

Parallel expression of macrophage metalloelastase (MMP-12) in duodenal and skin lesions of patients with dermatitis herpetiformis

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Abstract

Background—Dermatitis herpetiformis (DH) is a specific dermatological manifestation of coeliac disease and 80% of DH patients have gluten sensitive enteropathy manifested by crypt hyperplasia and villous atrophy. Matrix degradation mediated by collagenase 1 (MMP-1) and stromelysin 1 (MMP-3) has previously been implicated in the pathobiology of coeliac intestine and cutaneous DH blisters.

Aims—To study expression of stromelysin 2, metalloelastase, collagenase 3, and matrilysin in the intestine and skin of DH patients.

Methods—In situ hybridisation using ³⁵S labelled cRNA probes was performed on duodenal biopsies of 15 DH patients, three samples each of control duodenal or jejunal mucosa, fetal ileal explants, lesional DH skin, and 19 serial biopsies of experimental DH blisters. Immunostaining was used to examine type IV collagen, macrophages (CD68), and 92 kDa gelatinase (MMP-9) in the specimens.

Results—Metalloelastase (MMP-12) was abundantly expressed by subepithelial macrophages in both coeliac intestine and spontaneous and induced DH rash. It was also upregulated in the experimental model of coeliac disease (staphylococcal endotoxin B stimulated fetal explants). The only other MMP detected was MMP-9 which did not localise with MMP-12.

Conclusions—Upregulation of metalloelastase is associated with T cell mediated immune responses both in the intestine and skin. In addition to modulating macrophage migration, it may contribute to degradation of proteoglycans or basement membrane components in the subepithelial mucosa.

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Keywords: coeliac disease; metalloproteinase; dermatitis herpetiformis

Dermatitis herpetiformis (DH) is a chronic cutaneous disorder characterised by a blistering rash on the elbows, knees, and buttocks, and granular IgA deposits in the basement membrane (BM) zone.¹ Although gastrointestinal symptoms in DH patients are rare, they all have gluten sensitive enteropathy manifested in 80% by crypt hyperplasia or partial villous atrophy, while the rest have normal villous architecture with increased counts of

intraepithelial lymphocytes.² The rash and enteropathy improve on a gluten free diet, implying that DH is a specific skin manifestation of mainly subclinical coeliac disease (CD).^{2,3}

Matrix metalloproteinases (MMPs) are a family of extracellular matrix (ECM) degrading enzymes that are collectively capable of degrading essentially all ECM components and that can further be subdivided into collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -7, -10, and -12), membrane-type MMPs, and other MMPs.^{4,5} The extracellular activity of MMPs is regulated by TIMPs 1-4.⁶ We have previously reported that expression of MMP-1 and -3 is enhanced in basal keratinocytes surrounding neutrophil abscesses in DH skin.⁷ Furthermore, when producing experimental DH blisters with 50% potassium iodide, urokinase plasminogen activator is upregulated at 12 hours, before blisters are seen, while both MMP-1 and MMP-3 are abundantly expressed in 24 hour biopsies of blisters.⁸ In DH, collagenase 1 (MMP-1) and stromelysin 1 (MMP-3) may contribute to formation of blisters by degrading BM components such as type VII as well as type IV collagen or laminin 1, respectively. Interestingly, Daum and colleagues⁹ have recently demonstrated that these same MMPs are expressed by subepithelial macrophages and fibroblasts in intestinal biopsy specimens from patients with CD.

Tissue transglutaminase (tTG) was recently identified as the endomysial antigen in patients with CD.¹⁰ Patients with DH have elevated immunoglobulin A autoantibodies to tTG confirming its pathogenic relation with CD.¹¹ tTG has been shown to be involved in the intermolecular cross linking of type VII collagen of anchoring fibrils of skin. Interestingly, type VII collagen is degraded, at least in experimental DH blisters, and expression of both MMP-1 and MMP-3 colocalise in these areas.⁸

The histological changes of CD are characterised by deepening of the crypts and flattening of the villi, and altered turnover of ECM has been implicated in these changes.⁹ Rapid collapse of the villus architecture can occur

Abbreviations used in this paper: DH, dermatitis herpetiformis; MMPs, matrix metalloproteinases; BM, basement membrane; CD, coeliac disease; ECM, extracellular matrix; SEB, staphylococcal endotoxin B; tTG, tissue transglutaminase; IFN- γ , interferon γ ; IL, interleukin; TNF- α , tumour necrosis factor α ; TGF- β , transforming growth factor β ; GM-CSF, granulocyte macrophage-colony stimulating factor.

