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J Immunol 1998; 160:5537-5545;
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Reversible Expression of Tryptases and Chymases in the Jejunal Mast Cells of Mice Infected with *Trichinella spiralis*1

Daniel S. Friend,2a‡ Namit Ghandiyal,1,§ Michael F. Gurish,1,§ John Hunt,†§ Xuzhen Hu,§ K. Frank Austen, †§ and Richard L. Stevens2†§

It has been established that mouse mast cells (MCs) can reversibly alter their expression of serglycin proteoglycans and the homologous granule chymases that have been designated mouse MC protease (mMCP)-1, mMCP-2, and mMCP-5 in vivo. Nevertheless, it remained to be determined whether these immune cells could modify their expression of other chymases and the granule tryptases mMCP-6 and mMCP-7. As assessed immunohistochemically, we now show that MCs reversibly change their expression of the recently described chymase mMCP-9 and both tryptases as these cells traverse the jejunal during the amplification and regression stages of the reactive MC hyperplasia. In noninfected mice, most jejunal MCs reside in the submucosa and express mMCP-6 and mMCP-7, but not mMCP-9 or the chymase mMCP-2. During the inductive phase of the helminth-induced inflammation, when the jejunal MCs move from the submucosa to the tips of the villus, the MCs briefly express mMCP-9, cease expressing mMCP-6 and mMCP-7, and then express mMCP-2. During the recovery phase of the inflammation, jejunal MCs cease expressing mMCP-2 and then express varied combinations of mMCP-6, mMCP-7, and mMCP-9 as they move from the tips of the villus back toward the submucosa. In other model systems, mMCP-6 elicits neutrophil extravasation, and mMCP-7 regulates fibrin deposition and fibrinogen-mediated signaling events. Thus, the ability of a jejunal MC to reversibly alter its tryptase expression during an inflammatory event has important functional implications. The Journal of Immunology, 1998, 160: 5537–5545.

In 1966, Enerbäck (1) made the seminal observation that the mast cells (MCs)1 that increase in number in the jejunum of helminth-infected rats and mice (2–8) differ histochemically from the MCs that reside in the peritoneal cavity. All MCs store large amounts of serglycin proteoglycans in their secretory granules (9, 10); and the proteoglycans that are present in the secretory granules of rat peritoneal MCs have predominantly heparin chains (11), whereas those in rat jejunal MCs have predominantly chondroitin sulfate E and/or chondroitin sulfate di-B chains (12, 13). Because jejunal and peritoneal rat MCs differ morphologically (14), biochemically (15–19), and functionally (20, 21), it was thought that these two populations of rat MCs (designated by some as mucosal MCs and connective tissue MCs, respectively) were developmentally unrelated.

The ability to induce a mature rat peritoneal MC to synthesize chondroitin sulfate E within minutes after exposure to β-d-xyloside (22, 23) led to the realization that rat peritoneal and jejunal MCs are closely related cell types. The β-d-xyloside experiments also revealed for the first time that a mature, nontransformed MC could quickly alter its histochemistry and proteoglycan expression in vitro. Although IL-3-developed mouse bone marrow (BM)-derived MCs preferentially express chondroitin sulfate E-containing serglycin proteoglycans when cultured alone (24), these in vitro-differentiated MCs preferentially express heparin-containing proteoglycans when cocultured in the presence of fibroblasts (25). The detection of hybrid mouse BM-derived MCs in the fibroblast co-cultures containing both safranin“ ” and safranin“ ” granules indicated that mouse MCs also can change their granule proteoglycan phenotype in vitro when exposed to different environments. Although adoptive transfer experiments conducted by Kitamura and coworkers on MC-deficient W/W” mice provided evidence that mouse MCs could change their histochemistry and proteoglycan expression in vivo (26–30), hybrid stainable granules were not routinely found in the MCs of the reconstituted animals. Thus, Kitamura and coworkers (31) speculated that MCs probably undergo “trans-differentiation,” dedifferentiating into unrecognizable MCs before acquiring their new granule proteoglycan phenotype. Besides serglycin proteoglycans, mouse MCs also store varied combinations of a carboxypeptidase activity and at least seven chymases (designated mouse MC protease (mMCP)-1 to mMCP-5, mMCP-8, and mMCP-9) and two tryptases (mMCP-6 and mMCP-7) in their granules (32–43). Using gene-specific probes and protease-specific Abs, at least five distinct populations of MCs have been detected in various tissues of the BALB/c mouse. For example, MCs that differ in their protease expression have been found in the peritoneal cavity (33, 34, 36–38), uterus (43), skin (44), spleen (45), and jejunal epithelium (8, 32, 35) of this mouse strain. Which chymase a MC expresses in the BALB/c mouse seems to be dictated by both the current and previous microenvironment of the cell. We recently reported that the MCs in the jejunum of *Trichinella spiralis*-infected BALB/c mice undergo time- and strata-dependent changes in their expression of the three chymases mMCP-1, mMCP-2, and mMCP-5 (8). Helminth-infected mice and rats experience a transient, but pronounced, T cell-dependent increase in the number of their jejunal MCs (2–8). Within 1 wk after BALB/c mice have been infected with *T. spiralis*

