Journal of Crohn's and Colitis (2009) **3**, 15–24

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Potential role of protease-activated receptor-2-stimulated activation of cytosolic phospholipase A_2 in intestinal myofibroblast proliferation: Implications for stricture formation in Crohn's disease☆

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Received 8 July 2008; Received in revised form 17 September 2008; Accepted 1 October 2008

KEYWORDS
Crohn's disease;
Intestinal myofibroblasts;
Phospholipase A_2;
Protease-activated receptor-2;
Tumor necrosis factor-α

Abstract

Background and aims: Myofibroblast hyperplasia contributes to muscularis mucosae thickening and stricture formation in Crohn's disease (CD). Protease-activated receptor-2 (PAR-2) and cytosolic phospholipase A_2 ($cPLA_2$) are known regulators of cell growth, but their significance in intestinal myofibroblast proliferation remain to be elucidated. The principle aims of the present study were to investigate if PAR-2 is expressed in the expanded muscularis mucosa in ileal CD specimens, if inflammatory cytokines may stimulate PAR-2 expression in intestinal myofibroblasts, and if PAR-2 and $cPLA_2$ may regulate intestinal myofibroblast growth.
Methods: Immunohistochemistry was used for detection of PAR-2 in ileal CD specimens. Studies on PAR-2 expression, PLA_2 activation and cell growth were performed in a human intestinal myofibroblast cell line, CCD-18Co. PAR-2 expression was investigated by RT-PCR and immunocytochemistry. PLA_2 activity was analyzed by quantification of released ^{14}C-arachidonic acid (^{14}C-AA). Cell growth was examined by ^{3}H-thymidine incorporation and cell counting.

Abbreviations: AA, arachidonic acid; $AACOCF_3$, arachidonyl trifluoromethyl ketone; BEL, bromoenol lactone; CD, Crohn's disease; MAFP, methyl arachidonyl fluorophosphonate; PAR-2, protease-activated receptor-2; PLA_2, phospholipase A_2; $cPLA_2$, cytosolic phospholipase A_2; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; PMA, phorbol myristate acetate; TNF-α, tumor necrosis factor-α.

☆ Conference presentation: Parts of this work were presented at the World Congress of Gastroenterology, Montreal, 2005, and at the 10th International Congress of Bioactive Lipids in Cancer, Inflammation and Related diseases, Montreal, 2007.

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1873-9946/$ - see front matter
doi:10.1016/j.crohns.2008.10.002

Results: The thickened muscularis mucosae of the CD specimens showed strong PAR-2 expression. In cultured myofibroblasts, tumor necrosis factor-α (TNF-α) up-regulated PAR-2 mRNA and protein, and potentiated PAR-2-stimulated ^{14}C-AA release by two known PAR-2 activators, trypsin and SLIGRL-NH_2. The release of ^{14}C-AA was dependent on $cPLA_2$. Trypsin stimulated the proliferation of serum-starved cells, and inhibition of $cPLA_2$ reduced normal cell growth and abolished the growth-promoting effect of trypsin.
Conclusions: The results suggest that PAR-2-mediated $cPLA_2$ activation might be of importance in intestinal myofibroblast proliferation. The results also point to the possibility that PAR-2 up-regulation by inflammatory cytokines, like TNF-α, may modulate this effect.

1. Introduction

Formation of strictures, leading to intestinal obstruction, is a common clinical problem in Crohn's disease (CD). Overgrowth and thickening of the muscularis mucosae is frequently seen in CD, and contributes to the stricture formation.[1,2] The expansion of muscularis mucosae is associated with an increased number of myofibroblasts, possibly as a result of transdifferentiation and proliferation of resident smooth muscle cells in this layer.[3,4]

Protease-activated receptor-2 (PAR-2) is a G-protein coupled receptor that is activated upon proteolytic cleavage by specific serine proteases, such as trypsin and mast cell tryptase.[5] PAR-2 is expressed by various different cell types in the intestine of mammals,[6–10] including intestinal myofibroblasts.[11] PAR-2 activation has been shown to increase normal cell proliferation,[12–15] and to augment growth of tumor cells.[16,17] However, the significance of PAR-2 in regulating growth of intestinal myofibroblasts has never been investigated.

