The Mouse Mast Cell–Restricted Tetramer-Forming Tryptases Mouse Mast Cell Protease 6 and Mouse Mast Cell Protease 7 Are Critical Mediators in Inflammatory Arthritis

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Objective. **Increased numbers of mast cells (MCs) that express β tryptases bound to heparin have been detected in the synovium of patients with rheumatoid arthritis (RA). The corresponding tryptases in mice are mouse MC protease 6 (mMCP-6) and mMCP-7. Although MCs have been implicated in RA and some animal models of arthritis, no direct evidence for a MC-restricted tryptase in the pathogenesis of inflammatory arthritis has been shown. We created transgenic mice that lack heparin and different combinations of mMCP-6 and mMCP-7, to evaluate the roles of MC-restricted tryptase–heparin complexes in an experimental model of arthritis.**

Methods. **The methylated bovine serum albumin/interleukin-1β (mBSA/IL-1β) experimental protocol was used to induce inflammatory monarthritis in different mouse strains. Mice were killed at the time of peak disease on day 7, and histochemical methods were used to assess joint pathology.**

Results. **Arthritis was induced in the knee joints of mBSA/IL-1β–treated mMCP-6$^+$/mMCP-7$^-$ and mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice, and numerous activated MCs that had exocytosed the contents of their secretory granules were observed in the diseased mice. In contrast, arthritis was markedly reduced in heparin-deficient mice and in mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice.**

Conclusion. **MC-derived tryptase–heparin complexes play important roles in mBSA/IL-1β–induced arthritis. Because mMCP-6 and mMCP-7 can compensate for each other in this disease model, the elimination of both tryptases is necessary to reveal the prominent roles of these serine proteases in joint inflammation and destruction. Our data suggest that the inhibition of MC-restricted tryptases could have therapeutic potential in the treatment of RA.**

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Rheumatoid arthritis (RA) is an inflammatory disorder of synovial joints characterized by damage to articular structures due to chronic inflammation of the synovium. Numerous cellular participants of innate and adaptive immunity contribute to the pronounced inflammatory processes seen in rheumatoid synovitis. Our group (1–4) and many other investigators (5–14) have obtained data implicating a prominent involvement of mast cells (MCs) and their mediators in RA and some animal models of this autoimmune disorder. On a weight basis, tetramer-forming β tryptases (15–19) are the most abundant proteins present in the secretory granules of human MCs. Three β tryptase complementary DNAs (designated hTryptase β1, β2, and β3) have been cloned.

They are 95–99% identical and are encoded by 2 adjacent genes on human chromosome 16p13.3 (20). Their murine equivalents are mouse MC protease 6 (mMCP-6) (21) and mMCP-7 (22).

As occurs with the β tryptases in humans, MCs are the only cells that express mMCP-6 and mMCP-7 in mice. These tryptases are stored in the secretory granules of the cell as homotypic and heterotypic tetramers, ionically bound to serglycin proteoglycans that contain heparin glycosaminoglycans (23,24). Tryptase–heparin complexes are released into the extracellular milieu when these IgE-bearing MCs encounter the appropriate antigen. However, MCs can be activated in other ways, including via IgG–complement complexes and the anaphylatoxins C3a and C5a. Complement (25) and IgE directed against cartilage proteins (26,27) have deleterious roles in RA and animal models of this disorder, thereby supporting the notion of a prominent role of MCs and their exocytosed mediators.

Animal models of inflammatory arthritis do not reproduce all aspects of RA in humans. Nevertheless, they provide powerful experimental in vivo systems to elucidate key pathogenic processes (28). Methylated bovine serum albumin (mBSA) is a proarthritic antigen in mice due to its ability to bind to cartilage after being injected intraarticularly. In mice that receive mBSA intradermally in the presence of adjuvant, inflammatory arthritis is induced several days after intraarticular mBSA challenge (29). However, inflammatory arthritis develops at a faster rate if the intradermal sensitization step is eliminated and the antigen is initially placed in the joints of mice that subsequently receive interleukin-1β (IL-1β) to stimulate innate immunity (30). The latter mBSA/IL-1β–induced arthritis model depends on CD4-positive T lymphocytes and IL-1β but does not require B lymphocytes or antibodies (30). In C57BL/6 mice, disease manifestations peak on day 7 and typically resolve over the next 14 days. This has advantages over other experimental mouse models of arthritis in that it is highly reproducible and allows detailed histologic quantitation of the severity of joint inflammation and destruction. This model induces moderate arthritis, allowing discrimination of a wide range of responses.