1 Abbreviations used in this paper: MC, mast cell; BM, bone marrow; AAK, Z-Ala-Ala-Lys-4-methoxy-2-naphthylamide; mMCP, mouse mast cell protease; AP, alkaline phosphatase.

Received for publication July 29, 1997. Accepted for publication January 14, 1998.

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This work was supported by National Institutes of Health Grants AI-23483, AI-22531, AI-31599, AR-07530, and HL-36110.

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larvae, increased numbers of MCs appear in the crypts at the base of the villi, and the number of MCs throughout the villi increases by >25-fold by wk 2. During the resolution phase of the helminth infection, MCs initially disappear from the tip of the villus, then from the mid-region of the villus, and finally from the lower villus. At the height of the helminth infection, only intraepithelial MCs possess stellate-shaped granules containing crystalline structures (8, 46). The retention of such granules with fragmented crystals in lamina propria MCs during resolution of the infection indicates that at least some MCs migrate through the jejunal strata during the different phases of the inflammation (8). At the height of the infection, MCs found in the muscle expressed mMCP-5 but not mMCP-1 or mMCP-2, even though most MCs in the epithelium at this time point expressed mMCP-1 and mMCP-2 but not mMCP-5. Accompanying these two MC populations were transitional forms in the submucosa that expressed mMCP-5 and mMCP-2 but not mMCP-1 and forms in the lamina propria that expressed mMCP-2 alone. In the recovery phase of the infection at wk 4, MCs sequentially cease expressing mMCP-1, express mMCP-5, and finally cease expressing mMCP-2 as they progressively move from the tip of the villus to the submucosa. BALB/c mouse BM-derived MCs that are developed in vitro with IL-3 reversibly change their chymase expression when cultured in the presence of different combinations of cytokines and glucocorticoids (47–51), primarily through a posttranscriptional mechanism (51, 52). Thus, it is possible that jejunal MCs alter their expression of mMCP-1, mMCP-2, and mMCP-5 in part by altering the stability of the individual chymase transcripts.

Because the v-abl-immortalized V3-MC line also changes its chymase expression following its adoptive transfer into BALB/c mice (45), it has been proposed that the chymase phenotype of a jejunal MC is the result of the dynamics of changing MC-regulatory factors. Although V3-MC can be induced to express mMCP-7 following its adoptive transfer into BALB/c mice, we and others have been unable to cytokine-regulate tryptase expression in IL-3-developed, mouse BM-derived MCs. Thus, it remained to be determined whether a mouse MC can alter its tryptase expression in vivo during an inflammatory event. We now show that jejunal MCs reversibly alter their expression of the two tryptases and the in vivo during an inflammatory event. We now show that jejunal MCs reversibly alter their expression of the two tryptases and the in vivo during an inflammatory event. We now show that jejunal MCs reversibly alter their expression of the two tryptases and the