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within hours of administering gliadin to CD patients in vivo.⁹ Daum and colleagues⁹ also recently demonstrated in vivo that MMP-1 may contribute to degradation of interstitial collagens and MMP-3 to epithelial cell shedding in CD. In an experimental model of human fetal small intestine, direct addition of activated MMP-3 in explant cultures caused collapse of the villi within 24 hours.¹² Furthermore, crypt hyperplasia and villous atrophy rapidly occurred following activation of lamina propria T cells with the bacterial superantigen *Staphylococcus aureus* endotoxin B (SEB).¹³ We have shown that various other MMPs such as stromelysin 2 (MMP-10), matrilysin (MMP-7), collagenase 3 (MMP-13), and macrophage metalloelastase (MMP-12) are upregulated in another intestinal disorder, inflammatory bowel disease, characterised by influx of T cells, eosinophils, and neutrophils, as well as degradation of the mucosa.¹⁴ MMP-10 has a nearly identical substrate specificity to MMP-3.¹⁵ MMP-12 is the most elastolytic of the MMPs but also degrades various other substrates such as type IV collagen, laminin 1, fibronectin, vitronectin, and proteoglycans.^{16, 17} Matrilysin cleaves in vitro collagen type IV, proteoglycans, gelatin, aggrecan, fibronectin, laminin, tenascin, and elastin.¹⁸ Collagenase 3 degrades fibrillar collagens, gelatin, type IV collagen, tenascin, and fibronectin.¹⁹ The aim of this study was to further characterise the patterns of MMP expression in DH intestine. We compared expression of various MMPs in the intestine and skin of patients with DH, and also used the fetal explant model of enteropathy to investigate the link between T cell activation and MMP production.

Methods

TISSUES

Fifteen specimens of formalin fixed paraffin embedded duodenal biopsies taken during upper gastrointestinal endoscopy were obtained from the archives of the Department of Dermatopathology, University of Helsinki, Finland. All patients had been diagnosed as having DH clinically, by demonstration of granular deposits of IgA in the BM zone using immunofluorescence, and by elevated values of IgA gliadin or reticulin antibodies. Eight of 12 patients were endomysial antigen positive at the time of endoscopy. Histologically, six of the samples demonstrated subtotal and the remainder partial villous atrophy (table 1). Cutaneous samples included specimens of lesional skin from patient Nos 13–15. In addition, 19 serial biopsies of experimental DH blisters were taken four, 12, or 24 hours after application of 50% potassium iodide, as previously described.⁸ Patients receiving dapsone (10/12) were asked to stop their medication 24 hours before the study. Samples taken from 50% potassium iodide test areas in four healthy volunteers were also examined for control purposes. Histologically normal duodenal (n=4) and jejunal mucosa (n=3) from non-coeliac patients was also analysed. The study was approved by the ethics committee of the

Department of Dermatology, Helsinki University Central Hospital. Informed consent was obtained from individual subjects for all procedures.

IN SITU HYBRIDISATION

The production and specificity of the antisense human stromelysin 2, macrophage metalloelastase, collagenase 3, and matrilysin probes have been described previously.^{14, 20–22} As a control for non-specific hybridisation, sections were hybridised with ³⁵S labelled sense RNA from a bovine tropoelastin cDNA. The validity of this probe as a negative control has been confirmed by northern²³ and in situ hybridisation²⁴ assays. The cDNAs were transcribed in vitro using a commercial kit (Promega Corp., Madison, Wisconsin, USA) and labelled with ³⁵S UTP, as previously described.²⁵

Following deparaffinisation and rehydration, 5 µm sections were pretreated with 1 mg/ml of proteinase K and washed in 0.1 M triethanolamine containing 0.25% acetic anhydride. Subsequently, sections were hybridised with probes (2.5–5×10⁴ cpm/µl of hybridisation buffer) and washed under stringent conditions, including treatment with RNase A, as described previously.^{25, 26} Autoradiography was carried out for 20–40 days. All samples were processed in at least two experiments and were independently analysed by two investigators. Samples previously positive for stromelysin 2 (chronic wounds), macrophage metalloelastase (sarcoid granulomas), collagenase 3 (squamous cell carcinomas), and matrilysin (sweat gland tumours) were used as positive controls.

