

Protease phenotype of constitutive connective tissue and of induced mucosal mast cells in mice is regulated by the tissue

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Mouse mast cells (MCs) express a large number of serine proteases including tryptases, mouse mast cell protease (mMCP)-6 and -7; chymases, mMCP-1, -2, and -4; and an elastase, mMCP-5; along with carboxypeptidase-A3 (CPA3). In helminth-infected mouse intestine, distinct protease phenotypes are observed for connective tissue MCs (CTMCs) (mMCP-4⁺-7⁺, and CPA3⁺) and mucosal MCs (MMCs) (mMCP-1⁺ and 2⁺). To determine whether the protease phenotype was regulated by the tissue, we compared the phenotype of constitutive CTMCs and induced MMCs in trachea and large airways in antigen-sensitized unchallenged and challenged mice to MCs in skin and helminth-infected intestine. We found that in the trachea, unlike in skin and intestine, CTMCs and MMCs both express all six serine proteases and CPA3 (mMCP-1⁺, -2⁺, 4⁺-7⁺, CPA3⁺). This phenotype also holds for the lung CTMCs in the proximal bronchi, whereas the induced MMCs express only four proteases, mMCP-1, -2, -6, and -7. Thus, the T-cell-dependent induction of MMCs in trachea, large bronchi, and small intestine provides numbers but does not determine the protease phenotype. Furthermore, the CTMCs, which are constitutive, also show striking differences at these tissue sites, supporting the view that the differences in expression are tissue directed and not dependent on inflammation.

A phenotypic difference between constitutive connective tissue mast cells (CTMCs) and helminth-induced T-cell-dependent mucosal MCs (MMCs) was first described in rats by Enerback, on the basis of their different fixation properties in the jejunum (1). Decades later, the constitutive CTMCs in small intestine, peritoneum, and skin of mice were shown to express the chymase mMCP-4, the elastase mMCP-5, and two tetrameric tryptases, mMCP-6 and -7, whereas the helminth-induced MMCs of the small intestine expressed only two chymases, mMCP-1 and -2 (2–4). However, it was noted that the MMCs in the lamina propria during the resolution phase of a *Trichinella spiralis* infection underwent a modulation of the protease phenotype so as to express mMCP-5 or -6 along with mMCP-2 (4, 5). A MC-specific carboxypeptidase, CPA3, is also associated with murine CTMCs in the serosal cavity and skin and absent in MMCs (6, 7). The mucosal protease, mMCP-1 has been shown to increase from a constitutive plasma baseline by 80- to 100-fold during expulsion of the adult *T. spiralis* in concert with a similar expansion of MMCs in WT mice and clearance of the adult worms is impaired in a mMCP-1-deficient strain, revealing a role in host defense (8). Physiologic roles for the chymase mMCP-4 include regulating intestinal permeability (9) and turnover of fibronectin (10). In a sensitization and pulmonary challenge model, a deficiency of mMCP-4 increased some features of airway remodeling (11). In a pharmacologic approach, administration of the tryptase mMCP-6 to WT mice was shown to enhance neutrophil extravasation and innate host defense against a *Klebsiella pneumoniae* infection of the lung and peritoneal cavity (12, 13). Thus, distinct *in vivo* functions of specific mouse MC proteases are now being recognized in models of host defense and inflammatory injury.

The human MC expresses a tryptase, beta 1, a chymase, and a carboxypeptidase that are homologous to mMCP-6, mMCP-4, and mouse CPA3, respectively (10, 14). These proteases are expressed in intestinal and skin CTMC (often termed MC_{TC}), but only tryptase is present in most MMCs (termed MC_T) of intestine and lung (15, 16). The MMCs are considered to be T-cell dependent, on the basis of their absence in the gastrointestinal mucosa of individuals with acquired severe immune deficiency (17). Recently, the reported protease exclusivity of these two phenotypes in human MCs has been challenged by the findings of increased transcripts for tryptase and CPA3 but relatively fewer chymase transcripts in bronchial mucosal biopsies from asthmatic patients showing increased MMCs and a high Th2 transcript phenotype (18). Similarly, in patients with eosinophilic esophagitis, the associated MC hyperplasia correlated with increased transcripts for tryptase and CPA3 but not chymase (19).

