Impaired Immunity to Intestinal Bacterial Infection in Stromelysin-1 (Matrix Metalloproteinase-3)-Deficient Mice


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Impaired Immunity to Intestinal Bacterial Infection in Stromelysin-1 (Matrix Metalloproteinase-3)-Deficient Mice

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Infection of mice with the intestinal bacterial pathogen *Citrobacter rodentium* results in colonic mucosal hyperplasia and a local Th1 inflammatory response similar to that seen in mouse models of inflammatory bowel disease. Matrix metalloproteinases (MMPs) have been shown to mediate matrix remodeling and cell migration during tissue injury and repair in the intestine. We have previously shown enhanced pathology in infected TNFrp55−/−, IL-12p40−/−, and IFN-γ−/− mice, and here we show that is associated with an increase in stromelysin-1 (MMP3) transcripts in colonic tissues. We have therefore investigated the role of MMP3 in colonic mucosal hyperplasia and the local Th1 responses using MMP3−/− mice. In MMP3−/− mice, similar mucosal thickening was observed after infection as in wild-type (WT) mice. Colonic tissues from MMP3−/− mice showed a compensatory increase in the expression of other MMP transcripts, such as MMP7 and MMP12. However, MMP3−/− mice showed delayed clearance of bacteria and delayed appearance of CD4+ T lymphocytes into intestinal lamina propria. CSFE-labeled mesenteric lymph node CD4+ T lymphocytes from infected WT mice migrated in fewer numbers into the mesenteric lymph nodes and colon of MMP3−/− mice than into those of WT mice. These studies show that mucosal remodeling can occur in the absence of MMP3, but that MMP3 plays a role in the migration of CD4+ T lymphocytes to the intestinal mucosa. *The Journal of Immunology*, 2004, 173: 5171–5179.

Infection of mice with the intestinal bacterial pathogen *Citrobacter rodentium* results in colonic mucosal hyperplasia and a local Th1 inflammatory response similar to that seen in mouse models of inflammatory bowel disease (IBD). In susceptible mouse strains, *C. rodentium* infection is associated with colonic crypt hyperplasia, goblet cell depletion, and mucosal erosion (1, 2). Infection of mice with live *C. rodentium* or intracolonic inoculation of dead bacteria also induces a large infiltrate of CD4+ cells into the colonic lamina propria and a highly polarized local Th1 response with increased transcripts for the type 1 cytokines IL-12, TNF-α, and IFN-γ (3, 4).

To elucidate the role of proinflammatory cytokines in host defense and mucosal pathology in vivo, mice deficient in TNF-α, IFN-γ, and IL-12 were orally infected with live *C. rodentium* (5, 6). These animals showed various degrees of impairment in their ability to clear infection, but remarkably, mucosal pathology was greater in these mice than in wild-type (WT) controls.

Matrix metalloproteinases (MMPs) are a family of proteases important in the turnover of extracellular matrix and cell migration (reviewed in Ref. 7). MMPs belong to the matrixin subfamily of the metzincin superfamily of metalloproteinases. To date, >23 different MMPs have been cloned, and additional members continue to be identified. Structurally, all MMPs share a similar prodomain and catalytic domain, which act on a broad spectrum of the extracellular matrix components. Most MMPs are secreted as proenzymes and require proteolytic cleavage for activation. Inhibition of MMPs is conducted by endogenous MMP inhibitors, tissue inhibitor of metalloproteinases (TIMP), which contain four members (TIMP1–4). MMPs have been implicated in tissue injury and matrix remodeling in various chronic inflammatory diseases such as IBD, asthma, and rheumatoid arthritis. In the intestine, MMPs are highly expressed at ulcer edges in patients with chronic IBD and play a crucial role in the tissue injury that follows T cell activation in explants of human fetal small bowel (8–10). In celiac disease, where a mucosal Th1 response to gluten drives mucosal remodeling and growth, MMP3 is overexpressed in fibroblasts immediately under the epithelium (11).

