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Short Communication

Influence of Inflammatory Bowel Disease on the Distribution and Concentration of Pancreatic Secretory Trypsin Inhibitor within the Colon

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Gastrointestinal epithelia contain a powerful protease inhibitor called pancreatic secretory trypsin inhibitor (PSTI). Patients with inflammatory bowel disease have changes in mucus structure suggestive of increased proteolysis. We therefore examined the distribution and concentration of PSTI in the colon of normal subjects and patients with inflammatory bowel disease. In normal subjects (N = 12), mucosal levels of PSTI were approximately 200 ng/mg protein in all regions of the colon and was localized to goblet and endocrine cells. Mucosal PSTI levels in the (affected) left side of the colon of patients with active (N = 12) or quiescent (N = 10) ulcerative colitis were reduced (approximately 80% of control in descending colon, 55% of control in sigmoid colon, and 50% of control in rectum, all P < 0.01), whereas levels in the (unaffected) right side of the colon were normal. PSTI levels were also reduced to approximately 65% of control in colonic tissue affected by Crohn's disease (N = 6, P = 0.01) and immunostaining showed PSTI positivity within the ulcer-associated cell lineage. As the mucous layer is important in preserving mucosal integrity, our finding of prolonged reduction in mucosal PSTI levels after an episode of ulcerative colitis probably represents a long-term reduction in a mucosal defense mechanism that could lead to increased susceptibility to episodes of inflammation. (Am J Pathol 1995, 146:310–316)

Mucosal integrity depends on a balance between aggressive factors and mucosal defense. A key component of colonic mucosal defense is the mucous layer, which forms a continuous viscoelastic gel that acts as a barrier to the passage of bacteria and large proteins[1,2] in addition to lubricating the passage of feces. Under normal circumstances, the mucous layer is in a dynamic state of equilibrium with the rate of production by colonic goblet cells balancing the loss from the luminal surface due to mechanical shearing by feces and proteolytic digestion from bacterial and pancreatic proteases. However, this balance is disrupted in inflammatory bowel disease with increased mucus turnover[3] and biochemical abnormalities of both carbohydrate and protein constituents.[4] These changes are particularly marked in ulcerative colitis in which histological examination shows mucin depletion and decreased thickness of the gel layer.[1,5] The digestion of colonic mucus *in vivo* occurs in two stages. The adherent gel layer is initially solubilized by a variety of serine proteases that is then further digested by enzymatic degradation of the oligosaccharide side chains.[6] The increased mucus turnover in inflammatory bowel disease is therefore likely to reflect an imbalance of protease/antiprotease activity acting on the mucous layer.

Pancreatic secretory trypsin inhibitor (PSTI) is a 56-amino-acid protein that potently inhibits trypsin and other serine proteases.[7] Recent studies have shown PSTI-like immunoreactivity to be present throughout the gastrointestinal tract and it is the only protease inhibitor known to be secreted into the intestinal lu-

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men.[8,9] We have recently shown that gastric conditions associated with abnormalities of mucus (atrophic gastritis and/or gastric ulceration) also have reduced tissue levels of PSTI.[10] As PSTI prevents digestion of gastric mucus by luminal proteases,[9] this has led us to suggest that PSTI plays an important role in preserving the gastric mucous layer from digestion by luminal proteases. A similar protective mechanism is present in respiratory mucus where a deficiency of α-1-antitrypsin results in lung disease.[11] In addition to its antiprotease activity, PSTI is a mitogen to several cell lines.[12,13] PSTI possesses genomic and sequence homology with epidermal growth factor (EGF),[14–16] and its mitogenic effects may be mediated via the EGF receptor.[17] PSTI may therefore be important in directly preserving mucosal integrity, in a similar way to EGF, in addition to its mucus-protecting role. We therefore examined whether inflammatory bowel disease affects the distribution and concentration of the protease inhibitor PSTI.

Materials and Methods

Ethical Approval

Colonic biopsies were collected during routine endoscopy performed for clinical reasons, and local ethical approval was obtained for the study. All subjects gave informed consent.