IMMUNOSTAINING

Immunostaining was performed using the avidin-biotin-peroxidase complex technique.²⁵ Diaminobenzidine or aminoethylcarbazole (CD20) were used as chromogenic substrates. Monoclonal antibodies included MMP-9 (DB-2211; Diabor, Finland)²⁷ and CD68 (KP-1, No M814; Dako, Carpinteria, California, USA) to identify macrophages and type IV collagen (M785; Dako, Glostrup, Denmark), and CD20 (L26, M0755) to identify mucosal B cells. Tissues were counterstained with haematoxylin. Staining for revealing elastic fibres was performed using Weigert's elastin stain (Weigert's Resorcin-Fuchsin.).

ORGAN CULTURE OF HUMAN FETAL SMALL INTESTINE

The study received ethics approval from the Hackney and District Health Authority (London, UK). Human fetal small intestine was obtained from the Medical Research Council Tissue Bank, Hammersmith Hospital, London, UK. All specimens used in this study were aged 15–16 weeks' gestation. Culture of human fetal small intestine explants in serum free medium was performed as described previously.^{28, 29} Mucosal T cells were activated by adding SEB (10 µg/ml; Sigma) at the onset of culture and tissues were cultured for four days before fixation in formalin and paraffin embedding.

Table 1 Clinicopathological information and in situ hybridisation results for patients with dermatitis herpetiformis providing duodenal specimens

Age	Sex	Histology	Rash	GFD	MMP-12	MMP-9
46	F	Subtotal	+	+	+	+ nf, mf
17	F	Subtotal	+	-/+	(+)	++
31	F	Subtotal	+	-	+	(+) mf
21	F	Partial	+	-	++	+ nf, mf
52	F	Partial	+	-	++	-
22	M	Subtotal	+	-	-	ND
41	M	Subtotal	+	+	-	(+) nf
30	M	Partial	-	-	+	ND
27	F	Partial	-	-/+	-	+ nf, mf
75	M	Partial	+	-	++	++ nf, mf
31	M	Subtotal	+	-/+	+	+ nf
28	M	Partial	+	-	+	(+)
59	M	Partial	-	-	+	++ nf, mf
33	F	Partial	+	-	(+)	(+)
36	M	Partial	+	-	+	+

ND, not determined; -, no detectable signal; (+), signal in a few cells; +, specific signal in moderate number of cells; ++, specific signal in high number of cells.
mf, macrophages; nf, neutrophils.

GFD, gluten free diet; +, strict diet; -/+, partial diet; -, no diet.

Results

INTESTINE

MMP-12 mRNA was detected in 12/15 intestinal biopsies from patients with DH and most

positive cells were detected in samples representing partial villous atrophy (table 1). The plump pale staining macrophage/activated fibroblast-like cells were localised subepithelially (fig 1A, B). At least some of the MMP-12 positive cells were macrophages, as assessed by CD68 immunostaining (fig 1C, D). Although T cells have been reported to express MMP-12, at least during experimental autoimmune neuritis,³⁰ the MMP-12 positive cells did not have histological characteristics of lymphocytes (size, dark nuclear staining). To assess the integrity of the BM in the vicinity of MMP-12 positive cells, type IV collagen immunostaining was performed. It revealed that staining was often blurred in association with the MMP-12 positive areas, suggesting that the BM was not intact (fig 1F, G). Weigert's stain did not reveal elastic fibres in the MMP-12 positive areas (data not shown). No MMP-12 mRNA positive cells were detected in normal duodenal or jejunal mucosa (fig 1E).

Matrilysin, stromelysin 2, and collagenase 3 were not detected in any of the biopsies. There