CD is associated with a diminished intestinal epithelial barrier to macromolecules,[18,19] and it seems likely, thus, that increased amounts of luminal trypsin may cross the bowel wall in CD. Moreover, activation of intestinal mast cells is a feature of CD,[20] and activated mast cells are known to release large amount of the PAR-2 activator tryptase.[21] Taken together, there are reasons to believe that CD is associated with an increased content of PAR-2 activating proteases in the bowel wall. In addition, CD is associated with increased concentrations of tumor necrosis factor-α (TNF-α) in the intestinal mucosa.[22,23] It is widely accepted that this cytokine is a major mediator in the pathophysiology of CD, and anti- TNF-α therapy is highly effective in the clinical management of CD.[24] TNF-α has been shown to up-regulate PAR-2 expression in experimental studies.[25,26] One may speculate, thus, that such a possible PAR-2 up-regulation in CD might render cells of the bowel mucosa more responsive to PAR-2-mediated activation by proteases.

Activation of phospholipase A_2 (PLA_2) and production of arachidonic acid (AA) metabolites have been implicated in the pathophysiology of various inflammatory diseases, including CD,[27,28] and also in the regulation of cell growth.[29–31] It has been reported that stimulation of PAR-2 may activate intracellular, high-molecular weight, PLA_2s,[32–36] and PAR-2-stimulated proliferation has been associated with an increased production of AA metabolites in some studies.[13,14,17,37] Indeed, there are several lines of evidence that PAR-2 may regulate normal intestinal processes, such as motility and secretion, via the production of AA metabolites.[38] It is still unknown, however, if PLA_2 activation affects intestinal myofibroblast proliferation, and if PAR-2 stimulation activates PLA_2 in this cell type.

Increased levels of several AA metabolites in the CD intestine positively correlate with disease activity, and it is generally accepted that AA metabolites are involved in the pathophysiology of CD (for reviews see Eberhart,[39] Mohajer[40] and Wang[41]). Most mammalian cells express two main types of intracellular, high-molecular weight, PLA_2s, commonly named cytosolic PLA_2 ($cPLA_2$) and Ca^{2+}-independent PLA_2 ($iPLA_2$).[42] However, $cPLA_2$ is the only PLA_2 type that shows selectivity toward AA,[43] and it is generally believed, therefore, that $cPLA_2$ plays a key role in the biosynthesis of AA metabolites.[43] No studies concerning PLA_2 activity in intestinal myofibroblasts have been reported, and the relative contribution of different PLA_2 types in the release of AA in this cell type remains to be investigated.

The principal aim of the present study was to obtain further information about cellular mechanisms involved in the myofibroblast hyperplasia of the muscularis mucosae in CD strictures. Based on the obtained results, we hypothesize that TNF-α -induced PAR-2 up-regulation and PAR-2-mediated $cPLA_2$ activation might be of importance in intestinal myofibroblast proliferation.

2. Material and methods

2.1. Intestinal tissue samples

Full-thickness ileal specimens with microscopically established muscularis mucosae overgrowth were obtained from four patients (three men and one woman, 36–56 years, mean 47) operated on for Crohn's ileitis at the university hospital of Linköping. All specimens were taken from macroscopically normal mucosa, but specimens from one of the patients showed signs of microscopic inflammation (neutrophil infiltration). At the time of operation, one of the patients was on medication with azathioprine, and one (the one with microscopically inflamed mucosa) was on medication with mesalasine. Full-thickness specimens of normal ileum were obtained from five patients operated on for colonic cancer (four women and one man, 70–92 years, mean 81), and used as comparative controls. Specimens were snap frozen, cryosectioned in 4 μm slices, and analyzed for PAR-2 by immunohistochemistry. The study was approved by the ethics committee of research on human subjects (the regional ethical review board in Linköping), and carried out in accordance with the Declaration of Helsinki.

2.2. Cells

The human cell line CCD-18Co (ATCC, no. CRL-1459) was obtained from LGC Promochem (Middlesex, UK). CCD-18Co cells exhibit many of the known characteristics of intestinal