Patients

The normal colonic biopsies were obtained from twelve patients who had histologically normal colonic mucosa. Ten of the subjects had a final diagnosis of irritable bowel syndrome and two had a previous history of a benign colonic adenoma. A total of thirty patients with a history of inflammatory bowel disease were studied. Twelve of these had active left-sided ulcerative colitis (UC), ie, limited to descending and sigmoid colon and rectum. Ten patients had previously had left-sided UC but had been in remission for at least six months, six had Crohn's disease affecting the colon, and two had Crohn's disease affecting the small bowel without colonic involvement. Age and sex distribution were similar in all the groups. Of the twelve patients with active colitis, eight had mild colitis (increased number of inflammatory cells) and the remaining four had moderate colitis (crypt abscess formation). None of the twelve had severe colitis with complete loss of architecture.

None of the normal controls were taking any medication. Salazopyrine was taken by three of twelve patients with active colitis, four of ten with quiescent UC, two of six with colonic Crohn's disease, and the two patients with Crohn's disease not affecting the colon. Two of the twelve with active UC were taking steroid enemas, but none were taking oral steroids.

Collection of Samples

Biopsy forceps FG15L (Keymed, Southend-on-Sea, UK) were used to obtain all specimens. The mean weight (±SEM) of biopsies were 10 ± 1 mg. Biopsies were taken from the midpoint of the ascending, transverse, descending, and sigmoid colon and the rectum. All subjects had biopsies taken for PSTI radioimmunoassay (RIA), routine histology, and immunohistochemical staining for PSTI-like immunoreactivity (PSTI-LI).

All the material for histological examination was fixed in formal-saline, processed routinely, and embedded in paraffin wax in the conventional manner. Fresh sections were cut and stained with haematoxylin and eosin and also alcian-blue/diastase periodic-acid schiff. Material for RIA was snap frozen and stored at −20 C.

Immunohistochemistry

Biopsies from the same patients were stained and analyzed for PSTI-LI-containing cells by using a streptavidin/biotin-peroxidase (DAKO, Glostrup, Denmark) technique.[18] Samples were microwaved in sodium citrate buffer, pH 6.0, for 10 minutes and then stained for PSTI-LI with antiserum T4 at a final concentration of 1/1000, as previously described.[10] This antibody does not cross-react with EGF or transforming growth factor-α.[9] A brown reaction product was obtained using a peroxidase substrate (diaminobenzidine and PBS in addition to 0.3% hydrogen peroxide). Streptavidin-peroxidase staining on microwaved sections gives greater sensitivity than our previously published method of peroxidase-antiperoxidase reaction on trypsinized sections.[8] This is because trypsinization reduces the antigenicity of PSTI, probably by forming a PSTI-trypsin complex.

RIA

The frozen biopsies were extracted on ice by homogenization in 200 µl of Tris buffer (10 mmol/L, pH 7.3) for 1 minute. Extracts were centrifuged at 16,000 × *g* for 1 minute and the PSTI-LI measured in the supernatants by RIA with the antiserum T4 as described

previously.[8] The detection limit of the assay was 0.05 ng/tube. Protein was assayed by a modification of Lowry's method.[19]

Statistical Analysis

Tissue levels of PSTI-LI in normal subjects and patients with UC were analyzed by two-way analysis of variance with site and disease (control, active UC, quiescent UC) as factors. As a significant interaction of site and disease was found ($P < 0.01$), this showed that the effect of colitis on tissue PSTI levels varied according to the site being examined. We therefore analyzed the data by one-way analysis of variance for each site followed by *t*-testing on the basis of means and mean square error of the residual. Linear regression analysis was used to determine the correlation coefficient of age against PSTI level. To achieve a satisfactory distribution, $\log_e$ (PSTI levels) were used for all analyses.

As PSTI levels were similar in all parts of the colon of normal subjects and patients with active Crohn's disease had a patchy distribution of inflamed tissue, we analyzed the effect of Crohn's disease on PSTI levels by one-way analysis of variance with values obtained from inflamed areas of patients with Crohn's disease, those from non-inflamed areas of the colon of the same patients, and the mean of each normal subjects values as factors.

Results

Influence of Left-Sided UC on Colonic Levels of PSTI

In normal subjects, there was no significant difference in the level of PSTI in any of the regions examined (Figure 1A). In addition, there was no correlation between the age of the subjects and tissue PSTI levels. Immunohistochemical staining showed granular PSTI positivity in the goblet cells and some of the endocrine cells (Figure 2A, B).