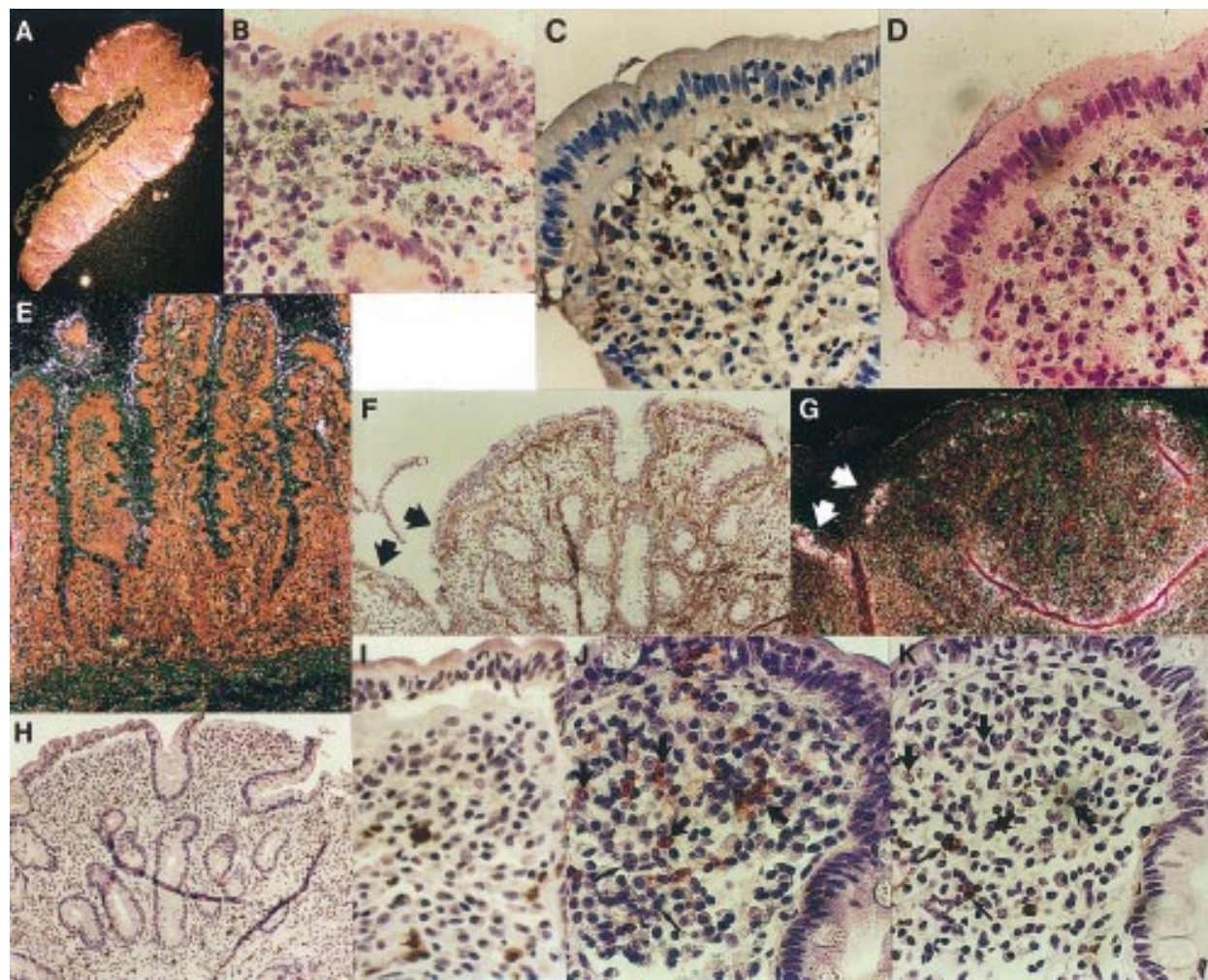


Figure 1 Expression of macrophage metalloelastase (MMP-12) in the subepithelial cell layer of coeliac intestine. (A) A dark field image showing MMP-12 expressed subepithelially in a duodenal biopsy from patient No 10. (B) Higher magnification bright field of the pale plump positive cells under the epithelium. (C) Immunostaining for CD68 macrophage marker. (D) MMP-12 positive cells in a serial section. Arrowheads depict corresponding spots. (E) Normal duodenum is negative for MMP-12 mRNA. (F, G) Serial sections showing MMP-12 in situ hybridisation and staining for type IV collagen. Note the blurred and faint staining in areas with a lot of positive macrophages. Arrows depict corresponding spots. (H) Immunostaining for MMP-9 showing positive cells in different regions than MMP-12. (I) Higher magnification of MMP-9 positive cells seen in (H). (J) Immunostaining for CD20 showing mucosal B cells. (K) Immunostaining for MMP-9 on a serial section. Small arrows show MMP-9 positive but CD20 negative cells, and large arrows depict cells positive for CD20 but negative for MMP-9. Original magnification $\times 20$ (A), $\times 100$ (E-H), and $\times 400$ (B-D, I-K).