An increase in lung MCs is well documented in mouse models of allergic pulmonary inflammation (20, 21); however, there has been no previous attempt to describe the overall protease phenotype of constitutive and induced MCs in the trachea and proximal airways. We now find that the definitions of constitutive CTMCs (mMCP-4⁺-7⁺ and CPA3⁺) and induced MMCs (mMCP-1⁺ and -2⁺), which are based on their protease phenotypes in intestine (4, 5) and supported by that of CTMCs in skin (6), do not hold for lung. Rather, in mouse trachea, both CTMCs and MMCs express both tryptases, mMCP-6 and -7; three chymases, mMCP-1, -2, and -4; an elastase, mMCP-5; and CPA3, revealing no distinction between constitutive and induced subclasses. In the proximal bronchi, the protease profile of CTMCs is like that of the trachea, whereas the induced MMCs express both tryptases and the chymases, mMCP-1 and -2, but do not express mMCP-4, mMCP-5, or CPA3. Most importantly, the induced profile of proteases by allergic inflammation in trachea or large airway is distinct from that of the *T. spiralis*-infected small intestine, indicating that in the BALB/c strain, Th2 inflammation alone does not define this expression. Rather, T-cell-driven inflammation predominantly determines the induction of the MMC hyperplasia from a negligible/minimal background number. Equally compelling is the finding that in sensitized, unchallenged mice, the T-cell-independent CTMCs in trachea and large airways express chymases not present in CTMCs in small intestine or skin. Together, these findings support a tissue lineage for MCs that, whether constitutive or expanded by T-cell host responses, assumes a secretory granule

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protease phenotype that is highly regulated by the host tissue site. Understanding the functional implications of these prominent tissue-specific MC phenotypes of constitutive and induced MCs is a compelling future direction.

Results

Increase in Lung MC Progenitor (MCp) Numbers and in Mature MMCs in the Trachea and Large Bronchi After Seven Daily Challenges with Aerosolized Ag in OVA-Alum Sensitized BALB/c Mice. In our previous studies focused on the recruitment of MCp to lung, we sought to avoid their maturation to induced MMCs by using only three challenges of sensitized mice with aerosolized Ag. We routinely assessed the MCp 1 d after the last challenge by limiting dilution and clonal expansion, a high throughput assay that we had confirmed by identification of MCp by flow cytometry (22). We did not find any mature MCs in the lung mononuclear cell (MNC) preparations by cytochemistry. We also did not observe any increase in MMCs in the large bronchi or trachea by histochemistry 1 d after the last challenge (22–24) but did note a small increase in tracheal intraepithelial MMCs 7 d after the last challenge (22). Although others have provided protocols of longer duration that increase numbers of pulmonary MMCs (20, 21, 25), we sought to develop a protocol of less than 1 mo in duration. Thus, in the same cohort of BALB/c mice, we monitored both the recruitment of MCp within the MNC population isolated from lung and the appearance of MMCs in the lung and trachea. We arrived at a protocol in which we again sensitized mice with OVA-alum on days 0 and 7 but followed with seven daily aerosolized Ag challenges (days 17–23) with assessments on days 24, 27, and 30 (Fig. 1A). We assessed for both MCp per 10^6 lung MNCs and for CTMCs and MMCs in trachea and large bronchi in parallel cohorts at each of the three time points. MCp were assessed by limiting dilution and clonal expansion and mature MCs by histochemistry with chloroacetate esterase (CAE) reactivity. Values from both assays for sensitized and challenged mice were compared with sensitized but unchallenged controls.