Although a prominent role for MCs in mBSA/adjuvant-induced arthritis has been shown based on studies carried out on MC-deficient WBB6F$_1$-Kit^W/Kit^{W-v} (W/W^v) mice (29), no definitive evidence has been obtained for the involvement of a tryptase in this or any other arthritis model. N-deacetylase/N-sulfotransferase (NDST-2) is essential for heparin biosynthesis. We and other investigators created NDST-2–null C57BL/6 mice and discovered that the MCs in the skin and peritoneal cavities of these mice cannot store appreciable amounts of many proteases in their secretory granules (24,31). The MCs in wild-type C57BL/6 mice constitutively express mMCP-6, as occurs in all other mouse strains examined (21). However, unlike MCs in most mouse strains, those in C57BL/6 mice lack mMCP-7 due to a naturally occurring point mutation at the exon 2/intron 2 splice site of the mMCP-7 gene (32,33). Using a homologous recombination knockout/knockin approach, we recently created a transgenic C57BL/6 mouse strain that lacks mMCP-6 but expresses mMCP-7 (34). We also recently created a transgenic C57BL/6 mouse strain that lacks both tryptases, using another homologous recombination approach (35). These NDST-2$^-$, mMCP-6$^+$/mMCP-7$^-$, mMCP-6$^-$/mMCP-7$^+$, and mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice now allow us the opportunity to evaluate the roles of MC-restricted tryptase–heparin complexes in a manner that previously was not possible. Using these mice, we now describe key roles for MC-restricted tryptase–heparin complexes in mBSA/IL-1β–induced arthritis.

MATERIALS AND METHODS

Mice. Inbred C57BL/6 mice were obtained from Taconic (Albany, NY). W/W^v mice and their control (+/+) littermates were obtained from the Walter and Eliza Hall Institute Animal Supplies (Kew, Victoria, Australia). Inbred mouse strains can differ substantially in their susceptibility to experimental arthritis and in their expression of the serpin family of protease inhibitors and some MC-restricted proteases. Therefore, our transgenic mice lacking NDST-2 and different combinations of mMCP-6 and mMCP-7 were all on a C57BL/6 mouse genetic background. All mice were ~8 weeks of age at the time of experimentation. Institutional Animal Care and Use Committee approval was obtained for all animal experiments.

Experimental inflammatory arthritis. The mBSA/IL-1β experimental protocol described by Staite et al (36) and Lawlor et al (30) was used to induce arthritis in the 6 mouse strains evaluated in our study. Briefly, mice were injected intraarticularly in each knee joint with 10 μl of a 20-mg/ml solution of mBSA (Sigma, St. Louis, MO). Recombinant human IL-1β (250 ng in 20 μl normal saline/0.5% normal C57BL/6 mouse serum) (PeproTech, Rocky Hill, NJ) was then injected subcutaneously into the rear footpad of each mBSA-treated mouse once daily on days 0, 1, and 2. Mice were killed 7 days after the initial mBSA injection, and the rear limbs were removed and fixed in Bouin's fixative (Ricca Chemical, Arlington, TX) or 4% paraformaldehyde for at least 2 days. The treated tissues were decalcified and processed for paraffin embedding. Frontal tissue sections (4 μm) were cut at 5 depths ~50 μm apart and stained with hematoxylin and eosin (H&E)
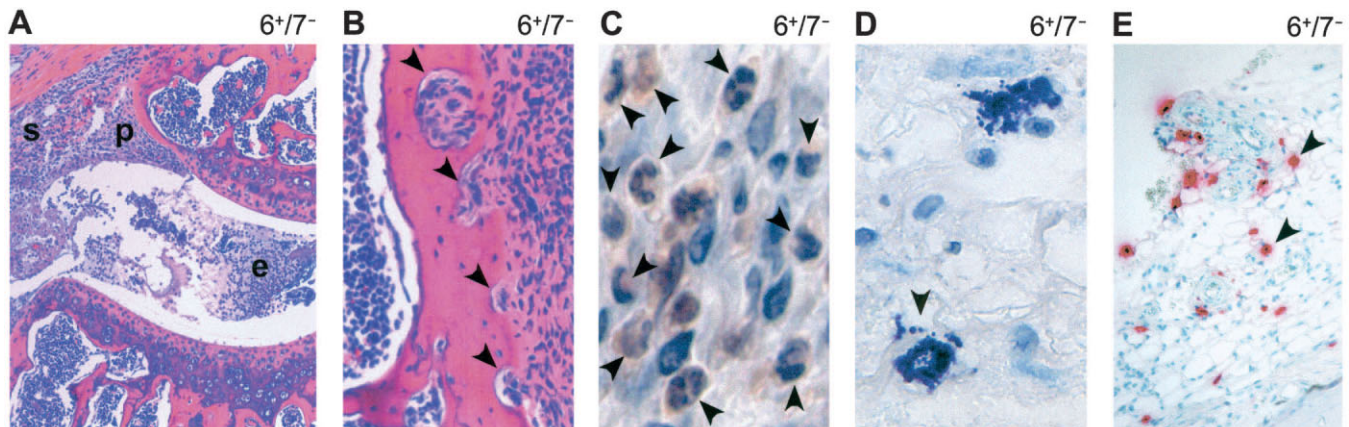
Figure 1. Characterization of the arthritic joints of methylated bovine serum albumin/interleukin-1β (mBSA/IL-1β)–treated C57BL/6 mice. **A** and **B**, Representative hematoxylin and eosin–stained frontal knee sections from mouse mast cell protease 6–positive (MCP-6$^+$)/mMCP-7$^-$ wild-type C57BL/6 mice on day 7 of mBSA/IL-1β–induced arthritis. Disease was severe, with prominent joint space exudate (**e**), synovitis (**s**), pannus formation (**p**), and bone erosion (**arrowheads**). **C**, Chloroacetate esterase activity. Many of the inflammatory cells infiltrating these joints were neutrophils (**arrowheads**), based on their enzyme cytochemistry and characteristic segmented nucleus. **D,** Toluidine blue staining. Activated mast cells (MCs) in the synovium of affected knee joints exocytosed their protease–proteoglycan macromolecular complexes (**arrowhead**). **E,** Immunohistochemical analysis of mMCP-6. The synovial MCs in affected knee joints also contained mMCP-6 (**arrowheads**).

