgE in 96-well culture plates, the medium was washed twice with 0.2 ml PIPES buffer (25 mM PIPES, 119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM MgCl₂, 1 mM CaCl₂, 40 mM NaOH, 0.1% BSA, pH 7.2). RBL-2H3 cells were stimulated after addition of 200 μl of 10 μg/ml ovalbumin for 1 h. Then 50 μl of supernatant was incubated with 100 μl 25 mM p-nitropheryl-2-acetamido-2-deoxy-β-D-glucopyranoside as substrate for enzyme hexosaminidase. Optical density (OD) of p-nitrophenol as a product after addition of 20 μl of 2 M KOH to the wells was measured at 405 nm in a microplate auto reader (Model 450; Bio-Rad, Hercules, CA, USA). The average OD of negative control sera at 1:10 dilution provided the reference value taken to determine the titer of the test sera. The reciprocal of the highest serum dilution giving an OD higher than the reference value was read as the titer. All analyses were performed in duplicate. The results were expressed as reciprocal log 2 titer.

Depletion of connective tissue mast cells

CTMCs were depleted according to Joos et al. (15), with a small modification. Briefly, one group of ovalbumin-sensitized rats was pretreated for 7 days by intraperitoneal injection of compound 48/80 at increasing doses ranging from an initial dose of 1 mg/kg to a final dose of 5 mg/kg. To reduce mortality due to excessive release of histamine, rats were also administered mepyramine i.p. at a dose of
Tissue preparation and measurement of tracheal contraction

Rats were killed by an overdose of pentobarbital sodium (100 mg/kg, i.p.). The neck was opened and the trachea quickly dissected. The trachea was carefully stripped of connective tissue and blood vessels, and then prepared as tracheal strips containing 3–4 cartilage rings, by cutting longitudinally through the cartilage.

The strips were mounted in 10-ml of organ bath solution containing Krebs-Henseleit buffer (118 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM NaH₂PO₄, 25.5 mM NaHCO₃, 5.6 mM glucose) containing 10⁻⁶ M indomethacin to reduce endogenous tissue tone and inhibit the effects of any cyclooxygenase products (18). The solution was maintained at 37°C and bubbled with 95% O₂ / 5% CO₂. The trachea strips were then allowed to equilibrate for at least 1 h, while exchanging the bath solution every 15–20 min at a resting tension of 1 g, which was found to be optimal for measuring changes in tension. Contraction was measured isometrically with TB-611T transducers (Nihon Kohden, Tokyo), with the signal being amplified by an AP-601G amplifier (Nihon Kohden).

To check the viability of preparations, 30 μM carbachol was added to the bath solution. This procedure was repeated once or twice at an interval of 30 min until the contraction was stable, i.e., less than 10% variation. The same was also done at the end of each experiment.

Contractile responses of rat trachea to stimulants

In a series of experiments, concentration-response curves to 5-HT were made cumulatively, using a range of 10⁻⁸ to 10⁻⁴ M on the same preparation from both types of rat. After measurement of the contractions induced by this agent, all preparations were evaluated in terms of the contractile response to ovalbumin. One tracheal strip was challenged by 100 μg/ml of ovalbumin, the concentration giving optimum contraction according to method of Lima and Silva (19).

In another series of experiments, contraction to ovalbumin was also evaluated in the presence of ketanserin, a specific 5-HT₂A-receptor antagonist, and ONO-1078, a leukotriene (LT) antagonist, which were exposed to the tracheal preparations for 20 min prior to ovalbumin challenge.

Enzyme histochemistry

Rat tracheas from +/- (with and without pretreatment with compound 48/80) and Ws/Ws rats were removed and fixed using 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 12 h at 4°C. The fixed specimens were immersed in 20% sucrose/0.1 M phosphate buffer overnight at 4°C, and then frozen in O.C.T. embedding medium (Tissue-Tek; Sakura Finetech, Tokyo) until studied. Sections were cut 4–5-μm thick using a cryotome.

Chloroacetate esterase activity of mast cells was visualized by enzyme histochemistry as described by Beckstead et al. (20). Briefly, the sections were incubated for 60 min at 30°C with chloroacetate substrate solution consisting of 0.5% naphthol AS-D chloroacetate and 0.02% hexazonitized pararosaniline as chromogen, then counterstained with hematoxylin. This procedure was also applied to trachea from control (+/-) rats after ovalbumin challenge. Four adjacent sections of each trachea were viewed at 200× magnification, and the number of mast cells in each section was counted. The average of four sections of each trachea was used for semi-quantitative analysis.
Statistical analyses

Results are expressed as the means ± S.E.M. Statistical analysis was carried out using a one-way analysis of variance followed by a Student’s paired t-test. P values less than 0.05 were considered to indicate significant differences.