### Immunohistochemistry

MCs in serial sections of jejunum from noninfected and *T. spiralis*-infected mice were evaluated immunohistochemically (8, 58) for their expression of mMCP-2 (49), mMCP-6 (59), mMCP-7 (56), and mMCP-9 (43) using previously described rabbit anti-peptide Abs. Briefly, collected tissues were fixed for 4 h at room temperature in 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.6). Alternately, selected samples were fixed in Carnoy’s solution. The preparations were washed twice with PBS containing 2% DMSO and then suspended in 50 mM NH$_4$Cl overnight at 4°C. The specimens were dehydrated and embedded in accordance with the JB-4 kit from Polysciences (Warrington, PA). Sections were cut on a Reichert-Jung Supracut microtome (Leica, Deerfield, IL) with glass knives and picked up on glass slides. The slides were incubated sequentially for 15 min at 37°C in 2 mM CaCl$_2$, containing 0.025% trypsin, for 15 min at room temperature in PBS containing 0.05% Tween-20 and 0.1% BSA, for 30 min at 37°C in PBS containing 0.05% Tween-20 and 4% normal goat serum, and then overnight at 4°C in 4% normal goat serum containing purified rabbit anti-mMCP-2 Ig (49), anti-mMCP-6 Ig (59), anti-mMCP-7 Ig (56), or anti-mMCP-9 Ig (43). Each section was evaluated immunohistochemically for the presence of just one mMCP. The mMCP-2-, mMCP-6-, mMCP-7-, and mMCP-9-specific Abs were obtained previously against synthetic peptides that correspond to residues 56 to 71, 160 to 178, 160 to 178, and 144 to 152 in the respective serine protease. Although the brush borders of the villus exhibit a nonspecific reaction in the immunohistochemistry procedure due to the endogenous intestinal alkaline phosphatase (AP), the Abs are mMCP-specific. The specificities of these rabbit anti-peptide Abs have been described in earlier publications using recombinant proteases and select MC populations. As assessed by the OD of the stock solutions at 280 nm, the affinity-purified Abs were generally used at a concentration of ~4 µg/ml with ~0.2 µg/slide. Samples were washed, incubated for 40 min at room temperature in buffer containing biotin-labeled goat anti-rabbit IgG, washed twice in 0.1% BSA and 0.05% Tween-20 in PBS, incubated for 40 min at room temperature in Vectastain avidin-biotin complex-AP reagent (Vector Laboratories, Burlingame, CA), and then incubated for 15 min in the dark at room temperature in an AP substrate solution. Tissue sections were counterstained with Gill’s hematoxylin in 20% ethylene glycol, and then coverslips with Immu-Mount (Shandon, Pittsburgh, PA) were applied.

### Results

Characterization of jejunal MCs during the development of and at the height of the helminth-induced MC hyperplasia

As assessed by immunohistochemical analysis of serial sections of jejunum, the MCs in the submucosa of noninfected BALB/c mice expressed mMCP-6 and mMCP-7 but not mMCP-2 or mMCP-9 (Fig. 1 and Table I). At 1 wk after helminth infection, MCs increased in number in the lower portion of the lamina propria, the lower portion of the villus epithelium, and between the epithelial cells in the crypts. Most of the MCs in the submucosa at this time point continued to express mMCP-6, but some MCs were found in this location that expressed all combinations of mMCP-2, mMCP-6, and mMCP-7, with the exception of mMCP-7 alone (Table I). MCs that expressed mMCP-9 were found in the submucosa at the height of the intestinal MC hyperplasia at wk 2, but mMCP-9 MCs were rarely found in the upper villus, mid-villus, lower villus, or crypts at this time point (Fig. 2). Nevertheless, the MCs in the submucosa at wk 2 did resemble those in the submucosa at wk 1 in terms of the diversity of their expression of the other three granule proteases (Table I). Most submucosa MCs continued to coexpress mMCP-6 and mMCP-7. While only a few submucosa MCs expressed mMCP-2 alone or mMCP-7 alone, numerous MCs could be found in the submucosa at this point that coexpressed mMCP-2 and mMCP-6 with (Fig. 3) or without
mMCP-7. Many of the MCs in the lamina propria region of the crypts and lower villus at wk 2 also coexpressed mMCP-6 and mMCP-7, but a greater percentage of the MCs in these locations just expressed mMCP-2 at that point. In addition, more MCs with a mMCP-2/mMCP-6/mMCP-7 phenotype were found in the crypt lamina propria than in the villus lamina propria, whereas