(4 sections) or toluidine blue (1 section), using standard histochemical methods to assess joint pathology.

The severity of arthritis in coded H&E-stained sections was assessed in a blinded manner and graded from 0 (normal) to 5 (severe) for 4 components that comprised joint space exudate, synovitis, pannus formation, and bone erosion, using a previously described method (30,36). The average score for the 4 sections analyzed from each joint was calculated for each component. A fifth component, cartilage erosion, was assessed by the degree of loss of toluidine blue staining of the patellofemoral joint articular cartilage and semiquantitatively scored from 0 (no loss of staining) to 5 (severe loss of staining). An overall mean histologic severity score (maximum possible score 25) for each joint was calculated by summing the scores for the 5 individual components.

Enzyme cytochemistry and mMCP-6 and mMCP-7 immunohistochemistry. Although neutrophils can be identified in H&E-stained tissue sections based on their segmented nuclei, these granulocytes also can be identified in fixed sections based on the ability of their granule proteases to cleave naphthol AS-D chloroacetate (Sigma). Thus, the chloroacetate esterase enzyme cytochemistry procedure developed by Leder (37) was used to evaluate the neutrophils in the synovial tissue and joint space exudates of the arthritic joints of mBSA/IL-1β–treated mice. Previously described immunohistochemical methods (32,38,39) also were used to evaluate the presence of mMCP-6 and mMCP-7 protein in the MCs found in the arthritic joints of mBSA/IL-1β–treated C57BL/6 mice. The antibodies used in these experiments were generated in rabbits against 19-mer synthetic peptides that correspond to the unique sequences at residues 160–178 in mMCP-6 (38) and mMCP-7 (32).

Statistical analysis. Student's 2-tailed t-test was used for direct comparisons of observations made in control and transgenic animals. P values less than 0.05 were considered significant.

RESULTS

MC involvement in mBSA/IL-1β–induced inflammatory arthritis in mice. Van den Broek and coworkers (29) noted that cartilage erosion was significantly reduced in MC-deficient W/W^v mice 14–35 days after these animals were sensitized with intradermal mBSA in the presence of adjuvant followed by intraarticular mBSA challenge. Because mBSA/IL-1β–induced arthritis is more acute and less severe than mBSA/adjuvant-induced arthritis, we evaluated the development of arthritis in C57BL/6 mice 7 days after administration of intraarticular mBSA and subcutaneous IL-1β. We also evaluated mBSA/IL-1β–induced arthritis in MC-deficient W/W^v mice (6 joints from 3 mice).