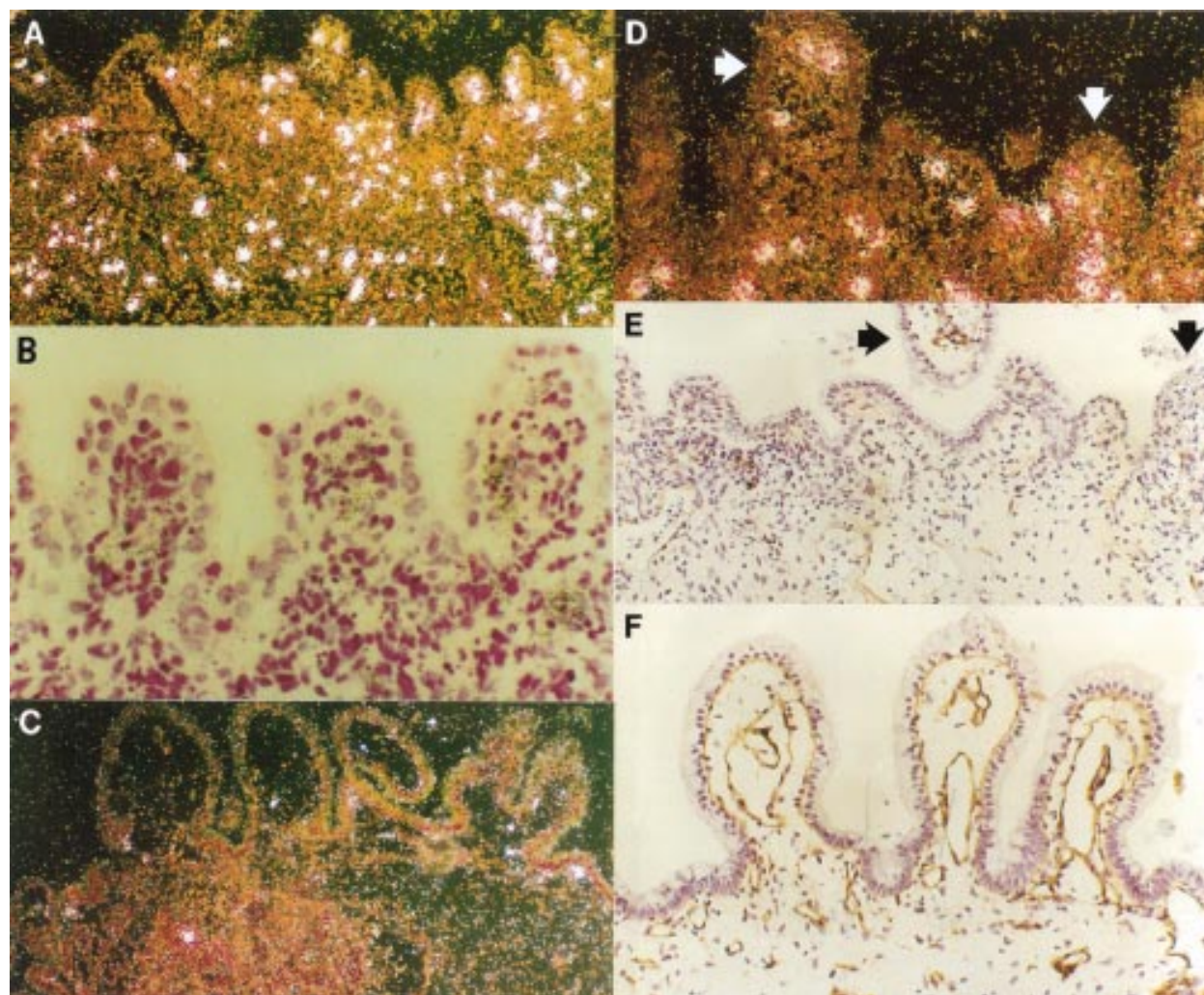


Figure 2 (A) Expression of macrophage metalloelastase (MMP-12) mRNA in a staphylococcal endotoxin B (SEB) stimulated explant cultured for four days. (B) Higher magnification of MMP-12 positive stromal cells under the epithelium. (C) Control explant cultured for four days has occasional MMP-12 mRNA positive cells. (D) MMP-12 mRNA in another SEB stimulated explant. (E) Type IV collagen staining is abnormal under the atrophic villi. Arrows depict corresponding spots. (F) Type IV collagen immunostaining in the control explant. Original magnification $\times 100$ (A, C), $\times 200$ (D–F), and $\times 400$ (B).

were MMP-9 immunopositive cells in all biopsies and they localised in deeper areas of the mucosa than MMP-12 (fig 1H, D). However, there did not appear to be any correlation between the amounts of MMP-12 or MMP-9 positive cells detected in individual samples (table 1). Staining for CD20 did not colocalise with MMP-9 (fig 1J, K), and hence cells expressing MMP-9 were not B cells, but macrophage-like cells and neutrophils. Occasional MMP-9 positive neutrophils were also detected in normal duodenum (data not shown).

