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Deletion of the Mouse Meprin \( \beta \) Metalloprotease Gene Diminishes the Ability of Leukocytes to Disseminate through Extracellular Matrix

Jacqueline M. Crisman,*† Binzhi Zhang, † Lourdes P. Norman,* and Judith S. Bond2*  

Meprins are metalloendopeptidases expressed by leukocytes in the lamina propria of the human inflamed bowel, that degrade extracellular matrix proteins in vitro implicating them in leukocyte transmigration events. The aims of these studies were to 1) examine the expression of meprins in the mouse mesenteric lymph node, 2) determine whether macrophages express meprins, and 3) determine whether deletion of the meprin \( \beta \) gene (Mep-1\( \beta \)) mitigated the ability of leukocytes to disseminate through extracellular matrix in vivo. These studies show that meprin \( \alpha \) and \( \beta \) are expressed in leukocytes of the mouse mesenteric lymph node, and meprin \( \alpha \), but not \( \beta \), decreased during intestinal inflammation. Deletion of Mep-1\( \beta \) gene decreased the ability of leukocytes to migrate through matrigel compared with wild-type leukocytes. Meprin \( \beta \), but not \( \alpha \), was detected in cortical and medullary macrophages of the lymph node. Thus overall, meprin \( \beta \) is expressed by leukocytes in the draining lymph node of the intestine, regardless of the inflammatory status of the animal, and is likely to contribute to leukocyte transmigration events important to intestinal immune responses. Thus, the expression of meprins by leukocytes of the intestinal immune system may have important implications for diseases such as inflammatory bowel diseases, which are aggravated by leukocyte infiltration.  


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3 Abbreviations used in this paper: IBD, inflammatory bowel disease; MMP, matrix metalloprotease; DSS, sodium dextran sulfate; MCP-1, monocyte chemotactic protein-1; MIP-1\( \alpha \), macrophage inflammatory protein-1\( \alpha \).
fibronectins), as well as growth factors, such as gastrins, TNF-α, and osteopontin (13, 14).

Defining the mechanisms regulating leukocyte extravasation is central to the understanding of the progression of IBD. The aim of this study was to determine the extent of meprin expression in the intestinal immune system, and whether inflammation alters the expression of these metalloproteases. For this purpose, intestinal inflammation was induced by orally administering sodium dextran sulfate (DSS), which generates an experimental model for IBD in mice (15). An additional aim was to determine whether deletion of the Mep-1β gene mitigates the ability of leukocytes from the mesenteric lymph node to migrate through extracellular matrix in vitro.

Materials and Methods

DSS-treatment of BALB/c mice

Male BALB/c mice (6–8 wk old) were purchased from Charles River Laboratories (Wilmington, MA). All animals were given chow ad libitum. Five percent DSS (TOB Consultancy, Uppsala, Sweden) was administered to mice in their drinking water (five mice) for 7 days, while untreated mice received plain drinking water (five mice). Body weights and the health of the animals were recorded every other day. After 1 wk, the mice were weighed, sacrificed, and the mesenteric lymph nodes were harvested for immunohistochemistry, immunofluorescence and RNA extraction. The ilium was also harvested and fixed for immunofluorescence.

RT-PCR to detect meprin α and β subunits

Mesenteric lymph nodes were macerated through wire mesh and suspended in RPMI 1640 to make a single cell suspension, and centrifuged at 800 × g. Poly(dI-dC):poly(dI-dC) was isolated using the MicroFast Track Kit per manufacturer’s specifications (Invitrogen, Carlsbad, CA). The RNA was checked for DNA contamination by performing standard PCR using primers to the housekeeping gene GAPDH (342 bp, upper primer 5′-GCAAGATGGAGATTGTTGCC-3′ and lower primer 5′-ATTTCTGCTGTTGCACACC-3′). Semi-quantitative RT-PCR was performed (One-Step RT-PCR kit; Life Technologies, Rockville, MD) to amplify the meprin α-transcript (161 bp, sense primer 5′-GTGCTGTTCTATGTTGTTGATC-3′ and antisense primer 5′-CCCACCAAAATGTTCACATAATCATC-3′) and β-transcript (63 bp, sense primer 5′-ACAAGGGTCTGGTCACTCAT-3′ and antisense primer 5′-TTTCCGTGCAGTC-3′) or CCR2 transcript (344 bp, sense primer 5′-TTACCTCAGTTATTCCTGCTCAACTG-3′; or antisense primer 5′-CCCACCAAAATGTTCACATAATCATC-3′). All RT-PCR were sampled every third cycle, between cycles 18–36. The relative abundance of the transcripts were calculated as the ratio of the optical densities of the meprin or CCR2 amplification products to that of the GAPDH, when the PCR amplification was in the linear range. These experiments were performed at least three times using animals from different DSS experiments.