Inflammation and joint destruction were reduced in treated MC-deficient W/W^v mice relative to their WBB6F$_1$$^{+/+}$ littermates (data not shown) and wild-type mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice (Figure 1). Methylated BSA/IL-1β treatment resulted in inflammatory arthritis in mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice, with an intense joint-space inflammatory infiltrate and marked synovial thickening (Figure 1A). Moderate pannus formation and mild-to-moderate bone erosion (Figure 1B) also were evident. As assessed histochemically (Figures
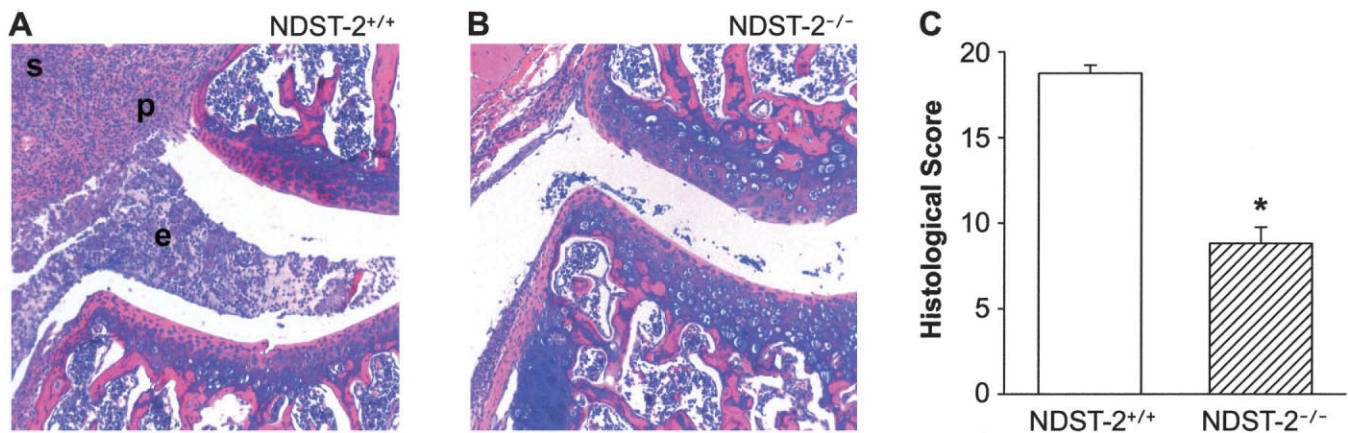
Figure 2. Reduction in the severity of mBSA/IL-1β–induced arthritis in heparin/*N*-deacetylase/*N*-sulfotransferase (NDST-2)–deficient C57BL/6 mice. **A** and **B,** Representative hematoxylin and eosin–stained knee sections from an NDST-2–sufficient C57BL/6 mouse (**A**) and an NDST-2–null C57BL/6 mouse (**B**), showing reduced cellular joint space exudate, synovitis, and bone erosion in the absence of heparin on day 7. **C,** Total histologic scores for joints from control NDST-2–sufficient and NDST-2–deficient C57BL/6 mice. Values are the mean and SEM results from 3 separate experiments (n = 27 joints per group). ∗ = $P < 0.001$. See Figure 1 for other definitions.

1A and B) and by the chloroacetate esterase cytochemistry procedure (Figure 1C), many of the inflammatory cells in the affected joints of the mBSA/IL-1β–treated mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice were neutrophils. We noted that the majority of the synovial MCs in the knee joints of C57BL/6 mice had degranulated 7 days after the induction of mBSA/IL-1β–dependent arthritis (Figure 1D). In contrast, we observed little or no degranulation of the MCs that resided in the nonarthritic knee joints of normal mice (results not shown).

The discovery that the synovial MCs in the diseased C57BL/6 mice contained mMCP-6 protein (Figure 1E), as previously observed in the K/BxN serum-transfer model of arthritis (39), raised the possibility that 1 or more tryptase–heparin complexes exocytosed from activated MCs in joint tissue play a prominent role in neutrophil accumulation and/or cartilage destruction.

Reduced mBSA/IL-1β–induced inflammatory arthritis in heparin-deficient, NDST-2–null mice. In an attempt to understand the primary reason MC-deficient
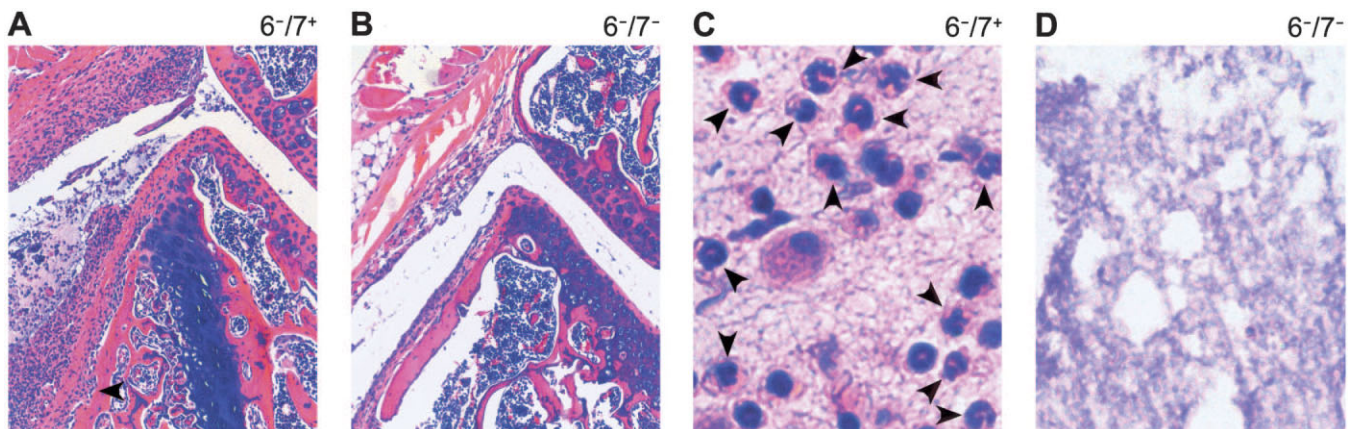