Table I. Quantitation of MCs exhibiting specific mMCP phenotypes in serial sections of jejunum of noninfected and T. spiralis-infected BALB/c mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>mMCP-2 Alone Mean (± SD)</th>
<th>mMCP-2 &amp; -6 Mean (± SD)</th>
<th>mMCP-6 Alone Mean (± SD)</th>
<th>mMCP-2, -6, &amp; -7 Mean (± SD)</th>
<th>mMCP-6 &amp; -7 Mean (± SD)</th>
<th>mMCP-7 Alone Mean (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninfected mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Villus epithelium</td>
<td>4.0 (2.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Villus lamina propria</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt epithelium</td>
<td>5.3 (2.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt lamina propria</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.7 (0.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Submucosa</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.0 (1.0)</td>
<td>18 (9.3)</td>
<td>0.3 (0.6)</td>
<td></td>
</tr>
<tr>
<td>1-wk-infected mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus epithelium</td>
<td>12 (5.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Villus lamina propria</td>
<td>4.3 (1.5)</td>
<td>2.3 (1.5)</td>
<td>2.3 (0.6)</td>
<td>2.3 (1.5)</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt epithelium</td>
<td>73 (19)</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt lamina propria</td>
<td>5.0 (3.6)</td>
<td>6.3 (2.1)</td>
<td>2.0 (1.0)</td>
<td>6.3 (2.1)</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Submucosa</td>
<td>0.7 (0.6)</td>
<td>2.7 (0.6)</td>
<td>2.3 (1.5)</td>
<td>8.3 (2.5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>2-wk-infected mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus epithelium</td>
<td>380 (87)</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Villus lamina propria</td>
<td>27 (9.6)</td>
<td>5.3 (3.5)</td>
<td>1.7 (1.2)</td>
<td>14 (4.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt epithelium</td>
<td>320 (53)</td>
<td>0.7 (1.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt lamina propria</td>
<td>19 (1.5)</td>
<td>1.7 (0.6)</td>
<td>4.3 (2.5)</td>
<td>21 (11)</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Submucosa</td>
<td>0.7 (0.6)</td>
<td>2.3 (2.1)</td>
<td>5.7 (2.5)</td>
<td>23 (5.0)</td>
<td>0.7 (0.6)</td>
<td></td>
</tr>
<tr>
<td>4-wk-infected mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villus epithelium</td>
<td>24 (7.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Villus lamina propria</td>
<td>22.7 (5.5)</td>
<td>24 (3.8)</td>
<td>22 (4.0)</td>
<td>19 (6.2)</td>
<td>0.3 (0.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt epithelium</td>
<td>110 (16)</td>
<td>0.7 (1.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Crypt lamina propria</td>
<td>7.0 (1.0)</td>
<td>24 (8.7)</td>
<td>12 (4.5)</td>
<td>37 (8.5)</td>
<td>1.0 (1.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Submucosa</td>
<td>3.3 (1.2)</td>
<td>9.7 (6.4)</td>
<td>2.7 (1.5)</td>
<td>4.7 (2.5)</td>
<td>2.3 (2.1)</td>
<td></td>
</tr>
</tbody>
</table>

*The data obtained are from 2.4- to 2.9-μm serial-sectioned tissue from three mice at each time point. Due to the limitations of the serial-section approach, the expression of only three granule proteases could be determined reliably in an individual MC. mMCP-6 and mMCP-7 were evaluated because tryptases are the focus of the study. mMCP-2 was selected as the third analyzed protease in order to link this study with our previous report, which monitored the changes in mMCP-1, mMCP-2, and mMCP-5 expression in jejunal MCs during helminth infection (8). MCs having an mMCP-2/mMCP-6/mMCP-7 phenotype were rarely seen.
more MCs having a mMCP-2⁺/mMCP-6⁺/mMCP-7⁻ phenotype were found in the villus lamina propria than in the crypt lamina propria (Table I). While MCs could be found in the upper villus that expressed nearly every combination of mMCP-2, mMCP-6, and mMCP-7, most of the MCs in this location and in the crypt epithelium expressed mMCP-2 alone (Fig. 3). In agreement with the immunohistochemistry data, the MCs in the submucosa of helminth-infected mice, but not those MCs in the crypt epithelium, contained tryptic proteases that cleaved the AAK substrate (Fig. 4). The MCs in the crypt epithelium that expressed mMCP-1 and mMCP-2, but not mMCP-6 or mMCP-7, did not exhibit AAK enzymatic activity.