RESULTS

Histamine and 5-HT content in +/+ and Ws/Ws rat tracheas

Since histamine is the major mediator of mast cells and is almost exclusively produced in and secreted from mast cells, we determined the histamine content in rat trachea as representative of the presence of mast cells. We also measured the 5-HT content in rat trachea as a preformed mediator released from rodent mast cells during degranulation. It was found that the histamine content of Ws/Ws rat trachea was only approximately 0.49% of that in tracheal tissue from +/+ rats, whereas the 5-HT content of tracheal tissue from Ws/Ws rats was about 1.27% of that from +/+ rats. Pretreatment with compound 48/80 for 7 days markedly reduced the histamine content to 9.31 ± 2.13% of the control level as well as 5-HT content in rat trachea. All data regarding histamine and 5-HT content in rat trachea are reported in Table 1.

IgE titer

Serum levels of ovalbumin-specific IgE were measured at different time points (days 0, 3, 7, 10 and 14). As shown in Fig. 1, the serum IgE titer showed a time-dependent increase with a peak at day 10 and then reached a plateau until day 14 (the day of the contraction study). No significant difference was found between +/+ and Ws/Ws rats with respect to the ovalbumin-specific IgE response measured using RBL-2H3 cell culture.

Enzyme histochemistry

Enzyme histochemistry analysis demonstrated the presence of both types of mast cells in the tracheal tissue from +/+ rats (control), but not in Ws/Ws rats (Fig. 2: a and b). Both CTMCs and MMCs/globule leukocytes are known to contain a chymotrypsin-like serine protease that can be detected by enzyme histochemistry, using chloroacetate as substrate (4). They can be distinguished from other cells in the trachea by the red reaction product produced by the catalytic action of protease on the substrate. In tracheal tissue, CTMCs were more abundant in the submucosal layer and on the abluminal surface, especially in the vicinity of smooth muscle cells and in the muscle itself, whereas MMCs were only found in the epithelium (Fig. 2a) and had a smaller and more rounded shape.

Semi-quantitative analysis of mast cell number was done, and the mast cell numbers are shown in Table 2. Pretreatment with compound 48/80 for 7 days in +/+ rats markedly reduced the CTMCs (Table 2), indicating that CTMCs had been depleted. It is of primary interest that the degranulation only occurred in CTMCs, whereas MMCs remained (Fig. 2c). After antigen challenge, both mast cells were degranulated, as indicated by the decrease in mast cell number and/or the degranulation process (picture not shown). Figure 2d showed clearly the degranulation of MMCs in the epithelial layer after ovalbumin challenge.

Contractile response of rat trachea to 5-HT

We have previously found that histamine does not induce the contractile response in rat trachea (5); therefore, we

Data are means ± S.E.M. in nmol/g tissue. *P<0.01, compared to the control. ND, not detected.
induced the contraction with 5-HT. Figure 3 shows the profile of the contractile response of sensitized-tracheal strips to 5-HT. The contractile effect of 5-HT was not significantly different between the two types of rats (+/Gb and Ws/Ws), with the average maximal tension being 0.133 ± 0.013 g/mg tissue (n = 12) that were induced by 30 μM of 5-HT. A similar value was observed concerning the contractile response to 5-HT in tracheal tissue from compound 48/80-pretreated +/+ rats, with the average maximal tension being 0.113 ± 0.016 g/mg tissue (n = 6). Carbachol (30 μM) was used to test the viability of tracheal preparations and induced contractions with the average tension of 0.205 ± 0.021 g/mg tissue (n = 12). The contraction elicited with carbachol at the start of the experiment was not significantly different from that at the end, indicating that the contractility of the preparation was well maintained during experiments.

### Table 2. Mast cell number in rat tracheas

| Mast cell number (cells/section) | Connective tissue mast cell | Mucosal mast cell |
|---------------------------------|-----------------------------|------------------|
| Control +/+ rats                | 30 ± 5 (n = 8)              | 112 ± 8 (n = 8)  |
| Compound 48/80 pretreated +/+ rats | 1 ± 1* (n = 8)              | 102 ± 6 (n = 8)  |
| Ws/Ws rats                      | 0 ± 0* (n = 8)              | 0 ± 0* (n = 8)   |

Data are means ± S.E.M. in cells/section (n = 8, 4 sections for each of 2 tracheas). *P<0.01, compared to each type of mast cells in the control +/+ rats.
Contractile response to ovalbumin and the effect of antagonists