Taken together, these results suggest that MMPs, especially MMP3, is involved in immune-mediated tissue injury in the intestine. MMPs probably play a role in degrading the matrix to produce mucosal ulceration, but they may also be important in controlling matrix turnover and in the mucosal thickening seen in experimental and clinical intestinal inflammation.

In this work, therefore, we first examined MMP3 in the colon of WT and TNF-α, IFN-γ, and IL-12 knockout (KO) mice infected with *C. rodentium* and subsequently investigated mucosal hyperplasia and the local immune responses in MMP3−/− mice.

**Materials and Methods**

**Mice**

Female 6- to 8-wk-old C57BL/6J and B10R111 mice were purchased from Harlan Olac (Bicester, U.K.) or Bantin and Kingman Universal (Hull, U.K.). TNFrp55−/−, IL-12p40−/−, and IFN-γ−/− mice (backcrossed to
C57BL/6 background at least 10 times) were originally purchased from The Jackson Laboratory (Bar Harbor, ME) and were maintained by homozygous matings under contract at Bantin and Kingman Universal. MMP3−/− mice were obtained from John Mudgett, Merck Research Laboratories (Rahway, NJ) and were maintained by homozygous matings. All mice came from specific pathogen-free colonies. They were kept in specific pathogen-free environment with free access to sterilized food and water. All experiments used five to six mice per group and were repeated at least twice.

**Bacterial strains and oral infection of mice**

A nalidixic acid-resistant isolate of *C. rodentium* (formerly *Citrobacter freundii* biotype 4280) was used in this study. DBS225 (pCVD438) is a *C. rodentium* cae (intimin α) strain, which expresses biologically active intimin and is virulent in mice (12). Bacterial inocula were prepared by culturing bacteria overnight at 37 °C in 10 ml of Luria broth containing nalidixic acid (100 μg/ml) plus chloramphenicol (50 μg/ml). Cultures were harvested by centrifugation and resuspended in 0.1 volume of PBS. Mice were orally inoculated with 200 μl of the bacterial suspension with a gavage needle. The viable count of the inoculum was determined by plating on Luria-Bertani agar containing appropriate antibiotics. In all experiments, mice received 1–4 × 107 CFU.

**Measurement of pathogen burden**

At selected times postinfection, mice were killed by cervical dislocation. The terminal 4 cm of the colon were removed, and the colon was weighed after removal of fecal pellets. In some experiments, 1-cm samples of distal colon were snap-frozen in liquid nitrogen for subsequent immunohistological analysis, cytokine RT-PCR, and MMP Western blotting. Specimens and colons were homogenized mechanically using a Seward 80 stomacher (Seward Medica, London, U.K.). The number of viable bacteria in organ homogenates was determined by viable count on Luria-Bertani agar containing 100 μg/ml nalidixic acid and 50 μg/ml chloramphenicol. The limit of sensitivity was 10 CFU/organ.

**Immunohistochemistry and measurement of mucosal thickness**

A three-step avidin-peroxidase staining was performed on 5-μm frozen sections as described previously (5) using mAbs YTS191 (anti-CD4), anti-β7 and anti-mouse MADCAM (both from BD BioSciences, Oxford, U.K.). Biotin-conjugated rabbit anti-mouse IgG (1/50) and avidin peroxidase (1/200; DAKO, Cambridge, U.K.) were diluted in Tris-buffered saline (pH 7.6) containing 1% (w/v) BSA. The slides were blocked by addition of 1.5% (v/v) BSA in PBS for 1 h. Plates were then washed twice with PBS-Tween 20 before adding 100 μl of the bacterial suspension with a gavage needle. The viable count of the inoculum was determined by plating on Luria-Bertani agar containing appropriate antibiotics. In all experiments, mice received 1–4 × 107 CFU.

**Protein extraction**

Colonic samples (2 cm) were snap-frozen at −70 °C and homogenized in ice-cold extraction buffer (50 mM Tris-HCl (pH 7.4), 10 mM CaCl2, 0.05% Brij 35, 0.25% Triton X-100) at maximum speed for 30 s using an IKA tissue homogenizer (Fisher Scientific, Loughborough, U.K.). The homogenates were centrifuged at 13,000 rpm for 10 min at 4 °C, and the supernatants were removed and assayed for protein concentration (Bio-Rad, Hemel Hempstead, Hertfordshire, U.K.). Homogenates were stored at −70 °C until required.