Figure 3. Reduction in the severity of mBSA/IL-1β–induced arthritis in the absence of both tetramer-forming MC tryptases. Shown are representative hematoxylin and eosin–stained knee sections from mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice (**A** and **C**) and mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice (**B** and **D**) on day 7. **Arrowheads** in **A** and **C** indicate prominent cellularity in the joint space exudate, synovitis, and bone erosion in the knee joints of mMCP-6$^-$/mMCP-7$^+$ mice and neutrophilic predominance in the inflammatory infiltrate from these mice, respectively. These changes were similar to those observed in mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice (see Figures 1A and B). In contrast, arthritis was less severe in the knee joints of mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice (**B**), and the joint space exudate contained almost no leukocytes (**D**). See Figure 1 for definitions.
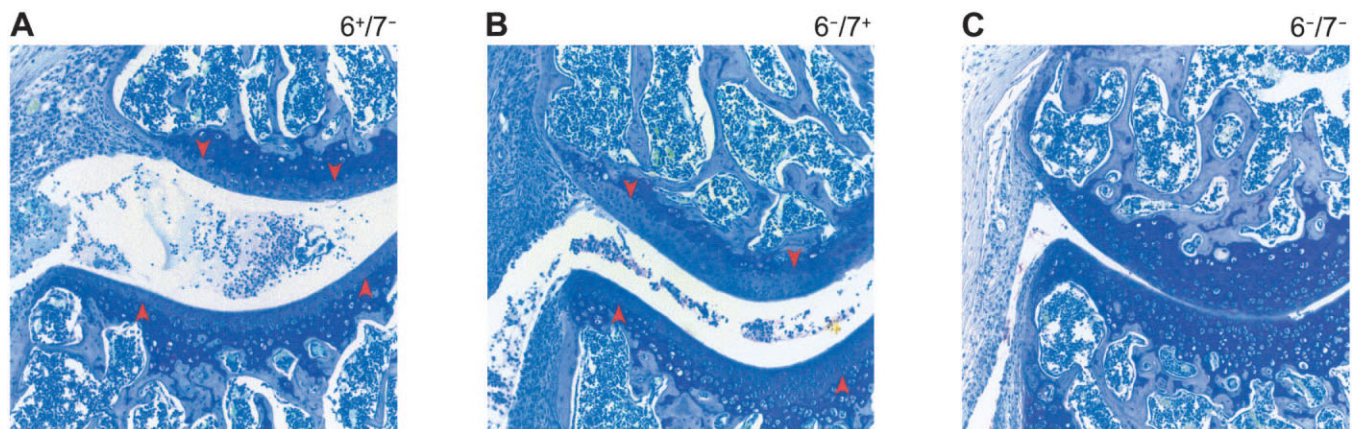
Figure 4. Reduction in the severity of cartilage degradation in the methylated bovine serum albumin/interleukin-1β (mBSA/IL-1β)–induced arthritis model in the absence of tetramer-forming mast cell tryptases. On day 7, knee sections from mBSA/IL-1β–treated mouse mast cell protease 6–positive (mMCP-6$^+$)/mMCP-7$^-$ (**A**), mMCP-6$^-$/mMCP-7$^+$ (**B**), and mMCP-6$^-$/mMCP-7$^-$ (**C**) C57BL/6 mice were stained with toluidine blue under conditions that stain cartilage proteoglycans. Diminished staining of the articular surfaces is clearly seen in **A** and **B** (**arrowheads** point to delineation), indicating loss of proteoglycans from the surface cartilage. In contrast, little or no loss of toluidine blue staining is seen in **C**, indicating minimal cartilage degradation in the knee joints from mMCP-6$^-$/mMCP-7$^-$ mice.

mice have less severe mBSA/IL-1β–induced arthritis than similarly treated MC-sufficient mice, we next studied transgenic C57BL/6 mice that lack heparin due to targeted disruption of the NDST-2 gene, which encodes an enzyme required for the biosynthesis of this glycosaminoglycan in MCs (24). Because heparin-containing serglycin proteoglycans are critical in the posttranslational processing and assembly of MC secretory granule proteases, the MCs in NDST-2–null C57BL/6 mice contain diminished levels of numerous granule proteases (24). When mBSA/IL-1β–dependent arthritis was induced in the joints of NDST-2–null mice, the mean severity of disease activity was reduced by ~50%, from a mean ± SEM histologic score of 18.2 ± 0.5 in control C57BL/6 mice to 8.8 ± 0.9 in NDST-2–null C57BL/6 mice (Figure 2).