Ovalbumin at the final concentration of 100 μg/ml markedly induced contraction in control sensitized +/+ rats. The contraction was transient, started in 1 min, reached a peak at 3 – 4 min, and then decreased over a period of 8 – 10 min. The average maximum contraction was 45.39 ± 4.23% of 5-HT maximal contraction (30 μM). The pattern of ovalbumin-induced contractions was different from those induced by exogenous 5-HT, probably due to the different concentration of 5-HT present in the organ bath solution. In ovalbumin contraction, mast cells released 5-HT in sufficient amount to induce contractions, however, they were degraded quickly, so the released 5-HT may be insufficient to maintain sustained contraction, as exogenous 5-HT did. No such contraction was observed in isolated trachea of non-sensitized +/+ rats or that of sensitized Ws/Ws rats. In compound 48/80-pretreated +/+ rats, only a negligible contraction was observed. The profile of ovalbumin-induced contraction and its comparison with 5-HT-induced contraction is represented in Fig. 4. The 5-HT$_{2A}$-receptor antagonist ketanserin completely blocked the contraction at a concentration of 0.1 μM, whereas 0.1 μM ONO-1078 did not affect contraction as shown in Fig. 5, indicating that ovalbumin induced the contraction via 5-HT, but not LTD$_4$, released from mast cells.

Release of mediators from mast cells

The results above showed that the rat tracheal contraction was mostly due to 5-HT, not to LTD$_4$ or histamine (5). We, however, failed to obtain a time-course curve of 5-HT release into the organ bath due to the presence of very low concentrations. Therefore, instead of 5-HT, we measured histamine release into the organ bath solution as indicator of exocytosis of mast cells. Histamine did not affect the tracheal contraction, but it was released from mast cells after ovalbumin stimulation. Since histamine and 5-HT release from mast cells show a linear and positive correlation with each other (21), we may assume that the pattern of histamine release from mast cells represents 5-HT release.

Figure 6 shows histamine release from mast cells into the organ bath solution after ovalbumin challenge. The significant increase in histamine release was observed after ovalbumin challenge in sensitized +/+ rats, but only basal/spontaneous histamine release was observed in control (saline-challenged) sensitized +/+ rats. Compound 48/80-
from shown in Table 1, the histamine content of tracheal tissue tyrosine kinase domain of the c-kit MMcs due to the deletion of 12-nucleotide bases in the Kitamura and co-workers, are devoid of both CTMCs and (cells in tracheal tissue. After depletion of CTMCs with our animal experiments. The cell-deficient (cell-deficient (5), one of the immediate responses of asthma. The DISCUSSION

In the present study, we investigated the possible role of MMCs in tracheal contraction. We previously reported that CTMCs make a significant contribution to tracheal contraction (5), one of the immediate responses of asthma. The functional differences between two types of mast cells are of interest to us since recognition of this functional difference is important for understanding mast cell biology and, potentially, for treating mast cell-associated disease, since both types of mast cells have different sensitivity to the drugs.

As in our previous study (5), we employed specific mast cell-deficient (W/Ws) rats and their congenic (+/+ ) rats for our animal experiments. The W/Ws rats, discovered by Kitamura and co-workers, are devoid of both CTMCs and MMCs due to the deletion of 12-nucleotide bases in the tyrosine kinase domain of the c-kit receptor gene (22). As shown in Table 1, the histamine content of tracheal tissue from W/Ws rats was approximately 0.50% that in control (+/+ ) rats. This was representative of the presence of mast cells in tracheal tissue. After depletion of CTMCs with compound 48/80, histamine content in tracheal tissue was markedly decreased in accordance with the decrease in number of CTMCs. The remaining histamine content in tissue is likely to be derived from the MMCs.

Compound 48/80 is known to activate mast cells (CTMC type) by directly stimulating trimeric G proteins, primarily the G subfamily (23, 24) to promote GDP-GTP exchange and dissociation into their constituent βγ- and α-subunits (25). In the cells, compound 48/80 penetrates the plasma membrane to stimulate membrane GTPase activity (26) and stimulates phospholipase C-mediated events (27). However, in MMCs, as studied in RBL-2H3 cells, the expression of G protein is very low (1:7, compared with that of the responsive cells) making it insufficient to stimulate necessary signal for exocytosis (27). This may explain why MMCs were not activated and still remained intact after compound 48/80 pretreatment, whereas CTMCs were degranulated.

It is also of important to take notice of the difference between the numbers of CTMC and MMC in the control condition. Our semi-quantitative analysis showed that there were 4 times more MMCs than CTMCs (see Table 2), and even as much as about 50 times more in whole mounts of rat trachea as reported by Tam et al. (4) in their quantitative study of mast cells in rat trachea. Taken together with the histamine and 5-HT content assay, it was found that with 4 times higher number, the total histamine content of MMCs was about 10% of that of CTMCs, yielding the proportion of about 1:40. This is in accordance with the finding of Tam et al. (4) that CTMCs contain approximately 44 pg histamine per cell, whereas MMCs contain about 1 pg histamine per cell.