**Western blotting**

Samples were denatured in reducing treatment buffer and loaded (200 μg/lane onto 10% (v/v) Novex Bis-Tris gels (Invitrogen, Paisley, U.K.). After electrophoresis, the proteins were transferred onto nitrocellulose membrane (Bio-Rad) using a Novex blotting apparatus (Invitrogen, Paisley, U.K.) and detected using goat anti-mouse MMP3 (1/250) (R&D, Abingdon, U.K.), HRP-conjugated rabbit anti-goat secondary Ab (DAKO, Cambridge, U.K.), and an ECL Plus kit (Amersham Biosciences, Little Chalfont, U.K.) according to the manufacturer’s instructions. The stripping was performed on ECL film (Amersham Biosciences). For control of protein loading, the blot was stripped with Tris-HCl buffer (62.5 mM, pH 6.7) containing 10 mM 2-ME for 30 min at 60 °C with gentle shaking. The membrane was then washed with PBS-Tween 20 for 10 min for 3 times at RT. After blocking with 5% skimmed milk in PBS-Tween 20, the membrane was re-probed with 1/10,000 rabbit anti-mouse β-actin (AbCam, Cambridge, U.K.) and 1/1000 HRP-conjugated goat anti-rabbit secondary Ab (DAKO, Cambridge, U.K.). The blot was then developed as described above.

**RNA extraction and quantitative RT-PCR**

Total cellular RNA was isolated from frozen colonic tissues by homogenization of the tissue in TRIzol (Invitrogen, Paisley, U.K.) followed by chloroform extraction and isopropanol precipitation. Total RNA was measured at 260 nm by spectrophotometric analysis (Beckman Coulter, High Wycombe, U.K.). Cytokine encoding plasmid (pCmQ2), kindly provided by M. F. Kagnoff (Department of Medicine, University of California, San Diego, CA), was used for competitive quantitative PCR for TNF-α transcripts as described in Ref. 13. To quantify mouse MMP mRNA levels, we constructed a plasmid that encoded a standard RNA using an approach similar to that described in Ref. 13, Sequences of the oligonucleotide primers used for PCR amplification, and the sizes of the predicted PCR products from the target and standard RNAs are given in Table I. All primers were synthesized and HPLC purified by Sigma Genosys (Sigma-Aldrich). Primers were designed using Primer 3 (Whitehead Institute for Biomedical Research, Cambridge, MA) from cDNA sequences available in PubMed (National Center for Biotechnology Information). The primers span exon-intron boundaries and do not amplify genomic DNA. Sequences are specific for mouse MMP using blast search (National Center for Biotechnology Information), and PCR products were checked and sequenced to verify that the fragment was MMP. The DNA insert containing different MMP primer sites was cloned into pSP64 poly(A) vector (Promega, Southampont, U.K.) to generate poly(A)1 transcripts in vitro. To generate RNA, plasmids were linearized with NotI for pCmQ-2 or EcoRI for mouse MMP plasmid and transcribed in vitro with either T7 or SP6 RNA polymerase under conditions recommended by the supplier (Promega). Serial