Patients with active left-sided UC had normal concentrations in unaffected regions, ie, ascending and transverse colon (Figure 1B) but levels were markedly decreased in the descending colon (74% of control), sigmoid colon (37% of control), and rectum (34% of control, all $P < 0.01$). Levels were also reduced in the left side of the colon of patients who had previously had left-sided colitis but were presently in remission (Figure 1C). Levels were 87% of control in the descending colon ($P < 0.05$), 59% of control in the sigmoid colon, ($P < 0.01$), and 53% of control in the rectum ($P < 0.01$). The distribution and intensity of immunohistochemical staining was normal in the right side of the colon but was markedly reduced in the left side of the colon of patients with active or quiescent UC (Figure 2C).

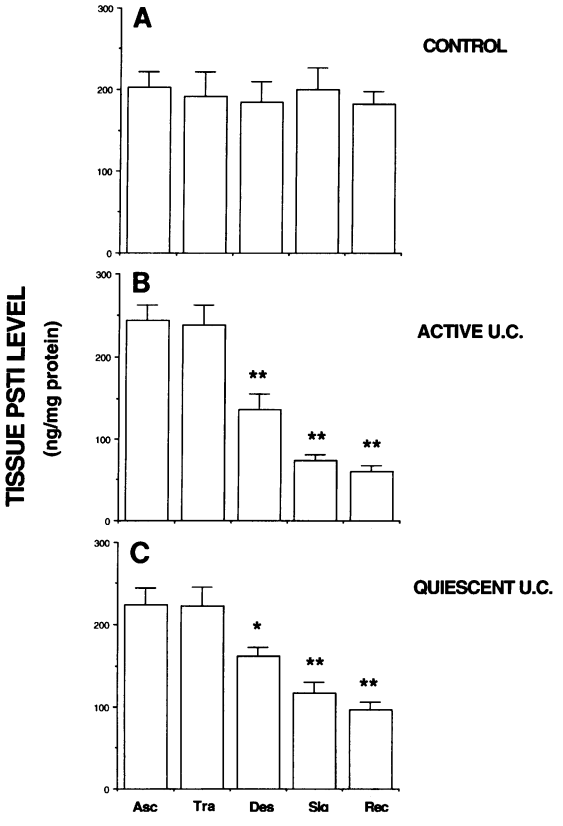
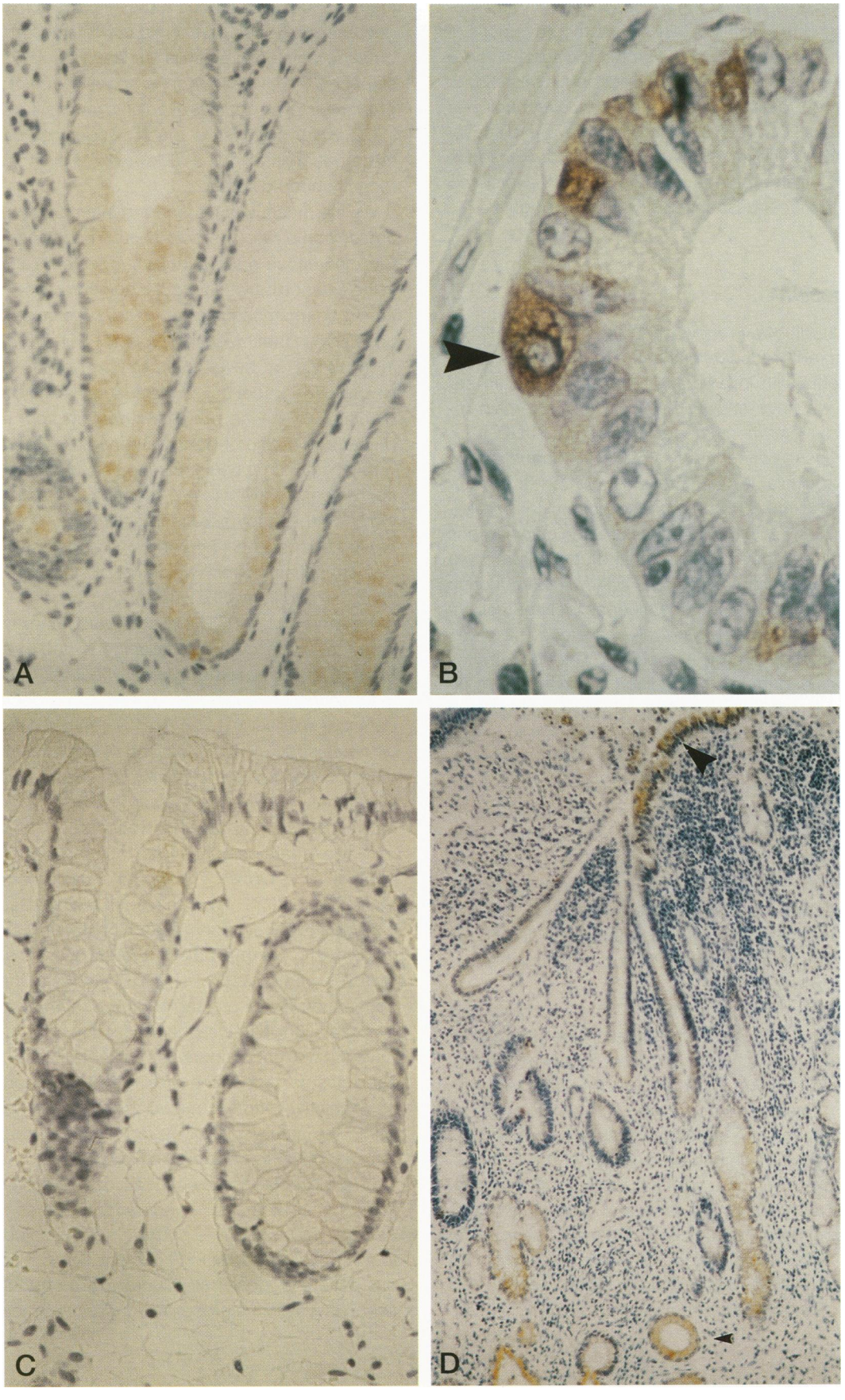


