Increased expression of protease-activated receptor-2 in mucosal mast cells in Crohn's ileitis

Ulrika Christersona, Åsa V. Keitab, Johan D. Söderholmb, Christina Gustafson-Svärd

a School of Pure and Applied Natural Sciences, University of Kalmar, SE-391 82 Kalmar, Sweden
b Department of Clinical and Experimental Medicine, Division of Surgery, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden

Received 18 July 2008; received in revised form 4 November 2008; accepted 5 November 2008

KEYWORDS
Crohn's ileitis;
Mast cells;
Protease-activated receptor-2;
Tumor necrosis factor-α

Abstract

Background and aims: Activation of protease-activated receptor-2 (PAR-2) may stimulate various events of importance in inflammatory processes, including release of inflammatory mast cell mediators. PAR-2 is frequently up-regulated during inflammatory conditions, but it is not known if the expression is altered in Crohn's disease. The aim of the present study was to investigate the ileal mucosal PAR-2 expression in Crohn's ileitis, with particular emphasis on the expression in ileal mucosal mast cells.

Methods: Surgical specimens from the distal ileum were collected from patients with Crohn's ileitis and patients with colonic cancer as controls. The overall expression of PAR-2 was investigated by Western blot, and the presence of PAR-2 expressing mucosal mast cells by immunohistochemistry and cell counting. The effect of tumor necrosis factor-α (TNF-α) on the PAR-2 expression in a human mast cell line (HMC-1) was investigated by RT-PCR and immunocytochemistry.

Results: In Crohn's specimens, the fraction of PAR-2-expressing mucosal mast cells was increased about 2.5 times (P < 0.001; n=14) compared with specimens from control patients (n=6). No difference was found between inflamed (n=6) and uninflamed Crohn's specimens (P > 0.05; n=8). Exposure to TNF-α for 48 h up-regulated PAR-2 mRNA and protein expression in the HMC-1 cell line.

Conclusion: PAR-2 is up-regulated on ileal mucosal mast cells in Crohn's ileitis, possibly due to the action of inflammatory cytokines, such as TNF-α. This may contribute to perpetuating the inflammatory process in the intestinal mucosa in Crohn's ileitis.

© 2008 European Crohn's and Colitis Organisation. Published by Elsevier B.V. All rights reserved.

1. Introduction

Protease-activated receptor-2 (PAR-2) is a G-protein coupled receptor that is activated by proteolytic cleavage by specific serine proteases, such as trypsin and mast cell tryptase. PAR-2 is widely distributed among different cell types.
throughout the human intestine, and is thought to modulate various intestinal functions in both health and disease. In particular, increasing clinical and experimental evidence shows that PAR-2 activation is of importance in inflammatory processes, also within the gastrointestinal tract. And PAR-2 expression is frequently up-regulated during inflammatory conditions. There are, however, no previous published reports on the expression of PAR-2 in the intestinal mucosa of Crohn's disease.

Mast cells are considered to be involved in various aspects of the pathogenesis of Crohn's disease, such as stricture formation, and may also play a role in disruption of the intestinal epithelial barrier. Moreover the effects of TNF-α on PAR-2 expression were previously demonstrated that most of the TNF-α-expressing cells in the ileal mucosa in Crohn's disease are mast cells.

Although several lines of evidence point to the possibility that PAR-2 activation of mast cells may play a role in the pathophysiology of Crohn's disease, it is still unknown to what extent ileal mucosal mast cells express PAR-2, and if this expression is altered in Crohn's ileitis. The principle aim of the present study was therefore to investigate PAR-2 expression in ileal mucosa, in particular mucosal mast cells, in patients with Crohn's ileitis and in control patients. Moreover the effects of TNF-α on PAR-2 expression were studied in a human mast cell line.

2. Materials and methods

2.1. Subjects

Full-thickness sections of ileal wall and/or mucosa were collected from 26 patients operated upon at the University Hospital of Linköping. Fifteen of the patients were operated on for Crohn's ileitis and 11 for colonic cancer, serving as controls. Eleven of the 15 Crohn's patients were taking medication, including steroids (prednisolone), azathioprine and mesalazine. Sampled specimens were submitted to the Department of Pathology for routine pathological evaluation, and specimens from Crohn's patients were considered as being uninfamed only if no signs of microscopic inflammation were observed. Ten out of 11 inflamed specimens were macroscopically inflamed. From five of the Crohn's patients, samples were taken from both uninfamed and inflamed areas. The specimens were analyzed for PAR-2 expression by western blot and/or immunohistochemistry. Details about the sampled ileal specimens and which analysis was performed are given in Table 1. The study was approved by the Regional Ethical Review Board in Linköping, and carried out in accordance with the Declaration of Helsinki.