Activation of lamina propria T cells with SEB results in villous atrophy and crypt hyperplasia, similar to CD.^{13–31} In human fetal ileal SEB stimulated explants, MMP-12 was abundantly expressed by macrophages of the stroma and by those trafficking up the villi (fig 2A, B). No expression was detected in the control sample harvested two hours after the culture was started on day 1 (data not shown). After four days of culturing, occasional stromal MMP-12 mRNA positive cells were detected in control samples (fig 2C) but their number

was significantly lower than in SEB stimulated explants. Staining for type IV collagen revealed that it was abnormal in the flat villi of SEB explants compared with controls (fig 2D–F).

SKIN

Spontaneous DH blisters from patient Nos 13–15 showed MMP-12 mRNA positive macrophages migrating from perivascular inflammatory infiltrates up towards the epidermis (fig 3A). The BM was partly disrupted in these areas, as assessed by type IV collagen immunodetection (fig 3B). Generally, there was already neutrophil accumulation in the BM zone (fig 3A).

To understand the spatial and temporal induction of MMP-12 in DH, we also studied serial samples from lesions produced by 50% potassium iodide that were clinically and histologically identical to spontaneous DH blisters, as previously described.⁸ At four hours, excess lymphocytes were seen in the dermis and by 12 hours their number had increased to form perivascular infiltrates, while some areas showed vacuolisation of basal keratinocytes. At