One day after seven challenges, there was a robust increase in pulmonary MCp concentration (MCp/ 10^6 MNC) (~ 19 -fold), in total lung MCp per mouse (~ 136 -fold) and in the total number of MNCs recovered from the lung (~ 6.2 -fold) relative to the sensitized but not challenged controls (Fig. 1B). In another cohort, 1 d after the seventh challenge, there was a striking appearance of intraepithelial MMCs in the trachea and large bronchi (200–250 μm) from a zero baseline in sensitized but unchallenged mice (Fig. 1C). The concentration of MCp and their numbers per mouse lung fell off significantly and selectively by postchallenge day 4, compared with the concomitant reduction of lung MNCs, which did not reach statistical significance even at day 7 postchallenge. At day 4 postchallenge, the numbers of induced MMCs in trachea and large bronchi were still comparable to the increment at day 1, and these numbers were still present at day 7 in another cohort (Fig. 1C). The significant decrease in the concentration of lung MCp/ 10^6 MNCs at the time of induction of intraepithelial MMCs in the trachea and large airways of parallel cohorts suggests that a rapid maturation of recruited MCp may account for the increase in the number of MMCs, which then persisted through the 7 d of study.

In contrast, there was no increment in numbers of submucosal CTMCs in the trachea at day 1 postchallenge but rather a slight decrease. The decrease is likely due to degranulation, although no extracellular granules were detected. Assessing MC degranulation by monitoring the appearance of extracellular granules is difficult even in passive cutaneous anaphylaxis and recording the response as reduced total MC numbers is reasonable (26). We cannot exclude the possibility that some of the decrease in CTMCs that occurs at the same time as the increase in MMCs may be due to migration of these CTMCs into the epithelial compartment. We

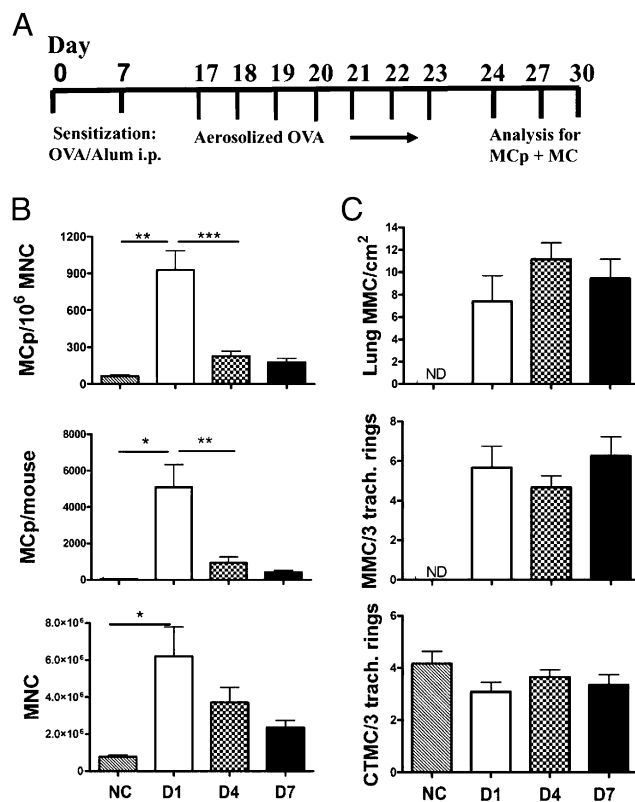


Fig. 1. Time course showing the changes in the numbers of lung MCp's and MCs in trachea and large airways of sensitized BALB/c mice on various days after the last of seven daily challenges. (A) The protocol of OVA-induced airway inflammation. BALB/c mice were sensitized with OVA adsorbed to alum on days 0 and 7 and either not challenged (NC) or challenged with aerosolized OVA daily on days 17–23. Mice from the original cohort were analyzed on day 24 (D1 postchallenge), day 27 (D4 postchallenge), or day 30 (D7 postchallenge). (B) The number of lung MCp's per 10^6 MNCs (Top), total lung MCp's per mouse (Middle), and isolated lung MNCs per mouse (Bottom) from NC mice or from challenged mice evaluated on D1, D4, or D7 postchallenge. The challenged values are the mean (\pm SE) from four separate experiments at each time point with 12 mice/group and the NC values are from 6 mice (two experiments). (C) The number of intraepithelial MMCs per cm^2 in large bronchi (greater than 200 μm , Top), the number of MMCs per three tracheal rings (Middle), and the number of submucosal CTMCs per three tracheal rings (Bottom). Values are the mean (\pm SE) from NC: 6 mice, two experiments; D1: 12 mice, four experiments; D4: 30 mice, nine experiments; and D7: 20 mice, six experiments. ND, none detected; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

