Localization and secretion of tissue kallikrein in peptidoglycan-induced enterocolitis in Lewis rats

ANTONI STADNICKI, JULIE CHAO, IWONA STADNICKA, ERIC VAN TOL, KUEI-FU LIN, FENGLING LI, R. BALFOUR SARTOR, AND ROBERT W. COLMAN

Localization and secretion of tissue kallikrein in peptidoglycan-induced enterocolitis in Lewis rats.

The Kallikreins are serine proteases, which are divided into two main types, plasma kallikrein and tissue kallikrein. A single gene codes for plasma kallikrein, which is synthesized in the liver, whereas tissue kallikrein is a member of a multigene family that shows different patterns of tissue-specific gene expression. Plasma and tissue kallikrein differ in their molecular weight, isoelectric point, immunological properties, and substrate preference. Plasma kallikrein cleaves kininogens to release bradykinin (BK), whereas tissue kallikrein acts mainly on low-molecular-weight kininogen (LK) to produce kallidin (lys-BK), which is rapidly converted to BK by an aminopeptidase (2). BK has a short half-life in the circulation. Kinins are rapidly destroyed by kininases, which are present not only in blood but also in tissues. Removal of the COOH-terminal arginine of bradykinin by kininase I forms an active metabolite, des-Arg9-BK, which is rapidly converted to BK by an aminopeptidase (2). BK has a short half-life in the circulation. Kinins are rapidly destroyed by kininases, which are present not only in blood (2, 23) but also in tissues. Removal of the COOH-terminal arginine of bradykinin by kininase I forms an active metabolite, des-Arg9-BK, which has a physical half-life in the circulation of ~2 h. Tissue metabolism further limits the duration of its pharmacological effects. BK and des-Arg9-BK, respectively, bind to constitutive B2 or inducible B1 receptors on endothelial cells, smooth muscle cells, epithelial cells, and fibroblasts, which are coupled to second messengers through G proteins (22). Regardless of their genesis, kinins are potent inflammatory mediators that increase vascular permeability. In the intestine, excess kinin may contribute to visceral pain and motility and alter electrolyte secretion (15). The major, newly identified inhibitor of active tissue kallikrein was recently cloned and named kallikrein-binding protein (KBP) in rats and kallistatin in humans (7, 42). This serine protease inhibitor has been demonstrated in plasma and various tissues by Chao et al. (9).

We have developed an experimental rat model of chronic, spontaneously relapsing, granulomatous enterocolitis that permits detailed examination of the mechanisms of intestinal inflammation (25, 26). In this model, intestinal inflammation is induced by a poorly biodegradable bacterial cell wall component, peptidoglycan-polysaccharide from group A streptococci (PG-APS). Genetically susceptible Lewis rats injected intramu-
In early experiments, House and associates (33, 34) incubated rat cecal tissue in the presence of PG-APS and observed a significant increase in tissue kallikrein (ITK) activity. The ITK levels and the total protein levels were measured by a radioimmunoassay originally described by Shimamoto et al. (32). 125I-labeled RUK was also used as the radiolabeled competing ligand in the assay. The protein level of prepared cecal extracts was measured using the bovine nonionic acid protein assay (Pierce Laboratories) according to the method of Bradford (3). In a separate experiment, Lewis rats were injected either with PG-APS (controls, n = 4) or human serum albumin (HSA; n = 16) and then killed after 24 h. Intestinal inflammation was scored grossly, and the cecal tissues were homogenized as described above. Samples from the midcecum from both inflamed and control rats were washed and weighed. Full-thickness cecal samples were put into tissue culture as described below. After 6 h of incubation, the ITK levels and the total protein levels were measured in the supernatant by methods described above. Immunochemistry for tissue kallikrein. A piece of cecum at the site of PG-APS injection was removed and washed in PBS containing penicillin (100 U/ml), streptomycin (100 U/ml), and amphotericin (GIBCO) (100 U/ml), then placed in RPMI 1640 medium with antibiotics on ice. The culture medium used did not contain serum. Minced cecal tissue (100 mg) was placed in 12-well tissue culture plates in 1 ml of culture medium. The tissue was cultured at 37°C in humidified room air plus 5% CO2. After 24 h, the supernatant was collected and stored at −70°C until assay.