In this study, although 5-HT content of MMCs is less than that of CTMCs, we assumed that MMCs might also play role in tracheal contraction via a different mediator from the previously reported one (5). Indeed, less contraction after compound 48/80 pretreatment was one possibility as the 5-HT content decreased, but we also could not rule out the possibility that MMCs also produce and release LTD, that has potent bronchoconstrictor activity in humans, even 3000 times more potent than histamine (28). However in fact, only MMCs alone were not sufficient to provide an appropriate condition to induce a significant contractile response as shown by compound 48/80-pretreated rats (Fig. 4).

In this present work, we studied the effect of several antagonists in +/+ rat tracheal contraction to clarify the mechanism underlying the contractile response to ovalbumin. Ketanserin, a 5-HT antagonist (0.1 μM), completely prevented contraction, whereas the LTD antagonist ONO-1078 (0.1 μM) did not, indicating that LTD was not responsible for the contractile response and that the contraction was mostly due to 5-HT. Since rat trachea was found...
to be insensitive to histamine (5), due to the lack of H1 receptors in rat trachea, the possible anti-histamine action of ketanserin might be excluded. ONO-1078 at the same concentration was reported to inhibit contraction of guinea pig trachea and lung parenchymal strips induced by LTC4 and LTD4 (29). It is interesting to note that LTD4 had no significant effect, since both types of mast cells could be stimulated by immunologic stimuli, and MMCs are capable of releasing leukotriene (6). We did not measure the release of LTD4 from tracheal tissue during contraction; however, Nishida and colleagues (30) reported the release of LTC4 from lung mast cells (MMC type) of +/+ rats after antigen challenge, suggesting that leukotrienes might also be released from MMCs of rat trachea after such antigen stimulation. Therefore, the possible explanation concerning the lack of leukotriene response is that there may be no or very few leukotriene receptors in rat trachea. These evidences show that even though there are more MMCs than CTMCs, and the capability of MMCs to produce leukotrienes, MMCs contribute less to rachetal contraction in rats.

There are several advantages of using Ws/Ws rats and their congenic +/+ in this study. First, while the number of mast cells was different in each type of rat, there was no significant discrepancy in IgE production following ovalbumin sensitization, as shown in Fig. 1. Similar evidence was found in the same strain of rats sensitized with nematode *Nippostrongylus brasiliensis*, as reported by Nishida and colleagues (30), in that there was no difference in the increase of IgE and IgG antibodies between +/+ and Ws/Ws rats. Since the rat models used in the present study induced IgE synthesis after sensitization regardless of mast cell number, the rats provide a good model for studying the role of mast cells in the IgE-associated immune response.

Second, our previous data showed that the contractile response to carbachol and 5-HT in both types of rats was relatively similar (5), and in this study, the contractile response induced by antigen challenge was significantly different between +/+ and Ws/Ws rats. This provides direct evidence that mast cells play important role in tracheal contraction. Third, we might study further the functional difference of individual types of mast cells (CTMCs and MMCs) on tracheal contraction by modulating the mast cells existence using compound 48/80. By using Ws/Ws rats and their congenic (+/+ ) rats, we could modulate several stages of mast cell existence: normal number with intact CTMCs and MMCs, the absence of CTMCs, and the absence of both types of mast cells as a negative control.

In fact, there is limited data available concerning the role of MMCs in respiratory airways. The lack of MMCs function in both early and late airway response was also reported by Du and colleagues (31). Treatment with recombinant human IL-3 increased mast cell number, particularly MMCs in airway regions, but no significant effect in magnitude of either the early or late airway response was observed.

Only one study by Yang et al. (32) reported the possible role of MMCs in antigen transport across tracheal epithelium in rats sensitized with horseradish peroxidase. They did not clearly mention the type of mast cells involved in their study, although, the cells seemed to be mucosal mast cells since the mast cells studied were located in the epithelial layer of tracheal tissue. They demonstrated that sensitization increases uptake of specific antigen initially via an endosomal transcellular pathway across tracheal epithelium and that after the hypersensitivity reaction, mast cell-dependent recruitment of the paracellular pathway further augment antigen influx into airway tissue, which was not observed in mast cell-deficient Ws/Ws rats.

Recent evidence regarding the ability of mast cells to recognize and aggressively react to a wide range of bacteria (33) suggested another possible role for MMCs. Being ideally positioned in contact with the external environment, MMCs may be one of the first inflammatory cells encountered by microorganisms invading the respiratory epithelium (33). This role, however, does not provide direct evidence for a relationship with asthmatic features.

From our findings, we conclude that MMCs, although superior in number than CTMCs, make little or less contribution to tracheal contraction, and they might have another function in airway response. These results support the body of evidence regarding the functional heterogeneity of CTMCs and MMCs, particularly in airway region. A study to investigate the role of MMCs in asthmatic disease is being carried out in our laboratory. Finally, we also conclude that Ws/Ws rats provide a good tool for studying the role of mast cells in the IgE-associated immunological response.

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