also saw no degranulation of MMCs as evidenced by extracellular granules around the cells, and the absence of any MMCs in the unchallenged mice eliminates the possibility of seeing a reduction in number. Overall, the findings for induced MMCs in our protocol were similar to those reported with extended protocols for inflammation, although the remodeling observed in these longer protocols in terms of smooth muscle hypertrophy/hyperplasia, subepithelial fibrosis, collagen deposition, and increased angiogenesis, was absent (20, 21).

Specificity of the Abs Used to Define the Protease Phenotype of CTMCs and MMCs. To determine the protease phenotype of the MCs found in trachea and large airways, we first evaluated the specificity of the various antipeptide IgGs using the appropriate protease-deficient mice that have become available since these Abs were first designed. We used dorsal skin from WT and protease-deficient mice to establish that our Abs directed to mMCP-4–7 did not recognize some other secretory granule

protease. All samples contained CAE-reactive MCs. As shown in Fig. 2A, anti-mMCP-4-6 reacted with CTMCs in C57BL/6 skin but not with CTMCs in the skin of mice specifically deficient in these proteases. The specificity of mMCP-7 was confirmed by showing the reactivity in skin of BALB/c mice, which express mMCP-7, whereas there was no reactivity in the skin of C57BL/6 mice, which do not express this protease (27). The specificity of mAb to mMCP-1 has been previously reported (28) and was confirmed by showing reactivity in the helminth-induced MMCs of the intestine of BALB/c mice but not in the CTMCs in the intestinal submucosa (Fig. 2B) or in skin of C57BL/6 mice. Mice lacking mMCP-2 are not available. For the helminth-infected intestine, the MMCs showed only mMCP-1, whereas the CTMCs of the submucosa showed mMCP-4-7 and CPA3, but not mMCP-1. These findings with reagents of proven specificity confirmed the earlier data of a segregated protease profile for MMCs and CTMCs in the helminth-infected BALB/c small intestine (4, 5).

Broad and Identical Protease Phenotype of CTMCs and MMCs in the Trachea. The trachea is a mucosal-lined hollow tubular structure consisting of 15-18 evenly spaced parallel C-shaped rings of hyaline cartilage within and separated by fibroelastic tissue. The free dorsal ends of the cartilages are connected by bands of smooth muscle and connective tissue fibers. The wall of the trachea consists of mucosa, submucosa, cartilage-associated smooth muscle, and surrounding adventitia. The adventitia is loose connective tissue that is primarily composed of blood vessels, nerves, and adipose tissue surrounding the trachea.

In sensitized but not challenged BALB/c mice, the CAE positive MCs of the trachea were located in the connective tissue of the submucosa and adventitial layers and there were none in the mucosa. The submucosal tracheal CTMCs of unchallenged mice expressed each of the seven proteases studied, namely the chymases mMCP-1, -2, and -4; the elastase mMCP-5; both tryptases, mMCP-6 and -7; and the carboxypeptidase, CPA3 (NC, not

challenged; Fig. 3). The protease phenotype of CTMCs in adventitia of these unchallenged mice was the same as that in the submucosa. The CTMC in the submucosa and adventitia in the challenged mice showed the same seven proteases as in unchallenged mice (CH, challenged; Fig. 3). Thus, CTMCs of the trachea of sensitized BALB/c mice with or without challenge express the chymase mMCP-1, which is not seen in submucosal CTMCs of the small intestine or skin of the BALB/c strain and has been considered the hallmark of the jejunal MMCs (28, 29).