Immunohistochemistry to detect meprin α or β subunits

Mesenteric lymph node tissues from DSS-treated or untreated mice were prepared as described above. After deparaffinization and rehydration, the sections were blocked with Background Boster (Vector Laboratories) and incubated with 10 µg/ml PE-conjugated F4/80 Ab or PE-conjugated isotype control Ab overnight at 4°C. Additional sections were incubated with fluorescein-conjugated neutrphil mAb (clone 7/4, Serotec). The sections were washed and incubated for 90 min at 25°C with rabbit meprin β antisera or nonimmune rabbit antisera (1:1000). Magnetic field sorting was performed to obtain CD11b-enriched and -depleted leukocyte populations (Miltenyi Biotec), which were lysed with Stuart’s buffer and used to perform immunoprecipitation experiments as described above. These experiments were performed twice.

Immunofluorescence to determine the leukocyte populations expressing meprin α and β

Mesenteric lymph node tissues from DSS-treated or untreated mice were prepared as described above. After deparaffinization and rehydration, the sections were blocked with Background Boster (Vector Laboratories) and incubated with 10 µg/ml PE-conjugated F4/80 Ab or PE-conjugated isotype control Ab overnight at 4°C. Additional sections were incubated with fluorescein-conjugated neutrophil mAb (clone 7/4, Serotec). The sections were washed and incubated for 90 min at 25°C with rabbit meprin β antisera or nonimmune rabbit antisera (1:1000). FITC-conjugated donkey anti-rabbit antisera (0.75 µg/ml) was used to detect meprins in the immunoprecipitates. These experiments were performed twice.

Chemotaxis and invasion assays

Meprin α null and wild-type mice were generated on a C57BL/6:129/SvJ back background and characterized (19). Leukocytes from the mesenteric lymph node from 3-mo-old meprin β null mice and strain/age-matched wild-type mice were isolated as described above. Chemotaxis assays were performed as described in Crismon et al. (20), methods that have been previously well characterized for the migration of leukocytes (21–23). Briefly, cells were suspended in Gey’s balanced salt solution with 0.2% BSA at a concentration of 1 × 10⁵ and labeled with 2 µM calcein acetoxymethyl ester (Molecular Probes, Eugene, OR) for 30 min at 37°C. After washing, the cells were suspended to 1 × 10⁵ cells/ml. Variable concentrations of monocyte chemotactant protein-1 (MCP-1) or macrophage inflammatory protein-1α (MIP-1α; R&D Systems, Minneapolis, MN) were placed in a 96-well Polytronics viewplate inside a NeuroProbe chamber (NeuroProbe, Gaithersburg, MD). The plate was overlaid with a polycarbonate PVP-free framed filter (5 µm pore size) and the NeuroProbe chamber was closed. Two hundred microliters of cells were added to the top chamber and the assembly was incubated for 2 h at 37°C with 5.0% CO₂. The filter was removed and unbound cells were scraped from the top of the filter and
air-dried. The number of migrating cells was determined by reading the filter in a Fluoroskan Ascent fluorescence plate reader (Thermo Environmental Instruments, Franklin, MA). In these studies, 1 fluorescent unit corresponds to roughly 30,000 migrating cells. These experiments were performed three times with three replicates per sample.