Figure 1. **A:** *Concentration of PSTI within the colonic mucosa of normal subjects. There was no significant difference in the level of PSTI in any of the regions.* **B:** *Patients with active left-sided UC, ie, affecting descending and sigmoid colon and rectum only, had markedly reduced levels in actively inflamed areas.* **C:** *Levels were also reduced in previously affected areas of subjects with quiescent colitis. Values are mean ± SEM of N = 10 to 12 per group. Asc, ascending colon; Tra, transverse colon; Des, descending colon; Sig, sigmoid colon; Rec, rectum.* *P < 0.05 *and* **P < 0.01 versus *values found at same sites in control subjects.*

Influence of Colonic Crohn's Disease on Mucosal Levels of PSTI

The two patients who had Crohn's disease affecting only the small bowel had normal colonic levels of

Figure 2. *Immunohistochemical staining for PSTI. In the normal colon PSTI positivity was located within goblet cells* (**A**) *and some of the endocrine cells* (*arrow,* **B**). *The intensity of staining was markedly reduced in active UC and in the previously affected areas of patients with quiescent colitis* (**C**). *In patients with Crohn's colitis, actively inflamed areas also had reduced levels of PSTI although there was strong PSTI positivity within the ulcer-associated cell lineage* (**D**), *notably in the acinar component* (*small arrow*) *and the surface portion* (*large arrow*).


A
B
C
D

PSTI. Patients with Crohn's disease affecting the colon had normal levels of PSTI in unaffected regions of the colon (208 ± 20 ng/mg protein *versus* 191 ± 28 ng/mg protein in controls) but reduced levels (120 ± 14 ng/mg protein, approximately 70% of control, $P = 0.01$ *versus* control values) at sites of active disease. Immunostaining for PSTI was generally decreased in actively inflamed areas but was strongly expressed within the ulcer-associated cell lineage (Figure 2D).

Discussion

These studies have shown that mucosal levels of PSTI are markedly reduced in affected regions of the colon in patients with inflammatory bowel disease. In addition, we have shown that PSTI levels remain reduced several months after an episode of UC.