Even more surprising was the finding of all seven of these proteases in the induced intraepithelial MMCs of the trachea (CH, Fig. 3). These MMCs were situated between tracheal epithelial cells above the basement membrane and were somewhat less granulated than the CTMCs, suggesting that they either had experienced some degranulation following Ag provocation or that they were newly arisen. Their expression of both tryptases, mMCP-6 and -7, the chymase mMCP-4, the elastase mMCP-5, and the carboxypeptidase CPA3, contrasts sharply with the prior characterization of mouse MMCs in the jejunum (Fig. 2B), which lack these five enzymes and express only the chymases, mMCP-1 and -2 (4, 5, 29).

Broad Protease Phenotype of the CTMCs and MMCs of the Large Airways in the Lung. The trachea terminates at a main bifurcation (the carina) and gives rise to two main extra pulmonary bronchi

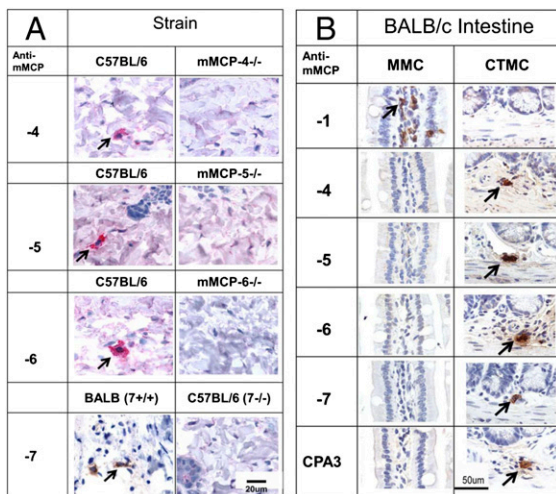


Fig. 2. Evaluation of the specificity of the Abs directed to mMCP-4-7 peptides and of the mAb to mMCP-1. (A) Demonstration of the immunodetection of proteases mMCP-4-6 in dorsal skin of C57BL/6 mice and the absence of each protease in the respective strain with a targeted protease deficiency. To evaluate the specificity of the anti-mMCP-7 IgG, dorsal skin from mMCP-7⁺ BALB/c mice was compared with that from the naturally mMCP-7-deficient C57BL/6 strain. Magnification is indicated by the bar in Lower Right corner. (B) Demonstration of the immunodetection of mMCP-1 in intestinal MMCs and its absence in the intestinal submucosal CTMCs of *T. spiralis*-infected BALB/c mice and conversely, the lack of mMCP-4-7 and CPA3 in the MMCs and their presence in the CTMCs. Arrows indicated MCs positive for the various proteases.

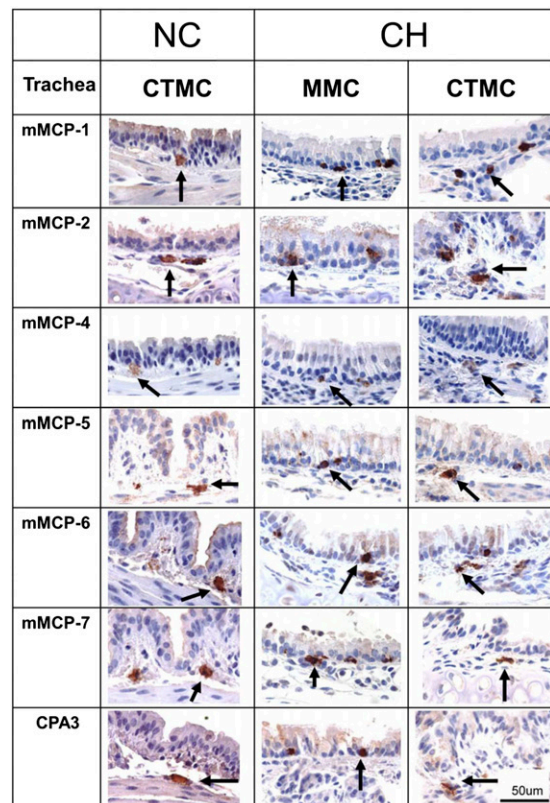


Fig. 3. Both the constitutive CTMCs and the induced MMCs of the trachea express all seven secretory granule proteases. The Left column indicates the antiprotease specificity used to define the protease phenotype of tracheal MCs. Mice were either sensitized and not challenged (NC) or challenged (CH) for 7 d with analysis on day 4 postchallenge. Arrows indicate the protease reactive CTMCs in the submucosa of the trachea from NC and CH mice (Left and Right, respectively) and the induced intraepithelial tracheal MMCs from CH mice (Middle) that each expressed mMCP-1, -2, -4-7, and CPA3. No MMCs were found in the epithelium of trachea from NC mice. Images are representative of the results of analysis of three mice per group from three separate experiments. (Scale bar, 50 μ m.)