Reduced mBSA/IL-1β–induced inflammatory arthritis in mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice but not in mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice. To determine whether the amelioration of inflammatory arthritis seen in NDST-2–null mice (Figure 2) was attributable to a lack of a heparin-associated secretory granule protease, mBSA/IL-1β–induced arthritis was next evaluated in mMCP-6$^-$/mMCP-7$^+$ and mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice (Figures 3–5). The obtained phenotypes were then compared with that of similarly treated mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice (Figures 1, 2, 4, and 5).

As assessed immunohistochemically, the MCs in the joints of the diseased mMCP-6$^-$/mMCP-7$^+$

C57BL/6 mice contained mMCP-7 protein but not mMCP-6 protein, whereas the corresponding MCs in the joints of the diseased mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice lacked both tryptases (results not shown). As reported previously (30) and confirmed in this study, in which a total of 70 knee joints were evaluated in 8 separate experiments, we observed that >90% of knee joints from mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice affected by mBSA/IL-1β–dependent arthritis had total histologic scores of ≥15, with no joints scoring ≤10. Similar pathologic changes were observed in mBSA/IL-1β–treated mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice, with marked synovitis, joint space exudate, and pannus formation (Figure 3A). Considerable cartilage erosion (Figure 4B) and some bone erosion (Figure 3A) were also evident and comparable with that seen in mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice (Figures 4A and 1B, respectively).

There was no significant difference between the mean ± SEM histologic scores for the knee joints from arthritic mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice (17.0 ± 2.0) and those for the knee joints from mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice (16.2 ± 1.2 [$P = 0.75$]; n = 10 joints in each group in this one experiment). In contrast, the knee joints from mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice showed much milder mBSA/IL-1β–dependent arthritis (Figure 3B), with 16 of 33 joints assessed (48%) scoring ≤10 and only 21% (7 of 33 joints) showing histologic scores of ≥15. Joint space infiltrates were substantially less cellular, and thickening of the synovial
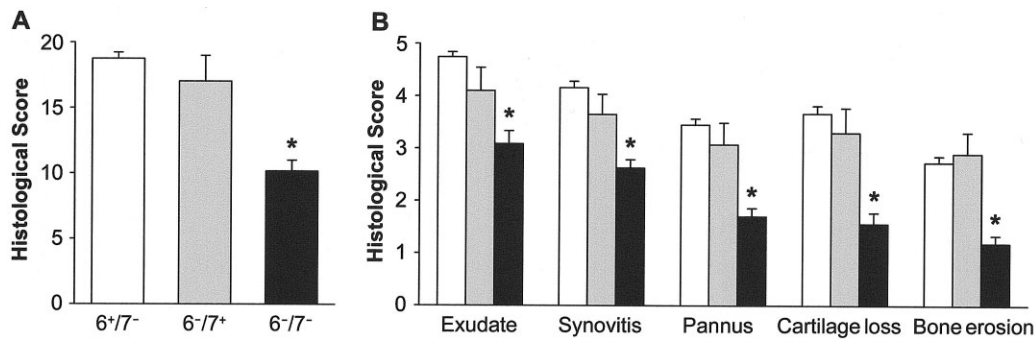
Figure 5. Histologic assessment of mBSA/IL-1β–induced arthritis in knee joints from various tryptase-deficient mouse strains. Knee sections from mBSA-IL-1β–treated mMCP-6$^+$/mMCP-7$^-$ (open bars; n = 33), mMCP-6$^-$/mMCP-7$^+$ (shaded bars; n = 10), and mMCP-6$^-$/mMCP-7$^-$ (solid bars; n = 33) C57BL/6 mice were assessed histologically for 5 features of inflammatory arthritis, each on a scale of 0 (normal) to 5 (severe). **A,** Total histologic scores, representing the sum of the scores for all 5 components. ∗ = $P < 0.01$ versus mMCP-6$^+$/mMCP-7$^-$ and mMCP-6$^-$/mMCP-7$^+$ mice. **B,** Histologic scores for each of the 5 components. ∗ = $P < 0.05$ versus mMCP-6$^+$/mMCP-7$^-$ and mMCP-6$^-$/mMCP-7$^+$ mice. Values are the mean and SEM results from pooled experiments. See Figure 4 for definitions.

membrane was much less marked and/or focal rather than generalized. When examined at high magnification, the joint space exudate typically was sparsely populated by leukocytes in mBSA/IL-1β–treated mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice (Figure 3D) relative to similarly treated mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice (results not shown) and mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice (Figure 3C). Many of the cells in the joint spaces of the arthritic mMCP-6$^-$/mMCP-7$^+$ mice were neutrophils (Figure 3C). Overall, the mean ± SEM total histologic severity score in mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice was 10.2 ± 0.8, compared with 18.8 ± 0.5 in mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice (n = 33 in each group from multiple experiments) (Figure 5A).