2.2. Antibodies and reagents

Mouse monoclonal antibody to human PAR-2 (FITC conjugated, subclass IgG2a), goat polyclonal antibody to human PAR-2 (N-19), and goat polyclonal antibody to human Actin (I-19) was obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). Mouse monoclonal antibody to human mast cell tryptase (clone AA1, subclass IgG1κ), normal rabbit serum, Negative control mouse antibody IgG2a,b, aprotinin, heparin, HRP-conjugated rabbit anti-goat was obtained from Jackson ImmunoResearch Laboratories, Inc (West Grove, PA, USA). Vectashield® mounting medium with propidium iodide was from Vector Laboratories, Inc (Burlingame, CA, USA). Heparin Leo 5000 IU/ml was bought from LEO Pharma A/S (Ballersum, Denmark). Precast trisglycine gels and nitrocellulose membrane from Invitrogen Life Technologies Ltd (Paisley, UK). ECL Plus Western blotting detection system and Hyperfilm ECL from GE Healthcare (Chalfont St Giles, UK). Iscoves modified Dulbecco's medium (IMDM) and foetal calf bovine serum (FCS) were from Gibco (Invitrogen Life Technologies Ltd, Paisley, UK).

2.3. Western blot

Human ileal tissue was homogenized in RIPA buffer containing PMSF (0.1 mg/ml), aprotinin (30 μl/ml) and sodium orthovanadate (1 mM). After centrifugation, total proteins were precipitated with a 10% trichloroacetic acid. After washing with cold 70% ethanol, the resulting crude protein pellets were re-suspended in sample buffer, heated at 60 °C for 10 min, and loaded onto a 15% SDS-PAGE gel. The separated proteins were transferred to nitrocellulose membranes. A mouse monoclonal antibody to human PAR-2 (FITC conjugated, subclass IgG2a) and a goat polyclonal antibody to human Actin (I-19) were used as the primary antibodies, and a goat polyclonal antibody to human Actin (I-19) was used as the secondary antibody. The proteins of interest were detected using ECL and autoradiography.

Table 1 Sources of sampled ileal specimens, and type of analysis performed.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age</th>
<th>Ileal specimens</th>
<th>Status</th>
<th>Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>F 94</td>
<td>Normal</td>
<td>WB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 72</td>
<td>Normal</td>
<td>IHC</td>
<td>WB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 86</td>
<td>Normal</td>
<td>IHC</td>
<td>WB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 78</td>
<td>Normal</td>
<td>IHC</td>
<td>WB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 56</td>
<td>Normal</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 73</td>
<td>Normal</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 76</td>
<td>Normal</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 85</td>
<td>Normal</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 74</td>
<td>Normal</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 48</td>
<td>Normal</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 82</td>
<td>Normal</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>M 45</td>
<td>ui</td>
<td>IHC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 24</td>
<td>ui</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 38</td>
<td>ui</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 56</td>
<td>ui</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 33</td>
<td>ui/i</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 40</td>
<td>ui/i</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 46</td>
<td>ui/i</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 20</td>
<td>ui/i</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 38</td>
<td>ui/i</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 58</td>
<td>i*</td>
<td>IHC</td>
<td>WB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 41</td>
<td>i</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 54</td>
<td>i</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 47</td>
<td>i</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 57</td>
<td>i</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 26</td>
<td>i</td>
<td>WB</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ui = Uninflamed, i = Inflamed (*macroscopically normal but showed signs of microscopic inflammation). IHC = Immunohistochemistry, WB = Western blot.
(10 µg) were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% normal rabbit serum in PBS containing 0.1% Tween 20 for 1 h at room temperature, and then incubated with polyclonal goat anti-human PAR-2 antibody (N-19, diluted 1:1000; 0.2 µg/ml) or polyclonal goat anti-human actin (I-19, diluted 1:10000; 0.02 µg/ml) at 4 °C for 16 h, and then with a HRP-conjugated rabbit anti-goat antibody (diluted 1:10000; 0.08 µg/ml) for 1 h at room temperature. Detection was undertaken using the ECL Plus Western blotting detection system and Hyperfilm ECL. Negative controls omitted the primary antibody. Densitometrical measurements was performed using NIH Image J software.