that enter the left and right lung lobes and ramify in a monopodial branching pattern. There are no cartilaginous rings in the walls of bronchi within the lung. The pulmonary arteries accompany the airways in a sheath of connective tissue, which is defined as a bronchovascular bundle. The wall of an intrapulmonary bronchus consists of mucosa, a narrow layer of smooth muscle, submucosa, and adventitia. In the lungs of sensitized but unchallenged BALB/c mice, the CAE-positive MCs were distributed in the adventitial connective tissue surrounding the primary left and right lobar bronchi of at least 200 μm diameter, as observed for large bronchi in the rat (30). These CTMCs in the bronchi of unchallenged (NC, Fig. 4) or challenged mice (CH, Fig. 4), were positive for mMCP-1, -2, -4-7, and CPA3. Again, the finding of the chymase mMCP-1 in CTMCs was not expected, on the basis of the literature for CTMCs in jejunum and skin.

In sensitized but not challenged mice, no intraepithelial MMCs were observed in the airways of the bronchovascular bundles in lung by either CAE reactivity or by immunohistochemistry using Abs specific to these seven different MC proteases. After challenge, the MMCs appearing in the epithelial lining of the large

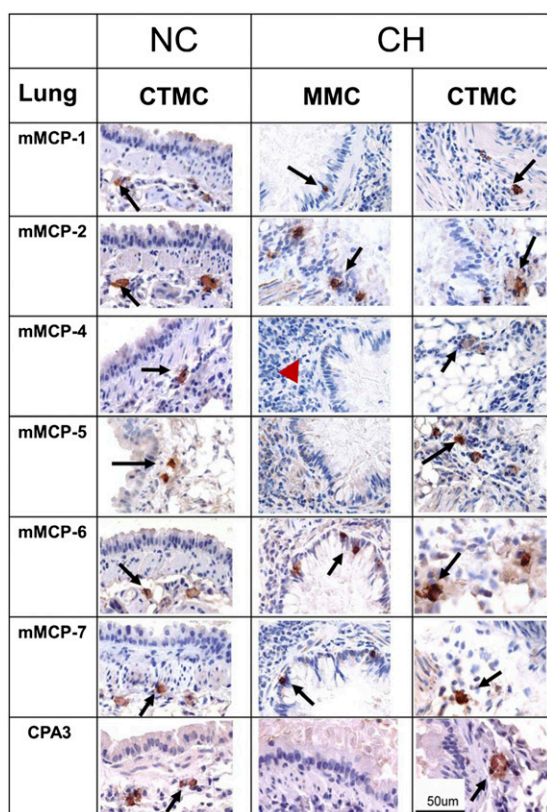


Fig. 4. The constitutive CTMCs of the large bronchi in the lung express all seven secretory granule proteases, whereas the induced MMCs do not express mMCP-4, -5, or CPA3. The *Left* column indicates the antiprotease specificity used to define the protease phenotype of the MCs in the large bronchi in the lung. Images are from the bronchovascular bundles from the lung of the same animals depicted in Fig. 3. Arrows indicate the MCs positive for mMCP-1, -2, -4-7, and CPA3 in CTMCs around the large bronchi in the lung of unchallenged (NC) and challenged (CH) mice (*Left and Right*, respectively). Lung MMCs in the epithelium of the large airways of CH mice (*Middle*) expressed mMCP-1, -2, -6, and -7, and do not express mMCP-4, -5, or CPA3. The cellular infiltrates (red arrowhead) found in the bronchovascular bundles of the CH mice are highlighted in a single panel. No MMCs were found in the bronchial epithelium of NC mice. Images are representative of the results of analysis of three mice per group from three separate experiments. (Scale bar, 50 μm .)