The tissue concentrations of PSTI in normal specimens is similar to that previously reported by ourselves and others.[8,20] Immunohistochemical localization of PSTI within the goblet cells of the colon agrees with the findings of Fukayama and co-workers,[21] although our finding of PSTI positivity within endocrine cells of the colon and in the ulcer-associated cell lineage has not been previously reported. The ulcer-associated cell lineage is specifically induced at sites of intestinal damage in conditions such as peptic ulcer and Crohn's disease.[22] It is formed from cells at the crypt base that then form their own glandular system and empty, via a duct, onto the edge of the ulcerated region. The ulcer-associated cell lineage produces many factors thought to be important in mucosal repair, such as EGF and trefoil peptides, and it has been suggested that it acts as a first aid kit to promote mucosal repair.[23] Our finding that PSTI is also produced by the ulcer-associated cell lineage would be in keeping with this idea as, in addition to its mucus-protecting role, PSTI acts as a growth factor to several cell lines.[12,13]

In the normal colon, mucus forms a continuous layer that is important in lubricating the passage of feces and preventing direct contact between the luminal bacteria and enzymes with the underlying epithelium. Gastric mucus, which has a similar structure to colonic mucus, retards the passage of molecules with molecular weight greater than 17,000.[2] The mucous layer exists in a dynamic state *in vivo* with the erosion of mucus by abrasion and proteolytic digestion being balanced by continued secretion from the goblet cells. In patients with Crohn's disease or UC, the mucus is abnormal and structurally weaker.[4,24] These changes are particularly marked in UC in which there is increased mucus turnover[3] and a markedly decreased mucous gel thickness.[1] The reason for this breakdown in equilibrium is unclear; Podolski and Isselbacher[25] have reported a specific defect in the production of a subclass of colonic mucus, fraction IV in UC, which suggests a specific underlying defect in the production of mucus. However, this finding has recently been disputed.[26]

As the mucous layer is in a dynamic state, an increase in the rate of production of mucus combined with decreased gel thickness suggests an alteration in the balance between protease/antiprotease activity acting on the mucous layer. In the present study, we have shown that mucosal levels of PSTI are reduced in inflammatory bowel disease. One of the earliest stages of degradation of colonic mucus is proteolytic digestion by serine proteases derived from luminal bacteria and also of pancreatic origin.[6,9,27] Patients with inflammatory bowel disease have increased fecal protease activity that is probably of bacterial origin.[6,27] These proteases increase mucus breakdown *in vitro* but can be inhibited by the addition of soya bean trypsin inhibitor,[6] which has a similar protease inhibitory profile to PSTI. Therefore, our finding that mucosal levels of PSTI are decreased supports the idea that this alteration in balance is due to a combination of increased aggressive factors (increased fecal serine proteases) combined with a decreased defense mechanism (mucosal PSTI levels). During an acute episode of UC, increased epithelial turnover[28] may not allow sufficient time for PSTI to be produced. In addition, our finding of lower levels of PSTI during active disease may have been affected by the inflammatory exudate contributing to the amount of protein present in the biopsy. This would cause an apparent dilution of the PSTI level as results are expressed as nanograms of PSTI per milligram of protein. However, this would not explain the low levels of PSTI seen in samples with quiescent colitis. Changes in the local concentrations of prostaglandins within the colonic mucosa might influence the production of PSTI, as we have shown that exogenous prostaglandin E_2 stimulates gastric production of PSTI.[9] It is important to note that, even though mucus and PSTI are produced within the same cells of the colon, their production may not occur in parallel. We have previously shown such a dissociation between mucus and PSTI output in the human stomach.[9] To pursue these ideas in more detail, it will be useful to perform dynamic studies on the rate of secretion of PSTI from the colon in the various patient groups. This will provide important additional data as in the present study we have measured

static mucosal levels assuming, possibly incorrectly, that they parallel changes in the rate of secretion.

As stated earlier, PSTI has sequence homology with EGF and is trophic to a variety of epithelial cell lines.[12,13] The reduced levels of PSTI seen in inflammatory bowel disease may therefore represent a lowering of a locally acting cytoprotective agent in addition to its mucus-protecting role. Although the alteration in PSTI levels are unlikely to be the primary cause of colitis, our finding that PSTI levels remain low many months after an attack of UC suggests a long-term reduction in one of the mucosal defense mechanisms. Once this has occurred, reduced levels of PSTI may predispose to further episodes of colitis.

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