When each of the 5 histologic components was examined, highly statistically significant differences were observed between the total scores for mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice and mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice ($P < 0.001$) and between mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice and mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice ($P < 0.01$) and for each of the histologic components between mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice and both other groups ($P < 0.05$). In contrast, there were no significant differences between the histologic scores for knee joints from mMCP-6$^-$/mMCP-7$^+$ and mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice ($P > 0.18$) (Figure 5B).

DISCUSSION

MC-deficient W/W^v mice are resistant to inflammatory arthritis induced by anti–glucose-6-phosphate isomerase antibodies from K/BxN mice (4). The ability to restore pathology in W/W^v mice by the adoptive transfer of in vitro–differentiated NDST-2$^+$/mMCP-6$^+$ MCs implicated critical roles for these immune cells and their exocytosed mediators in the K/BxN mouse serum transfer model. Van den Broek and coworkers also noted that W/W^v mice experience a milder form of erosive mBSA/adjuvant-induced arthritis compared with MC-sufficient WBB6F$_1^{+/+}$ mice (29). Consistent with the results of those earlier studies, we discovered that W/W^v mice additionally are less susceptible to mBSA/IL-1β–induced arthritis than their control (+/+) littermates (data not shown). The accumulated data implicate prominent proinflammatory roles for MCs in 3 different experimental arthritis models evaluated in W/W^v mice.

The subset of MCs that stores β tryptase and chymase (MC$_{TC}$) in its secretory granules predominates in normal human synovium (2,3). However, the MC subset that contains just β tryptase (MC$_T$) is preferentially increased in rheumatoid synovium. A significant correlation was previously observed between the histologic inflammation index and the number of MC$_T$, but not MC$_{TC}$, in tissue biopsy specimens. We also discovered that some of these expanded tryptase-positive/chymase-negative MCs physically interact with T lymphocytes in rheumatoid joints (3), thereby raising the possibility that MC-restricted tetramer-forming tryptases play adverse roles in RA.

Mouse MCP-6 (21) and mMCP-7 (22) are the only 2 tetramer-forming tryptases present in mouse MCs. Although their amino acid sequences are ~75%

identical, mMCP-6 and mMCP-7 are functionally distinct tryptases (40,41). Because recombinant hTryptase β1 (42,43) has a substrate specificity more similar to that of recombinant mMCP-6 (41) than recombinant mMCP-7 (40), mMCP-6 is the mouse ortholog of hTryptase β1. We previously showed that the MCs in the C57BL/6 mouse constitutively lack mMCP-7 due to a point mutation at the exon 2/intron 2 splice site of the gene (33). In the K/BxN mouse serum–transfer model of arthritis, the MCs in the arthritic synovial tissue of BALB/c mice express mMCP-6 and mMCP-7 (39). C57BL/6 mice are less susceptible to type II collagen–induced arthritis relative to other mouse strains.

Nevertheless, because arthritis can be induced in K/BxN mouse serum–treated C57BL/6 mice (44), we initially assumed that mMCP-7 would not play an important role in this mouse model of inflammatory arthritis. Mouse MCP-6 is one of the few proteases expressed in the IL-3–dependent mouse bone marrow–derived MCs (21) that were used in the adoptive transfer approach to restore pathology in W/W^v mice that subsequently received K/BxN mouse serum (4). As occurs in the K/BxN mouse serum–dependent arthritis model, the MCs in the affected joints of mBSA/IL-1β–treated wildtype C57BL/6 mice express mMCP-6 (Figure 1E).

The accumulated data raised the possibility that mMCP-6–heparin proteoglycan complexes might have a prominent role in experimental arthritis. In support of this possibility, Palmer and coworkers recently reported that joint swelling occurred 4 hours after mice received 1–5 μg of recombinant hTryptase-β complexed to heparin (14).

Using gene-targeting approaches, we created a C57BL/6 mouse strain that lacks heparin due to targeted inactivation of the NDST-2 gene (24). In more recent studies, we created a C57BL/6 mouse strain that expresses mMCP-7 but not mMCP-6 (34). We also created another C57BL/6 mouse strain that lacks both tryptases (35). Using these NDST-2$^-$, mMCP-6$^+$/mMCP-7$^-$, mMCP-6$^-$/mMCP-7$^+$, and mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice, we now show that MC-restricted tryptase–heparin complexes play prominent roles in the model of mBSA/IL-1β–induced arthritis. We discovered that inflammation and cartilage erosion were both markedly reduced in our treated NDST-2$^-$ (Figure 2) and mMCP-6$^-$/mMCP-7$^-$ (Figures 3–5) C57BL/6 mice relative to mMCP-6$^+$/mMCP-7$^-$ (Figures 1, 2, 4, and 5) and mMCP-6$^-$/mMCP-7$^+$ (Figures 3–5) C57BL/6 mice. The packaging of mMCP-6 in the secretory granules of MCs is dependent on heparin (24). Thus, the observation that arthritis is significantly reduced in NDST-2–