bronchi and assessed at day 4 expressed the chymases mMCP-1 and -2 and the tryptases mMCP-6 and -7 but not the chymase mMCP-4, the elastase mMCP-5, or CPA3 (CH, Fig. 4). The bronchovascular bundles were surrounded by cellular infiltrates with prominent eosinophils and lymphocytes and the bronchi showed desquamated epithelium, goblet cell metaplasia, and mucus plugging. The cellular infiltrate is noted by a red arrow in one of the panels showing MMCs (CH, Fig. 4). We did not observe any typical features of airway remodeling, such as thickening of basement membranes or hypertrophy of the bronchial wall muscle.

Discussion

Our findings that CTMCs and MMCs of lung and trachea exhibit a broad protease phenotype, which does not match that observed for CTMCs in mouse intestine and skin or for MMCs in intestine, imply several different lessons of which the foremost is that the protease phenotype of constitutive and T-cell-induced MCs is determined by the local host tissue. We had previously emphasized the distinction in protease profiles of intestinal CTMCs and MMCs as further defining the existence of these two subclasses by location and as reflecting the role of T cells in providing the MMCs but not CTMCs. However, we had recognized that these findings could reflect the broader role of the microenvironment on the basis of observing a mixed protease phenotype of MMCs in the intestinal lamina propria during resolution of a *T. spiralis* infection in which various lamina propria MMCs gained expression of mMCP-5 or mMCP-6 while retaining expression of mMCP-2 (4, 5). The role of the microenvironment was further supported by experiments using a cloned cell line developed by infection of bone marrow cells with a replication-deficient v-abl virus. In BALB/c mice injected i.v. with the transformed progenitor line expressing mMCP-5 and -6, the cells that developed into mature MCs in liver and spleen expressed these proteases and mMCP-2 and -7, whereas those expanding just below the intestinal epithelium in the small intestine turned off expression of mMCP-5 and -6 and expressed only mMCP-2 (7). The current findings now establish different protease phenotypes for CTMCs and induced MMCs at different tissue sites revealing local regulation at baseline and even in the presence of T-cell-dependent expansion. Thus, on the basis of comparative studies of gut and lung in the same strain, it appears that the immune response of T cells drives the increased appearance of MMCs, whereas the local tissue is the more important regulator of protease expression.

A possible second lesson relates back to the inability of investigators to derive intestinal-type MMCs expressing only mMCP-1 and -2 by culture of MCs from mouse bone marrow (BM). Immature mouse BM-derived MCs (BMMCs) developed by culture with stem cell factor, expressed mMCP-4-6 transcripts but not mMCP-1 and -2. Culture of BMMCs with IL-9 or IL-10 induced expression of mMCP-1 and -2, but without deletion of mMCP-5 or -6 (31-33), providing a protease phenotype not seen in the intestine at rest or with *T. spiralis*-induced inflammation. We now see that a similar complex protease profile holds for CTMCs of mouse trachea and large airways in lung and for MMCs in trachea. That mMCP-1 and -2 were present in the CTMCs of sensitized but unchallenged mouse trachea indicates that an induced local Th2 response was not needed for their expression. Miller and colleagues have found that TGF β 1 can induce mMCP-1 in BMMCs and that the inducing action of IL-9 for mMCP-1 is TGF β 1 dependent (34). As TGF β 1 is constitutively present in the lung, is up-regulated by allergic inflammation, and can be activated by the induced expression of $\alpha\beta$ 6 integrins, this may be the mechanism by which the CTMCs and MMCs in this tissue express this chymase (35). However, the sustained expression of the other proteases is likely a function of stem cell factor and other local cytokines. Early studies demonstrated that BMMCs cocultured

with a mouse fibroblast line increased heparin synthesis, histamine storage, and granule maturation, and this was ascribed to fibroblast production of stem cell factor (36, 37). More recently, a second fibroblast-derived factor, IL-33, which is an amplifier of innate mucosal immunity, was shown to augment mMCP-6 expression (38). Although T-cell-derived factors can generate and expand MMCs, it appears that stromal factors are the key regulators of the tissue protease phenotype. Addressing the mechanisms of the differential protease expression of pulmonary MCs is difficult, as the absence of the stromal factors implicated from in vitro studies, stem cell factor, and TGF β 1, results in a lack of all MCs and the presence of basal pulmonary inflammation, respectively (39, 40).