2.4. RT-PCR

Hexamer-primed cDNA was generated (Omniscript reverse transcript RT Kit, Qiagen, Solna, Sweden) from 1 µg isolated total RNA (Ultraspec II RNA Isolation system, Nordic Biosite, Täby, Sweden) and amplified using PuRe Taq RTG PCR beads (GE Healthcare, Buckinghamshire, UK) and primers (Invitrogen Life Technologies Ltd, Paisley, UK) specific for human PAR-2 (forward, 5′-CCA TCC AAG GAA CCA ATA G-3′; reverse, 5′-CTG AGG CAG GTC ATG AAG-3′; product size 436 bp) and for human 18S (forward, 5′-ACG RAC CAG AGC GAA AGC AT-3′; reverse, 5′-GGA CAT CTA AGG GCA TCA CAG AC-3′, product size 531 bp). Untranscribed total RNA was used as negative control. The amplification program used for PAR-2 was, 1 cycle 94 °C, 5 min; followed by 40 cycles of 94 °C, 1 min; 53 °C, 30 s; 72 °C, 45 s; and a final cycle of 72 °C, 5 min. The amplification program for 18S was as follows, 1 cycle 94 °C, 5 min, followed by 30 cycles of 94 °C, 20 s; 58 °C, 20 s; 72 °C, 45 s, and a final cycle, 72 °C, 5 min. Samples were resolved on a 1.6% (w/v) agarose gel and stained by ethidium bromide. The products were run against DNA ladder D7058 (Sigma-Aldrich, St. Louis, MO). The PCR products for PAR-2 were purified using a Qiagen gel extraction kit (Qiagen, Solna, Sweden) and DNA sequence was determined using ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) and BigDye Terminator Cycle Sequencing Ready Reaction DNA Sequencing kit (PE Applied Biosystems, UK).

2.5. Immunohistochemistry

To evaluate the localization of PAR-2 expressing cells in the ileal bowel wall, tissue slides were fixed in acetone in −20 °C for 5 min and then blocked with 50% normal rabbit serum for 1 h in room temperature. Prior application, to avoid interactions other than immunological, the anti-human mast cell tryptase antibody (1:200, 0.4 µg/ml) was pre-incubated with heparin 1000 IU/ml in PBS pH 6.0 as previously described. After application the slides were incubated at 4 °C for 16 h. Also, the secondary rabbit anti-mouse antibody (1:50, 10 µg/ml) was

Figure 1 Expression of protease activated receptor-2 (PAR-2) in ileal mucosal specimens. (A) Western blot analysis of PAR-2 in specimens from five control patients (Controls) and five Crohn’s ileitis patients (CD); Four of the Crohn’s specimens were macroscopically and microscopically normal, but one (line 7) showed signs of microscopic inflammation. A negative control (primary antibody omitted) showed no protein labeling (−). (B) Densitometric analysis of the band intensity and normalization to β-actin. NS (not significant) P>0.05.
pre-incubated with heparin 1000 IU/ml in PBS pH 6.0 and then applied to the slides to incubate for 1 h at room temperature. The FITC-conjugated anti-human PAR-2 antibody (SAM 11; 1:50, 4 µg/ml) was incubated at 4 °C for 16 h. Finally, the slides were mounted in Mowiol® 4–88 and placed in the dark at 4 °C until microscopic evaluation. As negative control to anti-PAR-2 antibody an irrelevant Negative control mouse antibody IgG2a, biotin conjugated secondary antibody and FITC conjugated streptavidin was used. As negative control to the mast cell staining primary antibody was omitted.

2.6. Evaluation of immunohistochemistry and mast cell count

Sections were examined using NikonEclipse E600 Confocal Microscope (Nikon, Japan) and Nikon Confocal Microscope EZ-C1 Software version 2.0. The total number of ileal mucosal mast cells, and the PAR-2+ mast cells was counted at a magnification of ×600. At least 400 cells/patient were counted. The number of mast cells/mm², and the number of PAR-2+ mast cells/mm² were evaluated. Additionally, the proportion of PAR-2+ mast cells was examined. Any mast cell showing immunological staining with the PAR-2 antibody was considered as being PAR-2+, regardless of the labeling intensity.