null C57BL/6 mice relative to heparin-sufficient wildtype C57BL/6 mice (Figure 2) is consistent with a prominent role for mMCP-6 in mBSA/IL-1β–induced arthritis. Nevertheless, our unexpected discovery that arthritis can be induced in the knee joints of mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice (Figures 3–5) suggests that the 2 MC-restricted tryptases have redundant proinflammatory activities in this experimental arthritis model, and that one must ablate both tryptase genes to fully reveal the prominent roles of these MC-restricted serine proteases in the disorder.

Although it is possible that mMCP-6 and/or mMCP-7 activate cells in the joint via the surface receptor protease-activated receptor 2 (PAR-2) (14), Masuko and coworkers reported that hTryptase-β induces PAR-2–independent release of vascular endothelial growth factor by cultured chondrocytes (45). Thus, the mechanism(s) by which mMCP-6 and mMCP-7 control the severity of the arthritis in mBSA/IL-1β–treated C57BL/6 mice at the molecular level remain to be determined. Mouse MCP-7 is able to induce the recruitment of eosinophils into the peritoneal cavity (42) and neutrophils and eosinophils in the conjunctiva by activating an IL-6–dependent pathway that eventually leads to the generation of other cytokines and chemokines (Miyazaki D, et al: unpublished observations). Mouse MCP-6 and its human homolog hTryptase-β1 induce neutrophil recruitment when injected into the mouse lung or peritoneal cavity (41,46). Moreover, transgenic mice lacking mMCP-6 have an impaired defense against bacterial infections due to a markedly reduced ability to recruit neutrophils into the infected tissue site (35). The discovery that recombinant mMCP-6 and hTryptase-β1 can induce increased expression of CXCL8-like chemokines by cultured cells (41,47) suggests that these serine proteases promote the accumulation of neutrophils (Figures 1C and 3C) in the rheumatoid joint by inducing bystander cells to increase markedly their production of chemokines that are recognized by the granulocytes.

Because large numbers of neutrophils and other leukocytes accumulate in the arthritic joints of mBSA/IL-1β–treated wild-type mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice but not transgenic mMCP-6$^-$/mMCP-7$^-$ C57BL6 mice, we conclude that MC-restricted tryptase–heparin complexes play prominent roles in the inflammatory aspect of the disorder. Nevertheless, our histochemical data also suggested greater loss of cartilage proteoglycans in the knee joints of mBSA/IL-1β–treated mMCP-6$^+$/mMCP-7$^-$ C57BL/6 mice than similarly treated mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice (Figure 4). Al-

though it is possible that MC-derived tryptases preferentially cleave aggrecan proteoglycans in cartilage in a direct manner, Gruber and coworkers (48,49) discovered that human β tryptases can activate latent metalloproteinases, thereby also implicating indirect effects of these MC-restricted proteases on cartilage turnover. In support of the notion that tryptases have a role in extracellular matrix turnover, the MCs in the rat peritoneal cavity express the ortholog of mMCP-6 and hTryptase-β1 (50), and we showed that the supernatants from activated rat peritoneal MCs can induce the rapid degradation of aggrecan proteoglycans in vitro (1). It also is possible that the reduced loss of cartilage in the diseased joints of mBSA/IL-1β–treated mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice relative to that in mMCP-6$^+$/mMCP-7$^-$ and mMCP-6$^-$/mMCP-7$^+$ C57BL/6 mice is attributable to decreased accumulation of collagenase/elastase/cathepsin G–rich neutrophils in mBSA/IL-1β–treated mMCP-6$^-$/mMCP-7$^-$ C57BL/6 mice.

Whatever the mechanisms by which tryptase–heparin complexes regulate inflammation and cartilage loss in the experimental model of arthritis induced by mBSA/IL-1β, we conclude that the earlier failure to appreciate the roles of mMCP-6 and mMCP-7 in mouse models of arthritis is attributable in part to their redundant proinflammatory activities in the diseased joint. Because we discovered that mMCP-7 can compensate for the loss of mMCP-6 in the experimental model of mBSA/IL-1β–induced arthritis, it is now apparent that one must carry out experiments on mice that lack both tryptases in order to uncover the prominent roles of MC-restricted tetramer-forming tryptases in inflammation and connective tissue remodeling. Finally, our data raise the possibility that inhibition of the β tryptases in MCs could have therapeutic value in the treatment of RA. However, on a cautionary note, the finding that our mMCP-6–deficient mice cannot efficiently combat bacterial (35) and helminthic (34) infections raises the possibility that patients with RA might be more susceptible to infectious organisms if their MC-restricted tryptases are inactivated systemically.

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