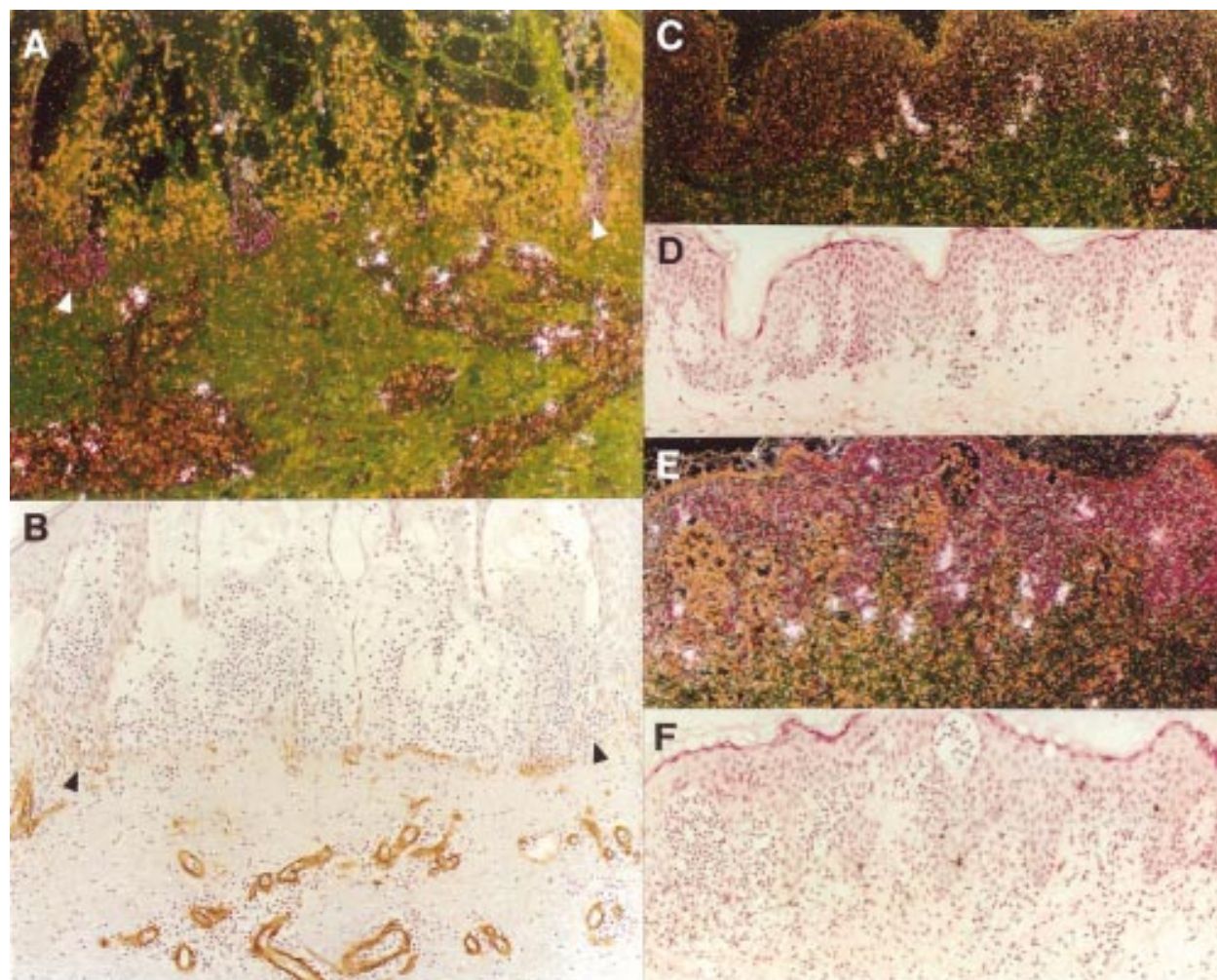


Figure 3 (A) Expression of macrophage metalloelastase (MMP-12) in a spontaneous blister of patient No 3. (B) Staining for type IV collagen in a serial section. Arrowheads depict corresponding spots. (C) MMP-12 positive cells are seen in an induced blister formation at 12 hours. (D) Corresponding bright field image. (E) Dark field image for MMP-12 in situ hybridisation in an induced blister biopsied at 24 hours. (F) Corresponding bright field image. Arrowheads depict corresponding regions. Original magnification $\times 100$ (A–F).

24 hours, typical features included inflammatory cell infiltrates, neutrophilic abscesses with various stages of multilocular blisters, and papillary oedema. The specimens taken from healthy volunteers at 24–48 hours showed mild eczema but no blister formation. MMP-12 mRNA was not detected in induced blisters biopsied at four hours (data not shown). However, expression was detected in 3/7 12 hour blisters in macrophages of inflammatory infiltrates and those migrating up towards the epidermis (fig 3C, D). In 6/7 24 hour blisters, subepithelial macrophages and some that had already migrated into the epidermis expressed MMP-12 mRNA (fig 3E, F).

Only one of four control specimens of healthy volunteers induced with potassium iodide had occasional MMP-12 mRNA positive cells. It has been reported previously that there are no MMP-12 mRNA positive cells in normal skin.²⁷ No MMP-12 positive cells were found in normal looking skin of DH patients (data not shown).

Discussion

Dermatitis herpetiformis is a specific dermatological manifestation of CD with small blisters

and pathognomonic IgA deposits in the papillary dermis.³² In this study, we have shown that, as previously demonstrated for MMPs-1 and -3,⁹ expression of MMP-12 was also enhanced in gluten sensitive enteropathy of patients with DH. Furthermore, MMP-12 was also abundantly expressed in macrophages migrating towards and into the epidermis in both spontaneous and induced DH blisters.

Macrophages are the major cell type known to express MMP-12 in adult tissues and this cell is known to contribute to ECM degradation in various inflammatory conditions. The rapid collapse of villus architecture, which can occur within hours of challenging CD patients with gliadin, has been suggested to be due to degradation of interstitial collagens.⁹ As shown in this study (fig 1), BM disruption also seemed to take place in the intestine of DH patients. This may be due to MMP-12 in these areas. Alternatively, MMP-12 may be degrading proteoglycans³³ or other BM components. MMP-12 has been implicated in elastin degradation in the pathogenesis of atherosclerosis and emphysema.^{34–35} No staining for elastic fibres was detected, even near MMP-12 positive areas, which makes it unlikely that

MMP-12 participates in elastin remodelling. However, MMPs have many other tasks than matrix remodelling: they function in intestinal mucosal defence by regulating the activity of defensins,³⁶ participate in activation of several cytokines, regulate apoptosis,³⁷ and may cleave adhesion molecules.³⁸