Invasion assays were performed to compare the ability of leukocytes from meprin β null and wild-type mice to migrate through extracellular matrix, using matrigel invasion chambers (BD Biosciences, Bedford, MA). Mesenteric lymph node leukocytes were obtained from 6-wk-old meprin β null and strain-matched wild-type mice, as described above. Matrigel-coated filters were hydrated in DMEM at 37°C for 2 h and immersed in a well containing DMEM only or DMEM with 5 or 10 nM MCP-1 or MIP-1α (in triplicate). Leukocytes (3.4 × 10⁶/well) were layered on top of the matrigel. After incubation for 18 h at 37°C with 5% CO₂, the filters were stained (Hema 3 stain set; Fisher Scientific, Pittsburgh, PA) and the migrating cells were counted in ×5–×20 fields and averaged. The number of cells migrating without MCP-1 or MIP-1α was subtracted from the number migrating in response to the chemoattractant. These experiments were performed four times.

Three additional matrigel assays were performed using MCP-1 as a chemoattractant, to determine whether meprin β is expressed by the wild-type leukocytes migrating through matrigel, as well as to determine whether these leukocytes were macrophages. The resulting filters were fixed in methanol and used to detect meprin β-expressing macrophages, as described above. Briefly, the filters were blocked with background buster, treated with meprin β or nonimmune antisera, followed by FITC-conjugated donkey anti-rabbit antisera. Macrophages were detected by incubating the filters with PE-conjugated F4/80 mAb or isotype control. The cells attached to the filters were treated with Horsch dye as a nuclear counterstain and examined by fluorescent microscopy, as described above.

**FIGURE 1.** Semi-quantitative RT-PCR to detect meprin α, β, or GAPDH transcripts in RNA samples isolated from the mesenteric lymph node of untreated or DSS-treated mice. RT-PCR was performed using RNA isolated from mesenteric lymph nodes of DSS-treated or untreated mice to detect meprin α (161 bp), meprin β (614 bp), or GAPDH (342 bp) transcripts. Representative results to detect meprin α transcripts and corresponding GAPDH in samples from untreated and DSS-treated mice are shown in A and B, respectively. C and D, Representative results from RT-PCR to detect meprin β transcripts and GAPDH transcripts from corresponding samples. E, Relative abundance of meprin α and meprin β transcripts calculated as described in Materials and Methods (■, DSS-treated; □, untreated). For meprin α, difference between DSS-treated and untreated, p < 0.05.
Superoxide anion release and phagocytosis assays

Peritoneal macrophages were isolated from 3- to 6-mo-old meprin β null/C57BL/6:129/SvJ and wild-type C57BL/6:129/SvJ mice by peritoneal lavage, as previously described (24). The percentage of macrophages (49.2 ± 9.1%) in each sample was determined by staining smears of each sample with PE-conjugated F4/80 Ab and Horscht stain, as described above. Macrophages from these two strains of mice were compared for their ability to release hydrogen peroxide and to phagocytose Escherichia coli. The release of hydrogen peroxide was measured using a luminol based kit (Lumimax Superoxide Anion Detection Kit; Stratagene, La Jolla, CA). Briefly, meprin β null/C57BL/6:129/SvJ and wild-type C57BL/6:129/SvJ macrophages (5 × 10^5/tube) were incubated with assay medium alone, or assay medium with 100 ng/ml PMA for 30 min. Luminol was added to each to a final concentration of 100 μM and the light intensity in each sample was measured on a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). These studies were repeated four times with six replicates per sample.

The ability of peritoneal macrophages from meprin β null/C57BL/6:129/SvJ and wild-type C57BL/6:129/SvJ mice to phagocytose FITC-conjugated E. coli K-12 (bioparticle) was also assessed using a commercially available kit (Vybrant Phagocytosis Assay Kit; Molecular Probes). For these studies, wild-type or meprin β null macrophages in DMEM/7.5% FBS were added to 12 wells of a 96-well plate. After 1 h at 37°C with 5% CO₂, the medium was removed from each well and 200 μl of bioparticles (1 mg/ml suspension) was added for 2 h at 37°C with 5% CO₂. The bioparticles were removed from the wells and 200 μl of trypan blue (500 μg/ml) was added to each well, to quench the fluorescence of extracellular bioparticles. The intracellular fluorescence was measured on a Fluroskan Ascent fluorescent plate reader (Labsystems, Helsinki, Finland). These experiments were performed 4 times with 6–12 replicates per sample.