2.7. Stimulation of PAR-2 expression in a human mast cell line

The human leukemia mast cell line HMC-1 (kind gift from Dr J.H. Butterfield, Mayo Clinic, Rochester, USA) was cultured in IMDM supplemented with 10% FCS, 1.2 mM α-thioglycerol, 100 µg/ml streptomycin and 100 U/ml penicillin. The cell line was maintained at 37 °C in a humidified atmosphere of 5% CO₂ in air. Cells were seeded, at a number of 5×10⁵, and cultured for 48 h with TNF-α (25 ng/ml) and then analyzed for PAR-2 expression using immunocytochemistry.

2.8. Immunocytochemistry

Cultured HMC-1 cells, were washed, smeared on poly-L-lysine coated glass slides and then allowed to air dry for at least 30 min.

Slides were fixed in 4% formaldehyde for 15 min at room temperature and then incubated with PBS containing 50% normal rabbit serum for 1 h. The samples were incubated with anti-human PAR-2 antibody (SAM-11; FITC 1:50, 4 µg/ml) for 16 h at 4 °C. Slides were washed and mounted with Vectashield® mounting medium with propidium iodide. As negative control to anti-PAR-2 antibody an irrelevant Negative control mouse antibody IgG2a, biotin conjugated secondary antibody and FITC conjugated streptavidin was used.

2.9. Statistics

If not otherwise indicated, results are expressed as mean ± SEM. Comparative statistical analysis was made with Wilcoxon rank sum test (between groups), and with Wilcoxon signed rank test in the case of paired samples (inflamed versus non-inflamed specimens within the same patients), with p<0.05 considered significant.

3. Results

3.1. No alteration in overall ileal PAR-2 expression in Crohn’s ileitis

Considering the frequently observed up-regulation of PAR-2 in various inflammatory conditions, we first investigated the overall ileal mucosal expression of PAR-2. As seen in Fig. 1, the overall expression of PAR-2 in ileal mucosal specimens from Crohn’s patients was not significantly different from the expression in control patients, as assessed by Western blot analysis. For methodological reasons, however, only macroscopically uninfamed mucosa could be investigated from the Crohn’s patients (it was not possible to properly separate the inflamed mucosa from the underlying tissues). In addition, therefore, we also investigated the PAR-2 expression in inflamed full-thickness ileal specimens from Crohn’s patients. As seen in Fig. 2, no difference in overall PAR-2 expression was found between full-thickness specimens from Crohn’s patients and control patients.

3.2. Increased number and proportion of PAR-2 expressing mast cells in Crohn’s ileitis

Since the high expression of PAR-2 in the human ileum²,³,⁵ may mask alterations confined to specific cell types within the mucosa, and considering the proposed role of PAR-2 in

![Figure 2](image-url)

**Figure 2**  Expression of protease activated receptor-2 (PAR-2) in full-thickness ileal bowel specimens. (A) Western blot analysis of PAR-2 in specimens from three control patients (Controls) and five Crohn’s ileitis patients (CD); The mucosa of the Crohn’s specimens were macroscopically inflamed. (B) Densitometric analysis of the band intensity and normalization to β-actin. NS (not significant) P>0.05.
autocrine and paracrine activation of mast cells in inflammatory conditions, our next step was to investigate the PAR-2 expression in ileal mucosal mast cells.

Cells positively stained with the tryptase antibody were found in the ileal lamina propria of both Crohn’s ileitis patients and control patients, and were considered as being mast cells. The total number of mast cells/mm² was increased in specimens from patients with Crohn’s ileitis, compared to specimens from control patients (87.8±10.9, n=14, versus 48.3±3.8, n=6; P<0.01; Fig. 3). No significant difference was found between inflamed and uninfamed Crohn’s specimens (71.9±5.6, n=6 versus 96.5±16.2, n=8; P>0.05; Fig. 3).

A fraction of the mast cells in both control and Crohn’s specimens were positive for the PAR-2 antibody. Mast cells

![Mast cell staining](image)

**Figure 3** Immunohistochemical analysis of the number of mast cells in ileal mucosal specimens. Control specimens (Ctrl; n=6), uninfamed Crohn’s ileitis specimens (CDui; n=8), and inflamed Crohn’s ileitis specimens (CDi; n=6). CDtot = CDui + CDi; n=14. Immunostaining with tryptase antibody and cell counting were performed as described in Materials and methods. Data about patients and specimens are given in Table 1. Horizontal bars represent median values. **P<0.01, *P<0.05, NS (not significant) P>0.05.

