Distribution of the matrix metalloproteinases stromelysin, gelatinases A and B, and collagenase in Crohn's disease and normal intestine

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Abstract

Aims—To investigate the role of the matrix metalloproteinases (MMPs) in the connective tissue changes seen in the intestine in Crohn's disease.

Methods—Indirect immunofluorescence microscopy using specific antibodies to the MMPs (collagenase, gelatinase A and B, and stromelysin) were used to assess the distribution of these enzymes in normal and diseased intestine.

Results—In normal intestine the matrix metalloproteinases were confined to a few isolated inflammatory cells, but in Crohn's disease, the inflammatory infiltrate was associated with increased numbers of polymorphonuclear leucocytes which stained positive for gelatinase B. Stromelysin was also detected extracellularly on the connective tissue matrix in regions of smooth muscle cell proliferation and mucosal degradation. Interestingly, in ulcerative colitis, another inflammatory bowel disease, stromelysin was localised in the lamina propria in regions of mucosal loss.

Conclusions—The increased numbers of inflammatory cells containing gelatinase B, and the localisation of extracellular stromelysin in regions of fibrosis and mucosal degradation, suggest that these enzymes have a role in the pathological changes seen in Crohn's disease. In cases of ulcerative colitis stromelysin was also detected on the lamina propria in regions of mucosal loss, and seems to be associated with the connective tissue changes that precede mucosal loss.

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Crohn's disease is a chronic inflammatory bowel condition that mainly presents in early adult life. The underlying aetiology remains unknown. Features of Crohn's disease include chronic transmural inflammation, fibrosis, and fistulae formation. Complications associated with intestinal obstruction are severe, but treatment is largely unsatisfactory, with stricture formation often recurring after surgical resection. In contrast to Crohn's disease, strictures are not observed in the other inflammatory bowel disease, ulcerative colitis, and inflammation is restricted to the mucosal layers of the colon and rectum.

Connective tissue degradation during both normal tissue remodelling and in pathological

conditions is mainly achieved by the action of the matrix metalloproteinases (MMPs). These are a family of zinc containing enzymes with distinct specificities for the individual components of the extracellular matrix.¹⁻³ The collagenases are the most specific of the MMPs, cleaving the triple helix of types I, II, and III collagens at a single site. This action causes the molecule to unwind⁴ and become susceptible to further proteolytic attack by gelatinases, a group of enzymes which also degrade types IV and V collagens.⁵ Two collagenases have been identified, a 55 kilodalton enzyme (MMP1), synthesised and secreted by connective tissue cells and macrophages, and a 75 kilodalton enzyme (MMP8), prepackaged in the specific granules of polymorphonuclear leucocytes. Two gelatinases have also been identified, a 72 kilodalton form (gelatinase A, MMP2) derived from mesenchymal cells, and a 95 kilodalton form (gelatinase B, MMP9) associated with macrophages and polymorphonuclear leucocytes (PMNLs) as well as some stimulated connective tissue cells and tumours.² The stromelysins (MMPs 3 and 10) have a much broader substrate specificity and degrade a range of extracellular matrix components, including proteoglycans, the non-helical regions of type IV collagen, laminin, and fibronectin.⁵

Methods

Resected lengths of intestine from six patients with Crohn's disease were examined. In each case Crohn's disease was confirmed by the presence of chronic transmural inflammation and macroscopically unaffected regions were confirmed histologically to be normal. Lengths of intestine from five patients with no evidence of inflammatory bowel disease were also examined. These included tissue from a patient with a stab wound, rectal or colonic cancer, severe constipation, colonic volvulus, and polypoid colon. The distribution of stromelysin was also examined in segments of intestine from five patients with ulcerative colitis.

Resected lengths of intestine were opened by a longitudinal incision through the wall and washed extensively. Sections, 5 mm in diameter, were taken at 5 mm intervals along the entire length of the resected tissue, including histologically normal regions at both ends. In tissue obtained from patients without Crohn's disease segments were taken from macroscopically normal regions only.

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All segments were snap frozen in isopentane and stored at -70°C .

