Matrix Metalloproteinase-7 and Premalignant Host Responses in *Helicobacter pylori*–Infected Mice

Seth R. Ogden¹,², Jennifer M. Noto¹, Shannon S. Allen¹, Dilan A. Patel¹, Judith Romero-Gallo¹, M. Kay Washington³, Barbara Fingleton³, Dawn A. Israel¹, Nuruddeen D. Lewis¹,², Keith T. Wilson¹,²,⁵, Rupesh Chaturvedi¹,²,⁵, Zhiguo Zhao⁴, Yu Shyr⁴, and Richard M. Peek, Jr.¹,²

Abstract

*Helicobacter pylori*-induced gastritis is the strongest singular risk factor for gastric adenocarcinoma. Matrix metalloproteinase-7 (MMP-7) is a proteolytic enzyme that can modify the intestinal microbial replicative niche as well as affect tumorigenesis, and *H. pylori* stimulates expression of MMP-7 in gastric epithelial cells in vitro. Utilizing a transgenic murine model of *H. pylori*-mediated injury, our experiments now show that gastric inflammation is increased within the context of MMP-7 deficiency, which involves both Th1- and Th17-mediated pathways. Enhanced gastritis in *H. pylori*-infected *mmp-7−/−* mice is strongly linked to accelerated epithelial cellular turnover. However, more severe inflammation and heightened proliferation and apoptosis are not dependent on MMP-7–mediated bacterial eradication. Collectively, these studies indicate that *H. pylori*-mediated induction of MMP-7 may serve to protect the gastric mucosa from pathophysiologic processes that promote carcinogenesis. Cancer Res; 70(1); 30-5. © 2010 AACR.

Introduction

*Helicobacter pylori* induces a gastric inflammatory response that persists for decades, which increases the risk for gastric adenocarcinoma (1, 2). A host molecule that may influence disease outcome in conjunction with *H. pylori* is matrix metalloproteinase-7 (MMP-7), a member of a family of proteolytic enzymes that play important roles in tissue destruction and remodeling (3). MMP-7 is one of a small number of MMPs expressed in polarized glandular epithelium (3), and in the intestinal tract, MMP-7 mediates the production of defensins which regulate microbial colonization (4). However, MMP-7 also influences cellular proliferation and apoptosis and is overexpressed in gastric malignancies (5–8). Furthermore, our group and others have shown that *H. pylori* increases the expression of MMP-7 in vitro (9–11). Therefore, we sought to more clearly define the role of MMP-7 within the context of *H. pylori*-induced injury in vivo by using a transgenic murine model of MMP-7 deficiency.

Materials and Methods

**Mice and *H. pylori* infections.** *H. pylori* strain SS1 was grown in Brucella broth with 5% fetal bovine serum (Life Technologies) for 18 h (11). C57BL/6 *mmp-7−/−* mice were generated by backcrossing *mmp-7−/−* heterozygous 129 mice (12) to C57BL/6 mice beyond the N12 generation. Six- to 8-wk-old wild-type and *mmp-7−/−* male C57BL/6 mice were used. Experiments were approved by the Vanderbilt Institutional Animal Care and Use Committee. Brucella broth containing 5 × 10⁸ *H. pylori* or broth alone was given to mice (13).

Mice were euthanized 12 or 36 wk post-challenge because infection of INS-GAS mice with *H. felis* increases MMP-7 expression by 3 mo, which persists to 9 mo post-infection (14). For one-half of the stomach, linear strips from the squamocolumnar junction through the duodenum were paraffin-embedded (13); one-fourth of the stomach was stored at −80°C for RNA extraction, whereas the other one-fourth was cultured. *H. pylori* colonization density was assessed by immunohistochemistry. Inflammation was graded on a 0 to 3 ordinal scale based on the Sydney System as follows: acute inflammation; grade 0, no polymorphonuclear (PMN) cells present; grade 1, focal mild neutrophil infiltration (<10 PMN/high-powered field); grade 2, focal dense neutrophil infiltration (10–20 PMN/high-powered field); grade 3, diffuse and dense PMN infiltration (>20 PMN/high-powered field). Chronic inflammation; grade 0, no inflammation (mononuclear cell infiltration independent of lymphoid follicles); grade 1, mild inflammation (slight increase in mononuclear cells); grade 2, moderate inflammation (dense but focal mononuclear inflammatory cells); grade 3, severe inflammation (dense and diffuse mononuclear inflammatory cells).