![Mast cell staining](image)

**Figure 4** Typical stainings of mast cells in ileal mucosal specimens upon double immunohistochemical labeling of mast cell tryptase and protease activated receptor-2 (PAR-2), illustrating mast cells with various degrees of PAR-2 staining. (A, C, E) A tissue slide from a control specimen. (B, D, F) A tissue slide from a Crohn’s disease specimen. (E, F) Tryptase labeling is shown in red. (C, D) PAR-2 labeling is shown in green. (A, B) Double labeling of mast cell tryptase and PAR-2 appears yellow. (B, D, F) A PAR-2 expressing mast cell showing intense PAR-2 staining is indicated by a thin arrow and a mast cell showing weak staining is indicated by a fat arrow. (A, C, E) A mast cell showing very weak PAR-2 staining (very close to detection limit) is indicated by a fat arrow. (A, C, E and B, D, F) a mast cell negative for PAR-2 is indicated by an arrow head. (A, C, E and B, D, F) a mast cell negative for PAR-2 is indicated by an arrow head. (A–F) Confocal images at a magnification of ×600; Bar=19.5 µm. (G, H) Confocal images at a magnification of ×200, showing intense PAR-2 staining of mast cells (three representative cells indicated by thin arrows) in a tissue slide from the same Crohn’s specimen as above (H; no mast cells negative for PAR-2 are seen in this slide), and weak PAR-2 staining of mast cells (three representative cells indicated by fat arrows) in a tissue slide from the same control specimen as above (G). Note the large difference in staining intensity of the PAR-2 labeled mast cells in the control specimen compared to the Crohn’s disease specimen, almost being invisible in the control specimen at this lower magnification. (I) Negative control showing no PAR-2 labeling with an isotype-matched irrelevant antibody. (J) Negative control showing no tryptase labeling when the primary antibody was omitted.
were considered as being positive for PAR-2 regardless of labeling intensity, and no attempt was done to compare the labeling intensity between different specimens. However, mast cells in Crohn’s specimens tended to be more intensely PAR-2 labeled than mast cells in control specimens. Typical immunohistochemical stainings of PAR-2 expressing mast cells showing various labeling intensity and mast cells negative for PAR-2, are shown in Fig. 4. The total number of PAR-2 expressing mucosal mast cells/mm² was significantly higher in specimens from Crohn’s disease patients, compared to controls (68.8 ± 10.1, n = 14, versus 14.1 ± 2.4, n = 6;  P<0.001; Fig. 5), with no difference between inflamed and uninflamed Crohn’s mucosa (58.2 ± 4.1, n = 6, versus 74.0 ± 15.5, n = 8 respectively;  P>0.05; Fig. 5).

To examine if the increased number of PAR-2 expressing mucosal mast cells in Crohn’s specimens was merely due to the increased mast cell number, the proportion of PAR-2 expressing mast cells was calculated. As seen in Fig. 6, the proportion of PAR-2 positive mast cells was significantly higher in specimens of Crohn’s ileitis (77.7 ± 3.9%, n = 14, versus 31.0 ± 7.2%, n = 6;  P<0.001; Fig. 5), with no difference between inflamed and uninflamed Crohn’s mucosa (81.9 ± 5.8%, n = 6, versus 75.3 ± 5.2%, n = 8;  P>0.05; Fig. 6).

In pair-wise comparisons of macroscopically inflamed and microscopically uninflamed areas obtained from the same five Crohn’s patients, no differences were found in total mast cell number/mm² (71.9 ± 5.6, versus 63.3 ± 8.1;  P>0.05), number of PAR-2 expressing mast cells/mm² (58.2 ± 4.1 versus 50.1 ± 6.4;  P>0.05), or proportion of PAR-2 expressing mast cells (81.9 ± 5.8%, versus 79.3 ± 1.6%;  P>0.05).

3.3. TNF-α up-regulates PAR-2 in a human mast cell line

Inflammatory cytokines are known to increase the PAR-2 expression in several cell types, and might be responsible for our finding that PAR-2 was up-regulated on mucosal mast cells in Crohn’s ileitis. Therefore, we next investigated if PAR-2 expression in human mast cells may be affected by TNF-α, an inflammatory cytokine of prime importance in the pathophysiology of Crohn’s disease.