Polyclonal antibodies raised in sheep against human stromelysin,⁶ human gelatinase A,⁷ and pig gelatinase B, which cross reacts with human gelatinase B,⁸ have been described before. Human interstitial collagenase (MMP 1) was purified according to the method of Whitham *et al.*,⁹ antibodies raised in sheep and the resulting serum characterised by western blotting, and inhibition and immunolocalisation were as reported in full for the other antisera. Pooled normal sheep serum (NSS) was used as a control and immunoglobulins (IgG) were isolated from all sera, as described before.¹⁰ Fluorescein isothiocyanate (FITC)-conjugated rabbit anti-sheep IgG was purchased from Sigma Chemical Co. Ltd, Poole, Dorset, England.

Frozen sections, $7\text{ }\mu\text{m}$ thick, were cut from each tissue block and fixed for 30 minutes with 4% paraformaldehyde in phosphate buffered saline (PBS). The sections were then treated with 0.1% Triton X-100 for 5 minutes to permeabilise the tissue and allow the antibodies to penetrate the cells. This was followed by a 20 minute incubation with 4-chloro-1-naphthol (2.8 mM in methanol/PBS, 20% v/v, with 0.01% H_2O_2) which prevents non-specific binding of fluorescein by inflammatory cells.¹¹ Sections were then incubated for 30 minutes, in a moist box, with either sheep anti-collagenase IgG, anti-gelatinase A IgG, anti-gelatinase B IgG, anti-stromelysin IgG ($5\text{ }\mu\text{g}$ in $100\text{ }\mu\text{l}$ PBS/section), or NSS IgG as a control for non-specific staining. Normal rabbit serum (5% v/v) was present in these incubations to prevent non-specific binding of the second antibody. Sections were incubated for 30 minutes with FITC-conjugated rabbit anti-sheep IgG ($2.75\text{ }\mu\text{g}$ in $100\text{ }\mu\text{l}$ PBS/section), washed between every stage of the procedure with three changes of PBS, each for five minutes, and treated with the nuclear counterstain and propidium iodide (10^{-6} M , five minutes) before washing in PBS.

All sections were mounted in glycerol/PBS mounting fluid containing additives to reduce fading (Citifluor Ltd., London) and observed using a Zeiss Axioscop photomicroscope, fitted with an FITC filter and epifluorescence. A Zeiss FITC filter system 9 (catalogue number 48-79-09) was used: this is specifically designed to permit simultaneous detection of FITC (bright green) and the nuclear counterstain propidium iodide (orange/red). The broader band of light detected with this filter system increased the background autofluorescence, muscle and connective tissue appearing as dull green and the mucosal epithelium as yellow. This autofluorescence was easily distinguished from the bright green specific FITC staining and permitted the precise orientation of fluorescence staining, whether intra- or extracellular, within sections. Photographs were taken on Agfachrome 1000 ASA film. After fluorescence studies the coverslips were removed, the sections stained with haematoxylin and eosin, examined with

bright field optics, and photographs taken on Kodak 50 ASA film.

Results

DISTRIBUTION OF THE METALLOPROTEINASES *Gelatinases A and B*

Sections stained with antiserum to gelatinase A had no specific immunofluorescence in either histologically normal or Crohn's diseased tissue. Sections of histologically normal intestine, from all patients studied, with and without Crohn's disease, stained with antibodies to gelatinase B, showed bright fluorescence in the few isolated PMNLs scattered throughout the wall of the intestine. When inflamed, strictured and fistulated regions from Crohn's disease tissue were examined and large numbers of brightly staining PMNLs were observed in all sections studied (fig 1A). These cells were detected throughout the intestinal wall: the greatest number were observed in the lamina propria, a substantial number in the submucosa, and a few in the muscularis propria. This distribution pattern was consistent throughout all sections of strictured, fistulated, and inflamed tissues.

Stromelysin

In histologically normal tissue from patients with and without Crohn's disease bright fluorescence was observed only in a small number of mononuclear cells, and only a slight increase in the number of these cells was observed in diseased tissues. Stromelysin was detected on the extracellular matrix in areas of smooth muscle cell proliferation in five of six patients (fig 1B), and in the lamina propria directly below the basement membrane in regions of mucosal damage in four of six patients (figs 1C and D).

Interestingly, in tissue sections from patients with ulcerative colitis extracellular stromelysin was detected in regions of mucosal damage in all five patients (figs 1E and F), but was never observed in histologically normal tissue from these patients.