<table>
<thead>
<tr>
<th>Target mRNA</th>
<th>5' Primer</th>
<th>3' Primer</th>
<th>Size of PCR Product (bp)</th>
<th>Standard</th>
</tr>
</thead>
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<tr>
<td>MMP3</td>
<td>cagttgtgtgttgctctgtag</td>
<td>egtaaagtgagacatccca</td>
<td>326</td>
<td>505</td>
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<tr>
<td>MMP7</td>
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<td>gaagagggagacagtgctag</td>
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<td>505</td>
</tr>
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<td>gcctgtaagactcatttc</td>
<td>303</td>
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</tr>
<tr>
<td>TIMP1</td>
<td>actggagctggtcataagggc</td>
<td>aagaggtcaggactgatt</td>
<td>268</td>
<td>505</td>
</tr>
</tbody>
</table>
10-fold dilutions of standard RNA (10^7–10^8 molecules) were co-reverse transcribed with total cellular RNA (1 µg) at 37°C for 50 min in a 20-µl reaction volume containing 50 nM Tris (pH 8.3), 75 mM KCl, 3 mM MgCl2, 3 mM DTT, 10 mM dNTP mix, and 0.5 µg of oligo-dexythymidylylate (Amersham Biosciences), using 100 U of Moloney murine leukemia virus reverse transcriptase (Invitrogen). PCR amplification was routinely conducted in 50-µl reaction volume (10 nM Tris (pH 9), 50 mM KCl, 1.5 mM MgCl2, 200 µM each dNTP, and 20 pmol of specific 5'- and 3'- primers), using 1 U of Tag polymerase (Amersham Biosciences). The temperature profile of the amplification consisted of 35 cycles of 45 s denaturation at 94°C, 75 s annealing at 56°C, and 75 s extension at 72°C. PCR products were then separated on a 1% agarose gel (Invitrogen), and the band intensities were quantified to 10^3 cytokine mRNA transcripts per µg of total RNA.

Flow cytometric analysis of dendritic cells (DCs)

Mesenteric lymph nodes (MLNs) were removed from infected B10R111 or MMP3^-/- mice 5 days postinfection and were washed in calcium-magnesium-free HBSS (Invitrogen). MLNs were then digested with 90 U/ml collagenase 1 and 2.5 U/ml dispase (Sigma-Aldrich) for 1 h at 37°C. The resulting cell suspension was passed through a 30-µm pore size cell strainer and washed in RPMI. DC were stained by FITC-conjugated B220, allophycocyanin-conjugated CD11c, and PE-conjugated CD8α (Caltag Laboratories, Towcester, U.K.) in PBS with 1% BSA at 4°C. Corresponding fluorochrome-conjugated isotype controls were also used as negative controls as described in the manufacturer’s instructions. After 3 washings in PBS with 1% BSA at 4°C for 10 min, cells were fixed in 1% paraformaldehyde in PBS. DC were defined as CD11c^+ B220^- cells and were further divided into CD8α^+ and CD8α^- populations. DC were then analyzed by FACSCalibur (BD Biosciences, Oxford, U.K.), and the results were analyzed by WinMDI 2.8 software (Scripps Research Institute, La Jolla, CA). The absolute numbers of DCs in the MLNs were calculated from the total number of collected mesenteric lymph node cells (MLNCs) and the proportion of CD11c^+ B220^- cells among them.

Adoptive transfer of CFSE-labeled cells

MLNCs were removed from infected MMP3^-/- mice or B10R111 mice 7 days postinfection and were passed through a nylon sieve to release lymphocytes. Cells were washed in RPMI 1640 containing 10% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma-Aldrich). The lymphocytes were labeled with 10 µM CFSE for 30 min at 37°C (Molecular Probes, Cambridge, U.K.). Labeling was stopped by adding a 5% final concentration of FCS, and the cells were immediately centrifuged and washed with ice-cold PBS. The CFSE-labeled cells from infected MMP3^-/- or B10R111 mice (3 × 10^7) were injected into naive B10R111 or MMP3^-/- recipient mice via tail vein (three recipients/group). One day after the cell transfer, mesenteric lymph nodes and colon were removed from B10R111 or MMP3^-/- recipient mice, and lymphocytes were prepared as described in Ref. 14 and stained with allophycocyanin-conjugated anti-CD4 mAb for MLNC or PE-conjugated anti-CD3 mAb (Caltag Laboratories). The lymphocytes were then analyzed by FACSCalibur (BD Biosciences) as described above. The absolute numbers of CFSE^+ CD3^- or CFSE^+ CD4^- T cells in the CD3^+ or CD4^- T cells were calculated from the total number of collected cells and the proportion of CFSE^+ CD3^- or CFSE^+ CD4^- T cells among them.

Statistical analysis

The significance of differences between means was determined using the paired t test and the Mann-Whitney U test. p < 0.05 was considered significant.