Results

Meprin α and meprin β mRNA were detected in the mesenteric lymph node of untreated and DSS-treated mice

To determine whether meprin expression in mesenteric lymph node was modulated by intestinal inflammation, BALB/c mice were treated with DSS for 1 wk. DSS treatment induced severe signs of intestinal inflammation including rectal bleeding in 86 ± 8% of mice. Furthermore, the DSS-treated mice from these experiments weighed ~22% less than control animals at sacrifice (e.g., body weight of DSS-treated mice, 17.9 ± 1.7 g, n = 31 vs 23.0 ± 1.5 g, n = 30 for untreated mice, p < 0.0001).

Polyadenylated mRNA from the mesenteric lymph node of DSS-treated and untreated mice was used to perform semi-quantitative RT-PCR. These studies measured the abundance of meprin transcripts relative to the abundance of transcripts for the housekeeping GAPDH gene. The expression of meprin α transcripts was 8-fold less in the mesenteric lymph nodes of DSS-treated group (n = 4) when compared with mRNA extracted from untreated group (n = 5, p = 0.0048, Fig. 1). The abundance of meprin β transcripts was similar in the mesenteric lymph nodes of DSS-treated (n = 4) and untreated animals (n = 3, p = 0.72). These results indicate that while meprin α and β are expressed to similar extents in these lymph nodes, the abundance of meprin α transcripts is markedly diminished during intestinal inflammation. Meprin β expression is unchanged by the inflammatory status of the intestine.
Meprins were detected in the mesenteric lymph nodes of untreated and DSS-treated mice

To localize the expression of meprins in the lymph nodes of BALB/c mice, immunohistochemistry was performed using rabbit antisera raised against recombinant rat meprin α or β proteins. In mesenteric lymph nodes of untreated mice, meprin α and β protein was detected in distinct leukocytes found in the medulla, trabeculae, and subcapsular space, as well as in the perifollicular regions of the outer cortex (Fig. 2). These data indicate that meprin α and β proteins are expressed by distinct populations of leukocytes in the lymph node under normal conditions. These studies were also performed with sections from the lymph nodes of C57BL/6:129/SvJ mice, yielding similar results.

Meprin α protein was decreased in mesenteric lymph nodes from DSS-treated mice compared with that from untreated mice

Consistent with the RT-PCR results, immunohistochemistry performed with mesenteric lymph node sections from DSS-treated mice demonstrated a marked decrease in the expression of meprin α protein, when compared with that from untreated mice (Fig. 3). Meprin β levels did not change after DSS treatment (data not shown).

Immunoprecipitation studies confirmed that after DSS-treatment the expression of meprin α in the mesenteric lymph node decreased. In samples prepared from the mesenteric lymph nodes of untreated animals, these studies detected a protein of ~90 kDa using meprin α antisera, consistent with the reported size of meprin α (Fig. 4, lane 2). However, little if any meprin α was detected in samples from DSS-treated mice (Fig. 4, lane 4). Immunoprecipitation studies also detected the expression of the meprin β subunit in mesenteric lymph node leukocytes from DSS-treated and untreated mice. A band of ~100 kDa was detected with meprin β antisera, consistent with the previously reported size for meprin β (data not shown).

Meprins are expressed in macrophages of the mesenteric lymph node

Previous studies detected F4/80+ macrophages in the subcapsular space and perifollicular regions of the lymph node and medulla (25, 26). Considering that a high density of meprin+ leukocytes

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**FIGURE 3.** Representative immunohistochemistry to detect the expression of meprin α protein in the mesenteric lymph node of DSS-treated mice. A and B, Sections of mesenteric lymph node from DSS-treated mice incubated with meprin α antisera. C, Sections from untreated mice incubated with meprin α antisera. D, Section stained with nonimmune antisera. Arrow indicates subcapsular space; T, trabeculae. Images taken for B–D were visualized by oil emersion.
were also detected in these regions of the mesenteric lymph node, the expression of meprins by macrophages of the lymph node was investigated. For this purpose, immunofluorescence was performed using F4/80 Ab and meprin β antisera. In these experiments, distinct F4/80⁺/meprin β⁺ and a few F4/80⁻/meprin β⁺ leukocytes were detected in the cortex and medulla of the mesenteric lymph node with and without DSS-treatment (Fig. 5, B and C). These studies did not detect meprin α expression in F4/80⁺ leukocytes of the lymph node. Finally, immunofluorescence studies using sections of mesenteric lymph node were performed with a fluorescein-conjugated Ab-specific for neutrophils (7/4 mAb). Neutrophils were not detected in these sections (data not shown).

