BASIC–ALIMENTARY TRACT

Myofibroblast Matrix Metalloproteinases Activate the Neutrophil Chemoattractant CXCL7 From Intestinal Epithelial Cells

LAURENS KRUIDENIER,* THOMAS T. MACDONALD,† JANE E. COLLINS,† SYLVIA L. F. PENDER,† and IAN R. SANDERSON*

*Research Centre in Gastroenterology, Institute of Cell and Molecular Science, Queen Mary's School of Medicine and Dentistry, University of London, London; and †Division of Infection, Inflammation and Repair, School of Medicine, University of Southampton, Southampton, England

Background & Aims: **The up-regulation of matrix metalloproteinases (MMPs) in the inflamed gut has mainly been associated with mucosal degradation and ulceration. However, their in vitro capacity to specifically cleave inflammatory mediators indicates that MMPs may have a profound immunoregulatory impact. We hypothesized that MMPs proteolytically modify intestinal epithelial chemokine signaling.** ***Methods:*** **Interleukin-1β–stimulated Caco-2 cells were exposed basolaterally to nanomolar concentrations of activated MMP-3 or cocultured with interleukin-1β–stimulated, MMP-producing, colonic myofibroblasts (CCD-18co). The conditioned media were subjected to chemotaxis assays. In addition, epithelial cells from patients with colitis were examined by real-time polymerase chain reaction, immunoblotting, and immunohistochemistry.** ***Results:*** **MMP-3 dose-dependently induced the neutrophil (up to 5-fold) but not monocyte chemoattractant capacity of Caco-2 cells. A similar Caco-2 chemotactic response was obtained in the Caco-2/CCD-18co cocultures. The principal mediator of these protease-related effects was identified as the potent neutrophil chemokine CXCL7 (neutrophil activating peptide 2), a proteolytic cleavage product of chemotactically inert platelet basic protein (PBP), not previously identified in the intestine. Antibodies against CXCL7 inhibited the MMP-induced chemotactic response by 84%, and PBP mRNA and protein were detected in stimulated Caco-2 but not in CCD-18co cells. Furthermore, PBP transcript and protein levels were low in the mucosa and in isolated epithelial cells from patients with Crohn's disease and from normal intestine but increased up to 13-fold in patients with ulcerative colitis.** ***Conclusions:*** **These findings identify a novel proinflammatory action of MMPs in inflammation and suggest that lamina propria myofibroblasts are required to achieve maximal intestinal epithelial immune activation.**

Matrix metalloproteinases (MMPs) are a large family of zinc-dependent extracellular matrix–degrading endoproteinases with pathogenetic significance in a broad range of disorders.[1] In intestinal inflammation, they are considered major end-stage effector molecules for mucosal injury. MMP production is dramatically increased in the gut of patients with the chronic inflammatory bowel diseases Crohn's disease and ulcerative colitis,[2,3] and functional studies in our laboratory have previously shown the involvement of certain MMPs in mucosal degradation on local immune activation.[4] In particular, addition of nanomolar concentrations of activated recombinant MMP-3 (stromelysin-1) to fetal human gut explant cultures caused tissue injury similar to that produced by T-cell activation.[4]

Recent in vitro studies have suggested functions for MMPs beyond tissue degradation or remodeling. Many bioactive molecules can be precisely and effectively cleaved by MMPs, and particularly cytokines have emerged as an important class of MMP substrates.[5] Chemokines are a family of about 40 chemoattractant cytokines that direct the migration, homing, and activation of leukocytes. The intestinal epithelium expresses several chemokines, and many are up-regulated in inflammatory bowel disease.[6,7] Because cytokine-activated subepithelial myofibroblasts are the main MMP source in the gut,[8,9] we hypothesized that interactions between MMPs and epithelial chemokines are likely to occur in intestinal inflammation. In this study, we investigated the conse-

Abbreviations used in this paper: ENA-78, CXCL 5; GRO, growth-related oncogene; IL, interleukin; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; NAP-2, neutrophil-activating peptide 2; PBP, platelet basic protein.

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quences of such interactions. Our work shows that recombinant and myofibroblast-derived MMPs regulate the recruitment of leukocytes by proteolytically activating the potent neutrophil chemoattractant CXCL7 (neutrophil-activating peptide 2; NAP-2) from the intestinal epithelium, showing that changes in MMP activity have a significant impact on local immune regulatory responses.