Seventy-five mice were used: uninfected wild-type (*n* = 5 at 12 wk and *n* = 4 at 36 wk), uninfected *mmp-7−/−* (*both* *n* = 5 at 12 and 36 wk), infected wild-type (*n* = 19 at 12 wk and *n* = 7 at 36 wk), and infected *mmp-7−/−* (*n* = 22 at 12 wk and *n* = 8 at 36 wk).
Real-time reverse transcription-PCR. RNA was extracted from mouse gastric tissues using the RNeasy Mini Kit (Qia-gen) and cDNA prepared using the iScript cDNA Synthesis Kit (Bio-Rad). Real-time PCR for cytokines was performed using the iQ SYBR Green Supermix (Bio-Rad) and the primer sequences: IFN-γ (F, 5′-ACTGGCAGAAGGATGGGAC-3′; and R, 5′-TGAGCTCATTGAATGCTTGG-3′), interleukin 17 (IL-17; F, 5′-GCTCCAGAAGGCCCTCAGA-3′; and R, 5′-CTTTCCCTCCGCATTGACA-3′), and IL-10 (F, 5′-CCAAGCCTTATCGGAAATGA-3′; and R, 5′-TCACTCTTACCCCGATTTG-3′) with data standardized to β-actin (F, 5′-CCAGAGCAAGAGAGGTATCC-3′; and R, 5′-CTGTGGTGGTGAAGCTGTAG-3′).

Immunohistochemical analysis. For MMP-7, sections were incubated with a rat anti–MMP-7 antibody (9, 15). For anti–H. pylori, anti-CD3, anti-CD20, anti-Ki67, and anti-caspase-3 staining, sections were rehydrated and placed in heated Target Retrieval Solution (Labvision). Endogenous peroxidase was neutralized with 0.03% hydrogen peroxide containing sodium azide, followed by a casein-based protein block (Dako). Sections were then incubated with either rabbit polyclonal anti–H. pylori (B0471; Dako; 1:100), rabbit polyclonal anti-CD3 (A0452; Dako; 1:50), mouse monoclonal anti-CD20 (M0755; Dako; 1:200) for 30 min, or rabbit polyclonal anti-Ki67 (VP-K451; Vector; 1:2,000) or rabbit anti-human cleaved caspase-3 (1:600; Promega) for 60 min, subjected to the Dako EnVision+ HRP/DAB System (Dako), and counterstained with hematoxylin. The number of positively stained cells was quantified per high-powered field examined.

Statistical analysis. Intergroup comparisons of histology scores were performed by two-tailed Fisher’s exact test and linear mixed effects models. Comparisons of immunostaining data and cytokine levels were performed by unpaired Student’s t test and ANOVA with Student’s Newman-Keuls test, respectively. Significance was defined as P < 0.05.

Results

H. pylori–induced inflammation is augmented in a background of MMP-7 deficiency. All mice challenged with H. pylori were successfully infected. Twelve weeks post-challenge, there was no evidence of MMP-7 expression in uninfected mice. In contrast, MMP-7 was detected in the stomachs of wild-type mice challenged with H. pylori. Immunolabeling localized exclusively to gastric epithelial cells (Fig. 1), which mirrors the pattern of MMP-7 expression previously observed in H. pylori–infected humans (9).