In addition, CD11b⁺-enriched and -depleted fractions were generated by positive selection using magnetic separation. CD11b is a specific marker for macrophages in the lymph node (27). Those cells that did not attach to the column during magnetic separation were found to be ≥96% depleted of CD11b⁺ cells by microscopically counting 20 separate ×40 fields (mean percentage of

![Image](53x605 to 293x742)
CD11b-depleted cells in two experiments 96.3 ± 1%). The positively selected CD11b population was found to be 64.6–72.7% enriched in 20 separate ×40 fields (mean percentage of CD11b-enriched cells in two experiments 68.7 ± 5.7%). To perform immunoprecipitation assays, these two leukocyte populations were disrupted with Stuart’s buffer. Meprin β protein was detected in both the CD11b-enriched and -depleted populations (Fig. 6A). Because the CD11b-depleted population was >96% depleted of CD11b+ cells (i.e., macrophages), it is likely that CD11b− leukocytes in the mesenteric lymph node express meprin β. Similar studies performed to detect meprin α in the CD11b-enriched and -depleted fractions indicated that meprin α is expressed in only the CD11b-depleted fraction (Fig. 6B). These studies overall indicate that macrophages contribute to the production of meprin β in the mesenteric lymph node. However, meprin α is expressed by leukocytes other than CD11b+ macrophages.

Leukocytes from meprin β null mice are deficient in their ability to invade into matrigel

Because meprin β is an integral membrane protein expressed by leukocytes in the mesenteric lymph node, it was hypothesized that meprin β is involved in leukocyte infiltration. To address this hypothesis, matrigel invasion assays were performed to compare these two leukocyte populations. Chemotaxis assays were also performed to compare the ability of meprin β knockout and wild-type mice to migrate in response to a chemoattractant. Chemotaxis assays comparing leukocytes from the mesenteric lymph nodes of meprin β null and wild-type mice demonstrated that leukocytes from both mouse strains migrated in response to the chemoattractant MCP-1 (Fig. 7), with peak chemotaxis occurring between 5 and 10 nM. Meprin β null leukocytes migrated in response to 5 and 10 nM MCP-1 similarly to age/strain-matched wild-type leukocytes (p = 0.7189 and 0.5162, respectively, n = 15). However, deletion of Mep-1β significantly diminished the ability of meprin β null leukocytes to infiltrate matrigel in response to MCP-1 (Fig. 8). However, when matrigel assays were performed using MCP-1 (10 nM) as the chemoattractant, the number of meprin β null leukocytes that migrated through the matrigel was decreased 8-fold compared with that from wild-type controls (i.e., mean number of wild-type leukocytes that migrated through matrigel 56.2 ± 10.2, n = 9 vs mean number of knockout leukocytes invading matrigel 7.4 ± 3.1, n = 9, p = 0.0013, calculated after subtracting the mean values of the negative controls). Studies performed with 5 nM MCP-1 reproducibly yielded similar results, specifically the number of meprin β null leukocytes that migrated through matrigel was decreased 3-fold compared with that from wild-typematched controls (i.e., mean number of wild-type leukocytes that migrated through matrigel 56.2 ± 10.2, n = 9 vs mean number of knockout leukocytes invading matrigel 8.8 ± 4.1, n = 6, p = 0.0025, calculated after deducting the mean values of the negative controls). Similar results were obtained in matrigel and chemotaxis assays using MIP-1α as a chemoattractant (Fig. 7 and 8). Specifically, deletion of Mep-1β diminished the ability of leukocytes 2-fold compared with wild-type leukocytes (p < 0.001), but did not alter their chemotactic response to MIP-1α.

Three additional matrigel assays were performed to determine whether the infiltrating wild-type leukocytes were meprin β+ macrophages. The leukocytes were allowed to migrate through the matrigel and onto the underlying filters. Afterward the filters were fixed and subjected to immunofluorescence using Abs to detect meprin β or the F4/80 macrophage-specific protein. These studies
determined that wild-type meprin β+ macrophages migrated through the matrigel (Fig. 8C).