FIGURE 1. MMP3 gene expression in colonic tissue of C57BL/6-, TNFαR55^-/-, IL-12p40^-/-, and IFN-γ^-/- deficient mice. Total RNA was extracted from colonic tissues of mice orally infected with C. rodentium 14 days previously. Data depict the mean number of transcripts (±SEM) encoding MMP3 in infected and uninfected C57BL/6 and cytokine KO mice. There were significantly more transcripts detected in all groups of infected mice compared with uninfected controls (p < 0.01 for all 4 groups, Student’s t test). Moreover, MMP3 transcripts were significantly more abundant in infected IL-12p40^-/- and IFN-γ^-/- mice than in infected TNFαR55^-/- or WT mice (p < 0.001).

FIGURE 2. MMP3 protein expression in colonic tissue of infected wild-type and MMP3^-/- mice. Total protein was extracted from colonic tissue of mice orally infected with C. rodentium 14 days previously. Expression of MMP3 protein was increased in infected B10R111 mice. MMP3 protein was absent in infected and uninfected MMP3^-/- mice. β-Actin was used as loading control.

FIGURE 3. Top, Mucosal thickness in infected MMP3^-/- mice. Data depict average colonic crypt length (±SEM) in uninfected and C. rodentium-infected WT and MMP3^-/- mice at different time points of infection. Average crypt length was significantly greater in infected WT and MMP3^-/- mice than in uninfected controls between days 14 and 35 postinfection (p < 0.05). However, similar increase in crypt length was observed in WT and MMP3^-/- mice. Bottom, Pathology of colonic tissue of infected mice. Colonic tissue was removed from the distal colon of B10R111 and MMP3^-/- KO mice 14 days after oral infection with C. rodentium, and frozen sections were cut and stained with H&E. The images depict tissues from uninfected B10R111 mice (A), infected B10R111 mice (B), uninfected MMP3^-/- (C), and infected MMP3^-/- (D). Infection caused mucosal thickening in WT and MMP3^-/- mice. H&E; original magnification, ×100.
Results

Enhanced pathology in infected TNFRp55−/−, IL-12p40−/−, and IFN-γ−/− mice is associated with increased MMP3 transcripts

Mice infected with *C. rodentium* develop colitis and mount a highly polarized intestinal Th1 response. A component of the response in colonic tissue of infected mice includes increased transcripts for IL-12, IFN-γ, and TNF-α. To determine whether type I cytokines are important in host defense and mucosal pathology, mice with targeted mutations in the IL-12p40 or IFN-γ or TNFRp55 gene were orally infected with *C. rodentium* strain DBS255 (pCVD438) (5, 6). These animals showed various degrees of impairment in their ability to clear infection (5, 6). Remarkably, however, mucosal pathology was greater in these mice than in WT controls. The enhanced pathology included increased crypt length and CD4 cell infiltrates. Therefore, we postulated the enhanced pathology in these cytokine KO mice is due to increased mucosal remodeling. Competitive PCR showed that MMP3 transcripts were significantly greater in infected colonic tissues of cytokine KO mice than infected WT controls (Fig. 1). In addition, MMP3 transcripts were higher in IL-12p40 or IFN-γ KO mice, which showed more crypt hyperplasia and enhanced pathology than did TNFRp55 KO mice.

Crypt hyperplasia in MMP3 KO mice infected with *C. rodentium*

To determine whether MMP3 is important in mucosal thickening, MMP3−/− mice were orally infected with *C. rodentium*. Infection of WT mice showed an increase in MMP3 protein in the mucosa, and as expected no MMP3 protein was seen in MMP3−/− mice (Fig. 2). Oral infection with *C. rodentium* caused a substantial increase in crypt hyperplasia in WT mice (Fig. 3, top). However, compared with infected WT controls, MMP3−/− mice showed a similar crypt hyperplasia at various times postinfection (Fig. 3, bottom).