A third point is that the expression of the tryptases in lung CTMCs and MMCs is consistent with a role for these proteases in previous studies of host defense to bacterial infection and allergic pulmonary inflammation. Both human beta 1 tryptase and its murine ortholog, mMCP-6, caused a selective influx of neutrophils when instilled into the lung of BALB/c or C57BL/6 mice, and MC-deficient mice treated with active but not inactive human tryptase beta 1 were protected when challenged with *K. pneumoniae* 24 h later (14). This host defense function of mMCP-6 was supported by the selective recruitment of neutrophils into the peritoneal cavity after direct instillation (12) and by the impaired resolution of *K. pneumoniae* infection of the peritoneal cavity in mMCP-6-deficient mice (13). In contrast, in an immune complex-based arthritis model, a target of tryptase cleavage with pathologic consequences was the proteoglycan of the articular cartilage, aggrecan (41). Further, in two different mouse models of antigen sensitization and challenge-induced pulmonary inflammation and in atopic asthmatic humans, tryptase inhibitors have been shown to reduce various aspects of the airway inflammation. In a model of OVA/alum sensitization followed by four intratracheal OVA challenges, administration of a selective tryptase inhibitor intranasally 30 min before the last two challenges reduced the influx of eosinophils and the levels of IL-4 and IL-13 in the bronchoalveolar lavage (BAL) (42). There was also a decrease in the cellular infiltrate in the bronchovascular bundles and in goblet cell hyperplasia and mucus secretion. In a model using mice sensitized and challenged with intratracheal house dust mite extract, DerP, systemic administrations of inhibitors of limited selectivity decreased the numbers of eosinophils and neutrophils in BAL

(43). Furthermore, Krishna et al. (44) using a selective tryptase inhibitor found decreased late-phase bronchoconstriction in allergen-challenged atopic asthmatics. Thus, our observation of a robust constitutive and induced expression of the tryptases in the pulmonary tract likely underlies the prominent role these proteases play in innate responses linked to microbial host defense and allergic inflammation in the mouse airways.

A final point from these studies relates to our findings for mMCP-1 in CTMCs in submucosa and adventitia of trachea and large airways. The seminal studies of Miller and colleagues identified expression of this protease in jejunal MMCs and its liberation into the serum with helminth infection was consistent with this interpretation (28, 29). More recently, it was noted that mMCP-1 could be found in the MMCs induced in the airways of mice subjected to chronic allergen challenge (20) and that allergic reactions occurring in the lung with conventional protocols also increased serum mMCP-1 levels (43). We even noted mMCP-1 accumulation on alveolar macrophages as an innate systemic effect of hind limb reperfusion injury, wherein there was complement activation and transient permeability changes in the lung (45). In the absence of MMCs, we now suggest that the mMCP-1 was derived from peribronchial CTMCs. Further, mMCP-1 may have contributed to the change in lung permeability in this trauma model by analogy to its role in the intestine during worm expulsion (46).

Materials and Methods

Animals. BALB/c and C57BL/6 mice were obtained from Taconic Laboratories. The protease-deficient mice have been described (8, 10, 13, 47). See [SI Materials and Methods](#) for more complete descriptions.

Ag Sensitization, Aerosol Challenge, *T. spiralis* Infection, and Pulmonary MCP Assessment. These procedures have been described previously (4, 23, 48).

Histochemical and Immunohistochemical Evaluations of MCs. For the histochemical evaluation of mature MCs, the tissues were prepared as described previously (4, 22). For immunohistochemical evaluation of the proteases, mMCP-1 was performed using a mAb from R&D Systems (28). The other proteases were detected using polyclonal antipeptide IgGs prepared in rabbits (7, 27, 49–52).

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