To assess whether the diminished infiltration into matrigel by meprin β null leukocytes resulted from decreased CCR2 expression (the MCP-1 receptor) compared with age/stained-matched wild-type, semi-quantitative RT-PCR was used. The relative abundance of CCR2 transcripts (the MCP-1 receptor) was similar when comparing pooled mesenteric lymph nodes of 10 meprin β null (mean value 0.46 ± 0.08, n = 2) and 10 wild-type mice (mean value 0.55 ± 0.03, p = 0.25, n = 2). In addition, the expression of meprin α was similar in the pooled mesenteric lymph nodes of 5 meprin β null mice (mean value 0.47 ± 0.02, n = 2) and pooled mesenteric lymph nodes of 10 strain/age-matched wild-type mice (mean value 0.60 ± 0.25, p = 0.52, n = 4).

To assess the impact of deletion of meprin β on other functional properties of macrophages, the ability of meprin β null and wild-type macrophages to undergo phagocytosis and superoxide anion release was compared. These studies indicated that meprin β null and wild-type macrophages were comparable in their ability to release hydrogen peroxide (wild-type 2470 ± 1419 vs meprin β null 2473 ± 1372, n = 12, p = 0.9989) and phagocytosis E. coli (wild-type 0.1641 ± 0.0583 vs meprin β null 0.1277 ± 0.0283, n = 12, p = 0.5949).

Macrophages of the ileum express meprin β
To determine whether meprin β+ macrophages were present during intestinal inflammation, immunofluorescence studies were performed using terminal ileum and colon sections from DSS-treated and untreated mice. Meprin β+/F4/80+ macrophages were detected in the lamina propria of DSS-treated, but not untreated, mice (Fig. 9). Finally, similar studies were performed with fluorescein-conjugated neutrophils mAb (clone 7/4, Serotec). Meprin β+ or meprin α+ neutrophils in the mesenteric lymph node, ileum, and colon from DSS-treated or untreated mice were not detected. (data not shown).

Discussion
The studies herein support the hypothesis that meprin β is an important mediator of leukocyte infiltration in the mesenteric lymph node and intestine. The results clearly demonstrate that for macrophages the functional responses and expression of chemokine receptors was not diminished by deletion of Mep-1β. Therefore, the cellular machinery of the macrophages needed for an immunological response was intact even without meprin β activity. Thus, the diminished infiltration of matrigel by meprin β null leukocytes can be directly attributed to the deletion of meprin β in leukocytes of the mesenteric lymph node.

FIGURE 8. In vitro matrigel assays to compare the ability of meprin β null and wild-type leukocytes to infiltrate matrigel. Meprin β null (▲), or age- and strain-matched wild-type (□) mesenteric lymph node leukocytes (1.7 × 10⁵ well) were incubated in the upper well of triplicate matrigel chambers, with 10 nM MCP-1 (A) or 10 nM MIP-1α (B) in the lower reservoir of the matrigel chamber. After 18 h, the filters were stained to reveal those cells that infiltrated through the matrigel and onto the filter. The infiltrating cells were counted in ×5×5 fields. (*, p < 0.05, n = 15). C, Matrigel assays were performed to detect meprin β and the macrophage-specific protein F4/80 on wild-type macrophages infiltrating matrigel. The filters were incubated with PE-conjugated anti-F4/80 mAb (red, C) or isotype control (red, D) and rabbit anti-meprin β antisera (C) or rabbit nonimmune sera (D), followed by FITC-conjugated donkey anti-rabbit IgG (green). Horscht stain was used as a nuclear counterstain (blue).
FIGURE 9. Representative immunofluorescence to detect meprin β⁺ macrophages in the ileum of DSS-treated mice. Sections were treated with PE-conjugated anti-F4/80 mAb (red) and rabbit anti-meprin β antisera, followed by FITC-conjugated donkey anti-rabbit IgG (green). Meprin β⁺/F4/80⁺ macrophages were detected in the lamina propria of the ileum after induction of DSS-mediated intestinal inflammation (red arrow, A and C). B, Meprin β⁺-expressing macrophages were not detected in the ileum of untreated mice. D, normal ileum stained with PE-conjugated isotype control (red) and nonimmune rabbit sera followed by FITC-conjugated donkey anti-rabbit IgG (negative control). All sections were counterstained with Horscht stain (blue).