Characterization of jejunal MCs during the resolution phase of the helminth infection

As previously determined (8), the number of jejunal MCs steadily decreased during the resolution phase of the helminth infection (Table I) and was near baseline by wk 8 (data not shown). MCs were rarely found in the tips of the upper villi at wk 4 (Fig. 5). During the recovery phase of the helminth infection, MCs could be found in the villus lamina propria that had nearly every combination of mMCP-2, mMCP-6, and mMCP-7 (Table I). However, most of the lamina propria MCs in the upper villus expressed either mMCP-2 alone or both mMCP-2 and mMCP-6, whereas most lamina propria MCs in the lower villus expressed either mMCP-6 alone or both mMCP-6 and mMCP-7 (Fig. 5). Although the MCs in the lamina propria of the crypts expressed every combination of mMCP-2, mMCP-6, and mMCP-7, a higher percentage of the cells in the crypt lamina propria and submucosa now expressed mMCP-7 relative to those cells in the villus lamina propria. At this time point, any MC that contained immunoreactive mMCP-6 or mMCP-7 in its granules cleaved the AAK substrate (data not shown). As illustrated in Figure 6 for one tissue section, 119 of the 122 analyzed MCs in the submucosa of three mice (~40 submucosa MCs analyzed in each

FIGURE 2. mMCP-9 expression in jejunal MCs at the height of the MC hyperplasia that occurs during T. spiralis infection. At 2 wk after helminth infection, serial sections of the upper villus (a–c), mid-villus (d–f), crypts (g–i), and submucosa (j–l) of the jejunum were analyzed cytochemically for their chloroacetate esterase activity (a, d, g, and j) or immunohistochemically for their expression of mMCP-2 (b, e, h, and k) or mMCP-9 (c, f, i, and l). Arrows indicate a MC in the submucosa (k and l) that expresses both mMCP-2 and mMCP-9. Although most jejunal MCs are mononuclear, the MC depicted in j–l has a bilobed nucleus.
mouse) and 37 of the 41 analyzed MCs in the crypt lamina propria of the same three mice expressed mMCP-9.

**Discussion**

Jejunal MCs in noninfected BALB/c mice tend to localize in the submucosa and express the chymase mMCP-5, but not the chymases mMCP-1 and mMCP-2 (8). We now show that the MCs in this location also tend to express the tryptases mMCP-6 and mMCP-7, but not the chymase mMCP-9 (Fig. 1). The fact that submucosa MCs fixed with paraformaldehyde still cleaved the AAK substrate (Fig. 4) established the Ags recognized by anti-mMCP-6 Ig and anti-mMCP-7 Ig as enzymatically active tryptases. Like the chloroacetate esterase cytochemistry procedure (53, 54) used to quantitate all chymase-expressing MCs in the jejunum (7, 8), skin (60), and spleen (8), the AAK enzyme cytochemistry procedure (55) can be used to quantitate MCs in the jejunum that express mMCP-6 and/or mMCP-7. The MCs in the upper villus generally express mMCP-2 but not mMCP-6 or mMCP-7.

**FIGURE 3.** mMCP-2, mMCP-6, and mMCP-7 expression in different populations of jejunal MCs at the height of the MC hyperplasia that occurs after *T. spiralis* infection. At 2 wk after helminth infection, serial sections of the upper villus (a–c and d–f), crypts (g–i and j–l), and submucosa (m–o) of the jejunum were stained with anti-mMCP-2 Ig (a, d, g, j, and m), anti-mMCP-6 Ig (b, e, h, k, and n), or anti-mMCP-7 Ig (c, f, i, l, and o). Arrows indicate immunoreactive MCs in the epithelium (d) that express just mMCP-2 alone or MCs in the submucosa (m–o) that express all three proteases. Arrowheads indicate immunoreactive MCs in the lamina propria that express mMCP-2 alone (d) or both mMCP-2 and mMCP-6 (j and k). Although most of the MCs in the submucosa at the height of the MC hyperplasia express all three proteases, some MCs can be found in the lamina propria region of the crypts that express mMCP-2 and mMCP-6 but not mMCP-7. The MCs in the upper villus generally express mMCP-2 but not mMCP-6 or mMCP-7.