Materials and Methods

Cell Culture

Caco-2 human colon carcinoma cells (passages 30–35) were maintained in Dulbecco's modified Eagle medium (Invitrogen, Paisley, Scotland) supplemented with 10% fetal calf serum (Invitrogen) as previously described.[10] Caco-2 cells were seeded at a density of 3×10^5 on polycarbonate culture plate inserts (0.4 μm pore size; Millipore, Watford, England), which were placed in 6-well plates and incubated in 2 mL of apical medium and 2 mL of basolateral medium. Monolayer formation was followed by measurement of transepithelial electrical resistance (Millicell-ERS; Millipore). For coculture experiments, human CCD-18co colonic myofibroblast cells (CRL-1459; American Type Culture Collection, Manassas, VA) were plated at passages 6–12 in 6-well plates (3×10^5 cells/well) and grown to confluence in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. Caco-2 and CCD-18co cells were incubated for 24 hours under serum-free conditions before being stimulated with human recombinant mature interleukin (IL)-1β (R&D Systems, Abingdon, England) and/or treated with the catalytic domain of recombinant human MMP-3 (Calbiochem, San Diego, CA) with the MMP inhibitors doxycycline (Calbiochem), CT1399 (Pender et al[4]), CT1847 (Pender et al[4]), or monoclonal anti-human CXCL7 (NAP-2) neutralizing immunoglobulin (Ig) G antibodies (R&D Systems).

Chemotaxis Assay

Sterile-filtered, conditioned cell culture media samples were added in duplicate to the bottom chambers of a disposable 96-well plate chemotaxis system (ChemoTx; Neuro Probe, Gaithersburg, MD). The 5-μm filter pore size membrane was placed onto the plate, and 5×10^4 peripheral blood polymorphonuclear cells or 5×10^4 mononuclear cells that had been freshly isolated from pooled whole blood samples from healthy individuals by Ficoll-Paque (Amersham Biosciences, Chalfont St Giles, England) gradient centrifugation were added. After 1 hour of incubation in humidified air with 5% CO_2 at 37°C, the cells that migrated through the membranes were counted microscopically. The chemotactic index of a sample was calculated as the number of cells that migrated to the sample over the number of cells that migrated to control medium. Recombinant human CXCL8 (IL-8; R&D Systems), recombinant human CXCL7 (R&D Systems), and recombinant human CCL2 (monocyte chemoattractant protein [MCP]-1; R&D Systems) were used as positive controls. In some experiments, the leukocytes were preincubated at room temperature for 30 minutes with monoclonal anti-human CXCR1 and monoclonal anti-human CXCR2 neutralizing IgG antibodies (R&D Systems). None of the (combinations of) stimuli, inhibitors, or antibodies had, in the concentrations used, nonspecific effects on chemotaxis, and neither did the addition of a protease inhibitor cocktail (Sigma, Poole, England), excluding any contribution of leukocyte proteases.

Patients

Representative parts of macroscopically normal (noninflamed) and inflamed intestinal mucosa were selected from paired resection specimens from patients with Crohn's disease (n = 5) or ulcerative colitis (n = 5) as previously described.[11] All resections were undertaken for persistent, severe intestinal inflammation that had not responded to corticosteroid and other immunosuppressive treatment. The diagnosis and extent of disease were established by defined clinical, laboratory, and endoscopic/radiologic criteria, and all samples were confirmed by histology. Control samples (n = 5) consisted of histologically normal mucosa from patients with colorectal cancer, taken at least 10 cm from the neoplastic lesion. All samples were snap frozen in liquid nitrogen and stored at −70°C until use. From another group of patients (10 controls, 5 patients with Crohn's disease, and 6 patients with ulcerative colitis), intestinal epithelial cells were isolated as previously described.[12] All patient samples were collected after approval by local human studies committees.

Reverse-Transcription Polymerase Chain Reaction

Total RNA was extracted from the cells/tissues using the TRIzol reagent (Invitrogen), according to the manufacturer's instructions. Complementary DNA was synthesized from 0.5 μg of total RNA by moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) using an oligo$(dT)_{15}$ primer (Promega) and subjected to polymerase chain reaction for 28–32 cycles to detect platelet basic protein (PBP; forward 5′-TCACCCTCACTCAGAGGTCTT-3′ and reverse 5′-AGAGGGTTGAAACCAGGCTTA-3′, amplifying a 666–base pair product), MMP-1 (5′-ATGCACAGCTTTCCTCCACT-3′, 5′-CAGGGTTTCAGCATCTGGTT-3′, 252 base pairs), MMP-2 (5′-CACTTTCCTGGGCAACAAAT-3′, 5′-GGACAGACGGAAGTTCTTGG-3′, 244 base pairs), MMP-3 (5′-AATCAATTCTGGGCCATCAG-3′, 5′-AGTGCCCATATTGTGCCTTC-3′, 395 base pairs), MMP-8 (5′-CTGCAAGGTTATCCCAAGGA-3′, 5′-TTGGTCCACTGAAGACATGG-3′, 250 base pairs), MMP-9 (5′-TTCATCTTCCAAGGCCAATC-3′, 5′-TGTCGCTGTCAAAGTTCGAG-3′, 274 base pairs), MMP-10 (5′-GCAGCGGACAAATACTGGAG-3′, 5′-CCCTATCTCGCCTAGCAATG-3′, 236 base pairs), MMP-12 (5′-GCTGTCACTACCGTGGGAAA-3′, 5′-TTGGGATAATTTGGCTCTGG-3′, 230 base pairs), and β-actin (5′-GATGCAGAAGGAGATCACTGC-3′, 5′-TAGTCCGCCTAGAAGCATTTG-3′, 201 base pairs). For quantitative reverse-