The human leukemia mast cell line, HMC-1, showed a basal expression of PAR-2, as demonstrated with RT-PCR (Fig. 7 A) and immunocytochemistry (Fig. 7 B). PAR-2 expression was significantly increased in cells incubated with 25 ng/ml TNF-α for 48 h (Fig. 7 A and 7 C).

4. Discussion

Increasing evidence shows that PAR-2 may have a role in inflammatory processes, both in the gastrointestinal tract, and at other locations. PAR-2 is known to be up-regulated in various inflammatory conditions, but to our knowledge, the present study is the first one addressing the expression of PAR-2 in Crohn’s disease. Although mast cells of the human colon and small intestine have been reported to express PAR-2, this is the first study specifically showing that human ileal mucosal mast cells do express this receptor. Moreover, our results suggest that PAR-2 is up-regulated in ileal mucosal mast cells in patients with Crohn’s ileitis, but that this increase is too low to be detected as an increase in overall ileal PAR-2 expression by Western blot. Consequently, although PAR-2 is widely expressed among different cell types in the human intestine, Crohn’s ileitis seems to be associated with a an up-regulation of PAR-2 restricted to one or few specific cell types, such as mast cells. However, further immunohistochemical studies investigating the PAR-2 expression in various cell types of the Crohn’s disease mucosa are needed to elucidate this issue. PAR-2 is increased in human neoplastic
Figure 7 Effect of 25 ng/ml tumor necrosis factor-α (TNF-α) for 48 h on the protease activated receptor-2 (PAR-2) expression in HMC-1 mast cells. (A) RT-PCR analysis showing increased PAR-2 mRNA expression upon TNF-α stimulation. Duplicate samples from one of seven experiments showing similar results. PAR-2, 436 bp; 18S, 531 bp. (B) Immunocytochemical analysis demonstrating basal PAR-2 expression (green) in unstimulated HMC-1 cells, and (C) increased PAR-2 expression upon TNF-α stimulation. Cell nuclei were visualized with propidium iodide staining (red). Right panels show the propidium iodide staining only. Results are representative of three separate experiments.
of luminal trypsin may be able to cross the bowel wall in this condition.

In conclusion, our results suggest that PAR-2 is up-regulated on ileal mucosal mast cells in Crohn’s ileitis, possibly due to the action of inflammatory cytokines, such as TNF-α. Considering that PAR-2 activation on mast cells may result in the release of several inflammatory mediators, one may speculate that up-regulation of PAR-2 on ileal mast cells contributes to perpetuating the inflammatory process in the intestinal mucosa in Crohn’s ileitis. Blocking of PAR-2 may thus have therapeutic potential in Crohn’s disease.

Finally, we have to emphasize that this is the first study investigating the expression of PAR-2 in Crohn’s disease, and that rather few patients were included in the study. Therefore, future studies including a larger number of patients are needed to confirm the present results. Especially the lack of difference in overall PAR-2 expression between Crohn’s specimens and control specimens, and in PAR-2 labeling of mast cells between inflamed and uninflamed Crohn’s specimens, might have been due to the low number of patients investigated.

Acknowledgements

This study was supported by grants from the Medical Research Council of Southeast Sweden (CGS), The Swedish Research Council (JDS), and the Natural Sciences Faculty, University of Kalmar, Sweden (CGS).

We thank Dr Lennart Mellblom (Kalmar County Hospital, Dept. of Pathology,) for help with a second-opinion pathological evaluation of the cryosections from CD specimens, and biomedical analyst Eva Sjödahl (Linköping University, Dept. of Clinical and Experimental Medicine) for help with the cryosectioning. We also thank Dr J.H. Butterfield (Mayo Clinic, Rochester, USA) for the generous gift of the HMC-1 cell line.

Statement of authorship. UC participated in the design of the study, performed the immunohistochemical analysis, carried out the experimental studies on the mast cell line, and participated in the draft of the manuscript. ÅVK participated in the design of the study, collected the ileal specimens, participated in analysis of immunohistochemistry, and participated in the draft of the manuscript. JDS participated in the design of the study, gave important intellectual content, and participated in the draft of the manuscript. CGS designed the study and drafted the manuscript. All authors read and approved the final manuscript.

References