There was no difference in the number of *C. rodentium* in the colon of MMP3−/− mice and WT mice on days 7 and 14 of infection but on day 21, when the WT mice had cleared the infection, bacteria were still present in the intestine of infected MMP3−/− mice (Fig. 4A). A similar pattern was seen in the spleen on day 21 (Fig. 4B). On day 35, all bacteria were cleared from the colon and spleen of MMP3−/− mice.

Also on day 21, there was no significant difference in the IgG, IgA, or IgM Ab response between infected WT and MMP3−/− mice (Fig. 5).

Reduced CD4+ cell infiltrates and TNF-α transcripts in infected colon of MMP3−/− mice

Infection with *C. rodentium* results in heavy infiltrations of CD4+ cells into the colon (Fig. 6). However, in MMP3−/− mice, the increase in CD4+ cell infiltrates in colonic lamina propria was significantly delayed as compared with WT controls (Fig. 6). In WT mice, the numbers of CD4+ cells increased steadily from day 7 to day 21 postinfection, but in MMP3−/− mice, the increase was significantly delayed for the first 3 weeks postinfection. The number of CD4+ cells in WT mice peaked at day 14 and subsequently declined, whereas the number of CD4+ cells in MMP3−/− mice remained elevated at day 21.

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Previous studies have shown that MMP3 is needed for the migration of Langerhans cells from the skin to the draining lymph nodes to initiate cutaneous sensitivity (15). We therefore reasoned that the delayed CD4+ cell response in MMP3−/− mice infected with C. rodentium might have a similar basis. In both WT and MMP3−/− mice, there was a modest increase in B220−CD11c+ cells in the MLNs 5 days after infection. There was no significant difference in DC number between infected WT and MMP3−/− mice (Fig. 9).

To determine whether CD4 T cells from MMP3−/− mice could migrate into the intestine, we performed transfer experiments. CFSE-labeled CD4+ T cells from infected WT or MMP3−/− mice migrated in equal number into the normal MLN or colonic lamina propria, showing no intrinsic defect in CD4+ T cell migration in MMP3−/− mice (Fig. 10, A and C). In contrast, CD4+ T cells from WT and MMP3−/− mice were impaired in their ability to migrate into the lamina propria and MLN of MMP3−/− mice (Fig. 10, B and D).

Other MMPs expressed in the colon of mice infected with C. rodentium

We also conducted a detailed analysis of the kinetics of various MMPs in WT and MMP3−/− mice throughout C. rodentium infection. The number of MMP3 transcripts was low in uninfected colon of WT controls (Fig. 8A). MMP3 transcripts increased rapidly (>4-fold) from day 7, peaked on day 14, and declined from day 21 to a very low number on day 35. As expected, there were no MMP3 transcripts in uninfected and infected colonic tissues of MMP3−/− mice.

Transcripts of other MMPs were also abundant in colonic tissues of infected mice. In WT mice, transcripts of MMP7 increased significantly on day 14 and reduced in number considerably on day 21. However, in MMP3−/− mice, transcripts of MMP7 increased remarkably from day 14 and declined steadily at high levels on days 21 and 35 (Fig. 8B). Transcripts for macrophage metalloelastase (MMP12) were also higher in MMP3−/− mice than in WT controls (Fig. 8C).

The activities of MMPs are also regulated by endogenous inhibitors TIMPs. TIMP1 transcripts were abundant in infected and uninfected colonic tissues infected with C. rodentium (Fig. 8D). The numbers of TIMP1 transcripts in infected colonic tissues increased significantly from day 7, peaked on day 14, and declined to a very low level on days 21 and 35. In addition, the number of TIMP1 transcripts increased significantly (>6-fold) in MMP3−/− mice compared with WT controls.
A possible explanation for the failure of cells to migrate into the intestines of MMP3−/− mice could be differences in MAdCAM expression. We therefore analyzed MAdCAM and β7 integrin expression in uninfected and infected mice (representative images shown in Fig. 11). Only a few β7+ cells were seen in uninfected colon from WT and MMP3−/− mice. In contrast, on day 14 of infection, the colon of WT mice was infiltrated with β7+ cells. In MMP3−/− mice, however, there was also an increase but much less so than in WT mice, consistent with the reduced infiltrate of CD4 cells. MAdCAM was also expressed on vessels of uninfected WT and MMP3−/− mice. On day 14 of infection, there was massive mucosal thickening and the striking appearance of long MAdCAM+ vessels in both groups of mice.