Collagenase

Fluorescence was observed in only a very few isolated mononuclear cells distributed throughout the intestinal wall (data not shown). There was no difference in the numbers or distribution of these cells staining positive for collagenase between diseased and histologically normal tissues.

Discussion

The inflammatory infiltrate, which is a feature of early lesions in Crohn's diseased intestine, is in part responsible for initiating and maintaining a number of the connective tissue changes seen in the intestine. These can include altered smooth muscle cell proliferation and migration, increased collagen, and changes in the activity of the matrix degrading enzymes. The increased numbers of PMNLs in this infiltrate, which stained for gelatinase B, were most numerous in regions

Figure 1 Distribution of gelatinase B (A) and stromelysin (B, C, E) in intestine from patients with Crohn's disease or ulcerative colitis. Resected intestine was frozen, sectioned, fixed and permeabilised. Sections were incubated with primary antibody followed by FITC-labelled anti-sheep IgG to localise the enzymes and propidium iodide to stain the nuclei orange. Sections D and F were subsequently stained with haematoxylin and eosin.

(A) Section of inflamed tissue from a patient with Crohn's disease stained for gelatinase B. Bright green granular fluorescence shows PMNLs staining positive for gelatinase B in a central blood vessel and in the surrounding submucosal tissue. Connective tissue of the submucosa autofluoresces dull green.

(B) Section of fibrotic Crohn's diseased intestine stained with antisera against stromelysin. Smooth muscle cell bundles (SM) are seen in the submucosa and bright green stromelysin staining can be clearly seen.

(C) Section of inflamed intestine from a patient with Crohn's disease stained with antisera against stromelysin. The arrow indicates bright green stromelysin fluorescence below the basement membrane of mucosal epithelial cells in a region of mucosal damage.

(D) The same section as in fig 1C stained with haematoxylin and eosin. The arrow indicates the region associated with fluorescence staining in fig 1C.

(E) Section of large intestine from a patient with ulcerative colitis. Green/yellow fluorescence shows stromelysin in the lamina propria below the basement membrane of mucosal epithelial cells.

(F) The same section as in fig 1E stained with haematoxylin and eosin. Arrows indicate an absence of connective tissue matrix in regions associated with stromelysin staining in fig 1E.

of connective tissue remodelling—namely, the mucosa and the submucosa. One possible role for gelatinase is the degradation of type IV collagen⁵ in the blood vessel basement membranes which permits the extravasation of cells and serum proteins into the surrounding tissue. Gelatinase has been shown to have such a role in metastatic tumour cell invasion.¹²

In contrast, in Crohn's disease stromelysin was predominantly associated with the extracellular matrix in regions of smooth muscle cell proliferation or mucosal damage. The presence of stromelysin, both surrounding and below smooth muscle cells in fibrotic

regions, may explain the ability of these cells to invade the submucosa. Stromelysin can degrade some basement membrane components, including type IV collagen and laminin^{13–15} and has been shown to have a critical role in potentiating the passage of cells across basement membrane barriers in other pathological states.^{16,17} The localisation of stromelysin around proliferating smooth muscle cells in Crohn's diseased tissues may implicate stromelysin in the degradation of smooth muscle cell basement membrane barriers, facilitating smooth muscle cell proliferation and aiding migration, the outcome of which is stricture formation.

There is little direct evidence concerning the basement membrane of smooth muscle cells and cellular activity, but modulation of cell behaviour seems to require interaction with, and passage through, the basement membrane of any potential cytokine. Disruption of smooth muscle cell basement membrane would therefore be a potential factor in altering cell migration or replication.

In inflamed tissue from patients with ulcerative colitis, a disease whose pathogenesis includes substantial loss of mucosal structures, stromelysin was consistently localised on the lamina propria in regions of mucosal loss. The presence of extracellular stromelysin in regions of mucosal degradation in two inflammatory bowel conditions points to an important role for this enzyme in the connective tissue degradation which precedes mucosal loss.

It is unclear if the inflammatory infiltrate is responsible for all the changes seen in the present report. Gelatinase B is clearly increased as a consequence of the PMNLs associated with the inflammation, and may be a contributory factor in potentiating this response. Stromelysin, on the other hand, may be a product of the smooth muscle cells, and therefore a critical determinant in facilitating their migration and proliferation.

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