The role of meprin β in leukocyte infiltration may be explained by a direct mechanism, whereby the membrane-localized meprin β degrades extracellular matrix and basement membrane to facilitate leukocyte infiltration. Alternatively, the decreased motility of meprin β null leukocytes may be attributed to indirect mechanisms (e.g., meprin β may activate another pivotal protease needed for leukocyte infiltration). In fact, meprin α is implicated in the cellular transmigration events of metastatic colon cancer cells, because these cell lines selectively express meprin α, and nonmetastatic colon cancer cells are meprin α⁻ (G. Matters, unpublished observations). Interestingly, active meprin α is localized with meprin β to the apical surface of the proximal tubular epithelium, while the secreted form of meprin α is inactive and forms meprin α homo-oligomers (5, 19). Whatever the mechanism, it can be concluded that meprin β is essential to leukocyte infiltration, at least in vitro in the mesenteric lymph node.

Many studies describe the expression of metalloproteases (e.g., gelatinase B) in inflamed and normal tissue, and their potential roles in leukocyte extravasation (2, 3). However, the role of proteases in leukocyte dispersion into the lymph node has not been extensively studied. Faveeuw et al. (28) demonstrated that a metalloprotease(s) is involved in leukocyte trafficking in the lymph node. These authors reported that a hydroxamate-based metalloprotease inhibitor (Ro 31-9790) inhibited leukocyte trafficking in the lymph node via the high endothelial venules. When Ro 31-9790 was administered to mice, a marked increase was noted in the number of leukocytes within the endothelial cell lining of the high endothelial venules. Because L-selectin shedding is also diminished by Ro 31-9790 in vitro, the authors concluded that this inhibitor diminished L-selectin shedding in vivo, and thus increased leukocyte transmigration across the high endothelial venules. However, it is also possible that Ro 31-9790 diminished the metalloprotease-mediated degradation of extracellular matrix, thereby slowing the migration of the leukocytes across the wall of the high endothelial venule. Ro 31-9790 efficiently inhibits the major classes of secreted MMP, including MMP1–3, MMP8, and MMP9, as well as the membrane-associated ectoenzymes, MMP14 and MMP17. However, the protease(s) inhibited by Ro 31-9790 in the lymph node is not likely to be meprin β, because few meprin β⁺ or meprin α⁺ leukocytes were found in or around the high endothelial venules of the paracortex.

The studies herein establish that meprin β is expressed by macrophages and implicate the protease in trafficking of macrophages through the lymph node. Furthermore, basement membrane of the subcapsular space is composed of primarily fibronectin, a known substrate for meprins (13, 14). This basement membrane effectively demarcates the subcapsular space from the rest of the lymph node, preventing even small particles of dye from entering the cortex (29, 30). Electron microscopy has shown that macrophages extrude through this basement membrane into the outer cortex, with little to no damage to the site of extrusion (29, 30). It is possible that membrane-bound meprins are involved in the migration of macrophages into the paracortex, where they comingle with T cells. This type of relationship would require elegant mechanisms to temporally and spatially regulate meprin activity. It is possible that the activity of proteases at the leading and trailing edge is highly regulated, by the selective targeting of proteases and/or selective activation and deactivation to minimize indiscriminate hydrolysis.

To date the bulk of the data support the expression of meprins by leukocytes to the intestinal immune system, implicating meprins in intestinal leukocyte infiltration. The expression of meprins is tissue-specific under normal conditions (i.e., primarily in kidney and intestine) (5, 10). Furthermore, renal leukocytes do not express meprins as demonstrated in rodent models of renal disease (e.g.,
ureteral obstruction (16) and acute renal failure (31)). These models are noted for having an extensive macrophage infiltrate that exacerbates the associated inflammation. However, interestingly, the macrophage infiltrate associated with these renal diseases does not express meprins.

During IBD, macrophages accumulate in large numbers in the intestine, where they function as APCs (27, 28, 30, 32). In addition, macrophages are a major source of important cytokines such as IL-18 and IL-12, which are instrumental to the development of the Th1 immune response associated with IBD (33). Considering that meprin α and β homooligomers: cleavage of basement membrane proteins and sensitivity to metalloprotease inhibitors. Biochem. J. 378:383.