Discussion

C. rodentium infection of mice produces features of T cell response and pathology in the colon, which are virtually identical with that seen in mouse models of IBD (3). It also represents an important animal model to study immune defenses against pathogens that colonize via attaching and effacing lesions (6). In the present study, we have shown for the first time that MMP3 plays a critical role in controlling pathogenic bacterial populations in the colon and the mobilization of T lymphocytes in the lamina propria. Moreover, mucosal remodeling can occur in the absence of MMP3, suggesting the redundancy of MMPs in tissue remodeling in vivo. In infected MMP3−/− mice, there is a deficiency of immune response to clear pathogenic bacteria in the colon. This is probably due to the delayed appearance of CD4+ T cells into the lamina propria and associated reduction of TNF-α expression in the colon. The reduced migration of CD4+ T cells from the donor WT or MMP3−/− mice into the lamina propria of recipient MMP3−/− mice strongly suggests that MMP3 is important in the tissue environment for migration of CD4+ T lymphocytes into the lamina propria.

The nature of the protective immune response needed for sterilizing immunity to this pathogen is still unclear although immunity requires T and B cells (16). We and others have previously shown that mice deficient in B cells or CD4 cells are highly susceptible to infection (17, 18). However, we were unable to clear bacterial infection from the intestine of B cell-deficient mice with immune sera, but recently Bry and Brenner (17) achieved immunity by transferring immune sera into infected CD4-deficient mice. Taken together, these results clearly indicated that immunity needs T cell-dependent Ab production. Nonetheless, we have also shown that cytokine-deficient mice are more susceptible to Citrobacter infection (5, 6). IL-12−/− mice, for example, have very delayed clearance of the bacteria and severe pathology; some mice die; however, serum Ab responses are the same as those in WT mice. Likewise, in this study we also could see no difference in Ab responses between WT and MMP3−/− mice. In the context of this study, our observation that MMP3−/− mice had delayed appearance of CD4+ T cells into the mucosa and reduced TNF-α transcripts again suggests a role of CD4+ T cells, perhaps not in
Then injected into B10R111 or MMP3 from the MLN or lamina propria of WT B10R111 mice when donor cells were taken from B10R111 or MMP3. However, no significant difference in DC frequency between infected B10R111 and MMP3 mice than from recipient B10R111 mice (19, 5177). In contrast, activated level of TIMP-1 expression on day 14 in infected MMP3−/− mice suggests the homeostatic mechanism to control the excessive activity of MMPs in matrix remodeling. Therefore, it would be interesting to study the phenotypes of MMP7−/− and MMP12−/− mice in C. rodentium infection.

The balance between MMPs and their inhibitors has been implicated in the tissue remodeling and ulceration in the intestines. In the intestine, stromelysin-1 (MMP3) has been shown to be expressed at high levels in ulcerated areas of clinical IBD and affected areas of celiac diseases (9, 11). In fetal intestinal explant model, MMP3 is the most critical molecule in tissue injury after T cell activation by PWM (19). Furthermore, in animal models, MMP3 is also expressed at high level in colitic samples of the SCID transfer model of colitis and transgenic mice defective in TGF-β signaling (20). Also in our study, elevated expression of MMP3 transcripts in colonic tissues is strongly associated with enhanced pathology in infected TNFRp55−/−, IL-12p40−/−, and IFN-γ−/− mice. However, crypt hyperplasia is induced in infected MMP3−/− mice to an extent similar to that in infected WT mice. The occurrence of crypt hyperplasia in the absence of MMP3 strongly suggests the redundancy of MMPs in tissue remodeling in vivo. The crypt hyperplasia in infected MMP3−/− mice is probably due to the compensatory increase of matrilysin (MMP7) and macrophage metalloelastase (MMP12) transcripts in the infected colon. Due to the similar and broad substrate specificity of different MMPs, matrix degradation could be conducted by other MMPs in tissues where they coexpress and coexert together. Specifically, MMP7 is strongly implicated in bacterial infections and cancers. It is highly expressed in epithelial cells and macrophages and can degrade proteoglycans, gelatins, and elastin (21). MMP12 is macrophage specific and preferentially degrades elastin as well as other matrix components. It is produced predominantly by infiltrating macrophages and appears essential for macrophage migration through the extracellular matrix. In disease, MMP12 has been strongly implicated in pathogenesis in emphysema (22).