We next infected wild-type and mmp-7−/− mice, and surprisingly, H. pylori–colonized mmp-7−/− mice developed more severe gastritis than infected wild-type mice (Figs. 1 and 2). Detailed analysis of inflammation revealed significantly increased levels of acute inflammation in the antrum (P = 0.008) and corpus (P = 0.03) 12 weeks post-infection, and in the antrum (P = 0.03) 36 weeks post-infection (Figs. 1 and 2).
Chronic inflammatory scores were significantly higher 36 weeks post-infection in infected mmp-7−/− mice in the antrum (P = 0.007) and corpus (P = 0.05), although there was a trend toward higher levels of chronic inflammation in the corpus (P = 0.08) 12 weeks post-infection (Figs. 1 and 2).

We next immunophenotyped inflammatory infiltrates within infected gastric mucosa harvested from wild-type or mmp-7−/− mice at the 36-week time point. Similar to the pattern for overall inflammation, gastric mucosa from infected mmp-7−/− mice contained significantly more T cells and B cells compared with mucosa from infected wild-type mice (Fig. 3A). To delineate potential differences in specific T-cell responses, we quantified the expression of Th1 (IFN-γ), Th2 (IL-10), and Th17 (IL-17) cytokines within the same samples of colonized mucosa. Although the levels of these cytokines were not altered in uninfect ed mmp-7−/− mice (data not shown), levels of IFN-γ and IL-17 were significantly lower in infected mmp-7−/− mice compared with infected wild-type mice (Fig. 3B).

To determine if differences in inflammation were due to altered levels of colonization, we compared colonization density in wild-type and mmp-7−/− mice infected for 36 weeks. There were no significant differences in levels of colonization (Fig. 3C). To ascertain whether altered levels of colonization might precede this time point, we examined colonization density in a subset of samples obtained from mice infected for 12 weeks and, similar to 36 weeks, there were no differences between infected wild-type or mmp-7−/− mice (Fig. 3C). Collectively, these results indicate that MMP-7 deficiency enhances the intensity of Th1- and Th17-mediated responses, independent of colonization density.

Cellular turnover is enhanced in infected mmp-7−/− mice. Because loss of MMP-7 augmented inflammation, we determined whether epithelial proliferation and apoptosis were differentially affected in a subset of colonized wild-type or mmp-7−/− mice. A trend (P = 0.1) toward higher proliferation levels was present in infected versus uninfected wild-type mice at 36, but not 12 weeks post-challenge (Fig. 4A and B). Infected mmp-7−/− mice exhibited significantly higher proliferation levels than either uninfected mmp-7−/− mice or infected wild-type mice at 12 (P < 0.001) and 36 (P < 0.001) weeks post-infection (Fig. 4A and B). In wild-type mice, Ki67+ epithelial cells were tightly clustered within the neck region of the gastric glands; however, staining in mmp-7−/− mice extended bidirectionally from the isthmus (Fig. 4A).

Figure 2. H. pylori challenge of mmp-7−/− mice results in enhanced inflammation. Representative images of H&E-stained sections from the antrum (A and C) and corpus (B and D) of wild-type (A and B) and mmp-7−/− mice (C and D) infected for 12 wk (+/+, wild-type; −/−, mmp-7−/−). Magnification, ×100.
Similar to proliferation, apoptosis levels were higher ($P = 0.05$) in infected versus uninfected wild-type mice at 36, but not 12 weeks post-challenge (Fig. 4C and D). Infected $mmp-7^{-/-}$ mice exhibited significantly higher apoptosis levels than either uninfected $mmp-7^{-/-}$ mice or infected wild-type mice at 12 ($P < 0.001$) and 36 ($P < 0.001$) weeks (Fig. 4D). In contrast to the topography of proliferation, apoptotic epithelial cells primarily localized to the upper one-third of the gastric foveolae (Fig. 4C). These results suggest that increased levels of cellular turnover in $H. pylori$–infected MMP-7–deficient mice may be influenced by paracrine signaling from infiltrating immune cells (14, 16, 17).