Gluten induced activation of T cells and secretion of cytokines are believed to be the stimuli for changes in the mucosa of CD and DH.³¹ Gluten exposure in patients with CD rapidly elicits high levels of interferon γ (IFN- γ), interleukins (IL) 2, 4, and 6, and tumour necrosis factor α (TNF- α).^{39, 40} In coeliac intestine, mRNA levels for IL-2, IFN- γ , TNF- α , IL-1 β , and transforming growth factor β (TGF- β) are elevated.⁴¹ Furthermore, positive immunostaining for TGF- β colocalises with MMP-12 in the subepithelial lamina propria.⁴² TNF- α , IL-1 β , macrophage colony stimulating factor, vascular endothelial growth factor, and platelet derived growth factor BB upregulated MMP-12 expression in human peripheral blood derived macrophages, and this induction can be inhibited by TGF- β .⁴³ In coeliac mucosa, TNF- α is elevated and activation of TGF- β may be impaired due to autoantibodies to tTG, both favouring upregulation of MMP-12 in macrophages. In murine peritoneal macrophages, only granulocyte macrophage-colony stimulating factor (GM-CSF), but not IFN- γ , IL-1 β , IL-2, IL-5, IL-6, or TNF- α , can induce metalloelastase production.⁴⁴ Interestingly, increased expression of GM-CSF has been reported in lesions of DH.⁸ Moreover, eosinophils in CD secrete GM-CSF⁴⁵ and could thus contribute to upregulation of MMP-12 in intestinal macrophages. In addition to cytokine stimulation, direct contact with T cells may also upregulate MMP-12 expression in mucosal macrophages.⁴⁶

In contrast with inflammatory bowel disease,^{14, 47} MMP-7, -10, and -13 mRNA positive cells could not be found in atrophic intestinal biopsies from patients with DH. MMP-7 and -10 were also not expressed in DH lesions. Furthermore, none of the MMPs investigated in this study was detected in mucosal epithelium. This suggests that mucosal re-epithelialisation is needed to induce MMPs in enterocytes.^{14, 47}

We also demonstrated that MMP-12 was markedly upregulated in an experimental model of CD—namely, fetal intestinal explants stimulated by SEB. Resident lamina propria cells are activated by SEB to produce T helper 1 (Th)-type cytokines and within 2–4 days there is crypt hyperplasia, a typical feature of CD.³¹ However, the ex vivo system does not contain neutrophils, B cells, mast cells, or eosinophils.¹² This suggests that proinflammatory cytokines can induce MMP-12 expression in resident lamina propria macrophages. SEB stimulation results in some loss of lamina propria glycosaminoglycans,⁴⁸ which are in vitro substrates, or both MMP-3 and MMP-12.^{17, 19} Furthermore, keratinocyte growth factor and TGF- α are known to be upregulated in SEB stimulated explants⁴⁹; however, there are no reports on the regulation of MMP-12 by these

cytokines. SEB activation of T cells also causes increased concentrations of MMPs-1, -2, -3, and -9, and TIMP-1,⁴⁷ as seen in CD in vivo.⁹

MMP-12 was abundantly expressed in both spontaneous and induced DH blisters. The mechanism by which potassium iodide provokes blistering in DH is not known but the reaction is characteristic of DH, and healthy controls do not get blisters.⁸ MMP-12 expression at 12 hours coincides with that of urokinase plasminogen activator in basal keratinocytes but precedes induction of MMPs-1 and -3.⁸ Based on our histological data on the distribution of MMP-12 mRNA and the integrity of the BM, as assessed by type IV collagen staining, it seems that degradation of the BM is initiated before MMP-12 positive macrophages have migrated into the epidermis (fig 3) and that it is associated with neutrophil accumulation.

Activated macrophages are generally thought to contribute to mucosal damage via secretion of cytokines such as IL-1 β and TNF- α . The results of this study suggest that by secreting MMP-12, they can contribute to BM changes and perhaps epithelial cell loss. MMP-12 may modulate macrophage influx into inflammatory sites by cleaving endothelial or epithelial BMs. Furthermore, MMP-12 is capable of activating TNF- α ¹⁵ and may thereby stimulate the inflammatory response in CD. tTG with plasmin is required for proteolytic activation of TGF- β .⁵⁰ If inhibition of tTG by coeliac autoantibodies indeed blocks tTG bioactivity and thus prevents TGF- β activation,⁵¹ it is probable that various MMPs are upregulated as TGF- β generally results in their down-regulation.⁴ In conclusion, our results demonstrate that in DH, both intestinal and skin manifestations could be partly caused by proteases secreted by activated macrophages. In the future, use of selective MMP-12 inhibitors or anti-TNF- α antibody therapy may prove beneficial in alleviating the cutaneous symptoms and intestinal manifestations of DH.

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