The deficiency in controlling the bacteria in the colon of infected MMP3−/− mice suggests that MMP3 is important in bacteria defense. MMPs are strongly induced in epithelial cells during bacterial infections. MMP7 is strongly induced in epithelial cells...
of inflamed mucosa of the stomach in Helicobacter pylori infection (23). MMP9 is strongly induced in human gingival epithelial cells infected with Porphyromonas gingivalis (24). MMP7 has been shown to activate antimicrobial peptides, α-defensins (cryptidins), in Paneth cells of mouse small intestine (25). MMP3−/− mice are more susceptible to infection with Salmonella typhi murium. The role of MMP3 in bacteria defense is emphasized in that proforms of MMP7 and MMP9 could be proteolytically activated by MMP3.

The reduction of CD4+ lymphocytes in the lamina propria in infected MMP3−/− mice suggests that MMP3 is important in migration of CD4+ lymphocytes into the lamina propria. There are two possibilities by which MMP3 affect the CD4+ T lymphocyte response. MMP3 is required for the migration of Langerhans cells from the skin to the draining lymph nodes to initiate cutaneous sensitivity (15). The first possibility is therefore that mobilization of DCs is impaired in infected MMP3−/− mice, but the similar increase in the number of DCs recovered from draining lymph nodes of infected WT and MMP3−/− mice suggests that there are no intrinsic defects of MMP3−/− mice to mobilize DCs from the intestine into draining lymph nodes. The second possibility of the immune defect of MMP3−/− mice is the inability of CD4+ lymphocytes to home to the intestine. It is evident in the CSFE cell transfer experiment there is a reduced migration of T lymphocytes taken from both WT and MMP3−/− mice into the mesenteric lymph nodes and lamina propria of MMP3−/− mice. Our data therefore suggest that the tissue environment is important in migration of T lymphocytes. Endothelial cells can make MMP3 (26, 27), and there is a possibility that when an intestine-homing CD4+ cell binds to a MAdCAM+ vessel in the colon, it signals to the endothelial cells to release MMP3 into the pericellular space to open up tight junctions, and the lymphocytes migrate through. To support this notion, it has been shown that treatment of mice with MMP inhibitors results in an accumulation of lymphocytes on the lymph node endothelium and reduced diapedesis (28). Whether the MMP inhibitors were acting via lymphocyte-derived MMPs or endothelial-derived MMPs was not established. We also emphasize that in the experiments shown here, recipient animals were not infected with C. rodentium and that endothelial changes induced by infection might add a further layer of complexity to the system. In this regard, it was striking that although there were fewer CD4 cells in the colonic lamina propria of infected MMP3−/− mice on day 14, on days 21 and 35, the numbers were the same. This would suggest that inflammation overrides this early block in lymphocyte extravasation.

Another explanation for the failure of cells to migrate into the intestine could be the absence of the intestine-homing integrin α7β1 on lymphocytes or endothelial MAdCAM expression in MMP3−/− mice. MAdCAM was expressed basally in MMP3−/− mice, and long, positively staining vessels were seen in the hyperplastic colonic mucosa of infected MMP3−/− mice, making the latter possibility unlikely. Cells infiltrating the colon of infected WT and MMP3−/− mice were also β1 integrin+.

The mechanisms by which the MMP controls T cell migration and bacterial defense remain virtually unexplored. The data presented in this study, using C. rodentium as a model system, add to our understanding of the implications of MMPs in tissue remodeling in the intestine as well as mucosal immunity in general. The identification of other factors in controlling tissue remodeling in vivo and control of T cell migration by MMP are the focus of our current studies.

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References