Discussion

Persistent inflammation induced by $H. pylori$ likely promotes the development of gastric adenocarcinoma; therefore, our results were somewhat unexpected. However, a potential mechanism through which augmented inflammation may occur in $mmp-7^{-/-}$ mice is via dysregulation of epithelial-derived chemotactant production. Gastric epithelial cells secrete the chemokine IL-8 in response to $H. pylori$ (18), which establishes a haptotactic gradient towards the epithelial surface. In mouse models of acute lung injury, MMP-7 promotes the formation of a transepithelial gradient of keratinocyte-derived chemokine, the murine functional homologue of human IL-8, via ectodomain shedding of syndecan-1, a heparan sulfate proteoglycan found on epithelial cell surfaces (19). Keratinocyte-derived chemokine associates with shed syndecan-1, establishing a gradient that drives immune cells toward the alveolar lumen. In $mmp-7^{-/-}$ mice, the ability of inflammatory cells to migrate from the interstitium into the alveolar compartment is impaired due to a lack of this gradient, effectively trapping immunocytes at the epithelial-matrix interface (19). $H. pylori$–infected $mmp-7^{-/-}$ mice may similarly lack the ability to establish proper chemotactant gradients within the stomach, thus preventing transepithelial migration of immune cells to sites of microbial replication, manifesting as a phenotype of increased inflammation.

$H. pylori$ persistence is likely due to inadequate adaptive immune responses characterized by insufficient Th1 and Th17 responses and inappropriate regulatory T-cell activation (18). We found that gastric mucosa from $mmp-7^{-/-}$ mice contained increased numbers of T cells, and a >2-fold increase in expression of the prototype Th1 and Th17 cytokines, IFN-$\gamma$ and IL-17, respectively, in conjunction with a concomitant decrease in the Th2/Treg cytokine IL-10. Although these effects would be expected to enhance T-cell function, there was no reduction in bacterial colonization, indicating that other immune components in $mmp-7^{-/-}$ mice may also be dysregulated. Consistent with this, we showed...
Figure 4. *H. pylori* challenge of *mmp-7−/−* mice increases the levels of cellular turnover. Representative images of anti-Ki67 (A) or anti–active caspase-3 (C) staining in *H. pylori*–infected wild-type and *mmp-7−/−* mice. The percentage of cells positive for Ki67 (B) or active caspase-3 (D) was calculated for 10 high-powered fields for each mouse. The average of these 10 fields is represented by a single dot in the vertical scatterplot (+/+; wild-type; −/−, *mmp-7−/−*.

***, $P < 0.001$; ****, $P < 0.0001$

infected *mmp-7−/−* versus infected wild-type. ##, $P < 0.01$; ###, $P < 0.001$

infected *mmp-7−/−* versus uninfected *mmp-7−/−*.

Magnification, ×400.
increased B-cell infiltration of the stomach, which has also been implicated in *H. pylori* persistence (18).

Functions that have been ascribed to MMP-7 include stimulation of apoptosis and proliferation (7, 8). Our results, however, indicate that both of these responses are paradoxically augmented in infected mice lacking MMP-7, similar to heightened levels of inflammation. *H. pylori* induces an influx of a myriad of immune cells that can stimulate apoptosis via increases in oxidative stress (18). In turn, cell loss reciprocally stimulates the proliferative response of epithelial cell precursors, to compensate for reduced cell mass. We speculate that, in *mmp-7*−/− mice, increased numbers of infiltrating immune cells further augments this process, accounting for differences in cell turnover.

We did not observe dysplasia or cancer in infected wild-type or MMP-7−/− deficient mice. However, previous data (20) have indicated that *H. pylori*-infected mice on a BL/6 background rarely develop gastric cancer prior to 15 months post-challenge. In contrast, *H. pylori* infection of hypergastrinemic INS-GAS mice (on a FVB/N background) leads to the development of premalignant lesions by 6 weeks and gastric cancer by 24 weeks (13). We are currently generating *mmp-7*−/− INS-GAS mice to more precisely define the role of MMP-7 in carcinogenesis, which will provide a framework for future studies.

In conclusion, MMP-7 expression is increased by *H. pylori* and negatively regulates inflammation and epithelial turnover. These findings may not only improve our understanding of *H. pylori*–induced carcinogenesis, but may also provide mechanistic insights into other malignancies that arise within the context of inflammatory states.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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