**FIGURE 4.** Enzyme cytochemistry and immunohistochemistry of jejunal MCs in *T. spiralis*-infected BALB/c mice. Serial sections (a–b and c–d) of the jejunum of a helminth-infected BALB/c mouse at wk 2 were incubated with the tryptase substrate AAK (a and c), anti-mMCP-2 Ig (b), or anti-mMCP-6 Ig (d). Arrows indicate MCs in the crypt epithelium that express mMCP-2 but fail to exhibit AAK enzymatic activity.
forms were present in the lamina propria of the crypts and lower villus that expressed mMCP-2 alone, mMCP-6 alone, mMCP-2 and mMCP-6, and even the combination of mMCP-2, mMCP-6, and mMCP-7. In an earlier study (8), we noted that at the height of the infection at wk 2, some of the MCs in the muscle and submucosa expressed mMCP-5 but not mMCP-1 or mMCP-2, whereas most intraepithelial MCs express mMCP-1 and mMCP-2 but not mMCP-5. Accompanying these two MC populations were transitional forms in the submucosa that expressed mMCP-2 and mMCP-5 but not mMCP-1 and forms in the lamina propria that expressed mMCP-2 alone. Because of the thickness of serially sectioned tissue, the practical evaluation of protease expression in a single MC is limited to three mMCPS. Nevertheless, the cumulative findings of current and previous studies now suggest that BALB/c mouse jejunal MCs that initially have a mMCP-1/mMCP-2/mMCP-5/mMCP-6/mMCP-7/mMCP-9 granule protease phenotype sequentially express mMCP-2 and mMCP-9; cease expressing mMCP-5, mMCP-6, mMCP-7, and mMCP-9; and finally express mMCP-1 as they progressively move from the submucosa to the mucosal lamina propria.

Essentially no intraepithelial MCs were present in the upper villus in the resolution phase of the MC hyperplasia at wk 4 (Fig. 5). We and others have previously showed that the MCs in the villus and crypt epithelium at the height of infection are the only population of MCs that contain stellate-shaped granules with crystalline structures (8, 46). The presence of granules with fragmented crystals in lamina propria MCs during resolution of the infection indicates that at least some MCs migrate through the thickness of the jejunum during this phase of inflammation (8). During the resolution phase of the reactive MC hyperplasia, the MCs in the lamina propria were able to express any combination of mMCP-2, mMCP-6, and mMCP-7 (Fig. 5). However, virtually all of the lamina propria MCs from the mid-villus to submucosal levels of the jejunum expressed mMCP-9 (Fig. 6). Based on earlier (8) and current studies, wk 4 is the point at which MCs stop expressing mMCP-1 and mMCP-2 and begin to express mMCP-5, mMCP-9, and various combinations of the two tryptases as they progressively move from the upper villus to the submucosa. Figure 7 shows a schematic representation of the expression of the four chymases and the two tryptases in the MCs that reside at different locations in the jejunum of noninfected and helminth-infected BALB/c mice.

These studies document for the first time that a mouse MC can alter its expression in vivo of multiple members of two distinct families of serine proteases. Although no other hematopoietic cell type has been found that undergoes such reversible changes in its differentiation pattern during an inflammatory event, the transformed but clonal MCs present in varied tissue sites of a patient with systemic mastocytosis also exhibit different protease phenotypes (61). Jejunal MCs presumably change their mMCP expression in response to their time- and strata-dependent location to modulate the inflammatory process in a purposeful fashion. The two exocytosed tryptases exhibit dissimilar
rates of dissociation from serglycin proteoglycans (59, 62). Even though the amino acid sequences of mMCP-6 and mMCP-7 are 71% identical (34, 38, 40, 42), the substrate-binding pockets of the two tryptases differ substantially (59, 63). Analysis of a tryptase-specific, phage-display peptide library has also revealed that the preferred amino acid sequences cleaved by mMCP-6 (63) are very different from those cleaved by mMCP-7 (64). Recombinant mMCP-6 can induce neutrophil extravasation and accumulation in tissues by inducing endothelial cells to selectively increase their expression of the IL-8 family of chemokines (63). Because fibrinogen is a physiologic substrate of mMCP-7 (64), this tryptase can acutely inhibit fibrin/platelet clot formation and alter fibrinogen/integrin-mediated signaling events. These recent studies on native and recombinant MC tryptases indicate that the ability of a jejunal MC to change its granule protease can have functional consequences in vivo.

References