Tissue culture of rat intestine. A piece of cecum at the site of PG-APS injection was removed and washed in PBS containing penicillin (100 U/ml), streptomycin (100 U/ml), and amphotericin (GIBCO) (100 U/ml), then placed in RPMI 1640 medium with antibiotics on ice. The culture medium used did not contain serum. Minced cecal tissue (100 mg) was placed in 12-well tissue culture plates in 1 ml of culture medium. The tissue was cultured at 37°C in humidified room air plus 5% CO2 for 6 h. The supernatant was collected and stored at −70°C until assay.

KBP, a major inhibitor of tissue kallikrein, was measured by an ELISA method (6). The functional levels of antithrombin activity were determined by the method of Scott (30).

Immunocytochemistry for tissue kallikrein. Rat cecal tissues injected with either PG-APS (control group) or HSA (control group) in the chronic phase of intestinal inflammation were used. The kallikrein in cecal tissue was detected using polyclonal sheep anti-RUK antibody as a primary

**Materials and Methods**

RUK antibodies were purified and characterized previously (8). The antibodies were raised against purified RUK as described previously (8). The specificity of the antibody has been reported (8, 18, 32). This antibody was found not to cross-react with plasma kallikrein, human or dog urinary kallikrein, collagenase, plasmin, trypsin, urokinase, or rat urinary elastase.

PG-APS polymers. Purified, sterile peptidoglycan-polysaccharide fragments from the cell walls of group A, type 3, strain D58 streptococci (Streptococcus pyogenes) were prepared as described previously (26) and quantified as equivalents of rhamnose, a sugar component of PG-APS.

Experimental protocol. Female inbred Lewis rats (mean weight 155 g; Charles River Laboratories, Raleigh, NC) were used at the University of North Carolina at Chapel Hill. Rats were housed in pathogen-free conditions and fed standard rat chow and water under the supervision of the Division of Laboratory Animal Medicine. Animals were anesthetized with 1.3 ml/kg body wt Innovar (Pitman-Moore, Washington Crossing, N.J.). The intestines were exposed by aseptic laparotomy. The rats were injected subserosally into the distal ileum and cecum with PG-APS (12.5 μg rhamnose equivalents/g body wt, experimental groups) or human serum albumin (37.5 μg/g body wt, control groups), as previously described (25, 26). Animals were killed by overdose inhalation of CO2 in the acute phase (day 2) (controls, n = 8, experimental animals, n = 16) and in the chronic phase (day 19) (controls, n = 5; experimental animals, n = 7). This method of euthanasia has an approved assurance from Research Risks Assurance no. A3410–01. The Animal Care and Use Committee of the University of North Carolina, Chapel Hill, approved all protocols involving vertebrate animals in this study. Cardiac blood was aspirated from the right ventricle by an 18-gauge needle into a syringe containing 3.8% sodium citrate (9:1, vol/vol). Citrated blood was twice centrifuged at 23°C to obtain the plasma for protein assays and then stored at −70°C. Intestinal inflammation was quantified grossly by a blinded observer according to criteria validated for this model (26). Gross gut score was calculated using the sum of zero to four determinations of four independent parameters, including intestinal wall thickening, adhesions, mesenteric contractions, and serosal nodules (granulomas). For measurement of ITK concentration, total protein, and Western blot analysis, the cecal tissues were homogenized in 20 mM potassium phosphate buffer, pH 7.4, at 4°C and centrifuged for 20 min at 3,000 g. The supernatants were collected and stored at −70°C.

Immunoreactive tissue kallikrein and total protein levels. Immunoreactive tissue kallikrein in intestinal homogenates was measured using a direct radioimmunoassay originally described by Shimamoto et al. (32). 125I-labeled RUK was also used as the radiolabeled competing ligand in the assay. The protein level of prepared cecal extracts was measured using the bovine nonionic acid protein assay (Pierce Laboratories) according to the method of Bradford (3). The specificity of the antibody has been reported (8, 18, 32). This antibody was found not to cross-react with plasma kallikrein, human or dog urinary kallikrein, collagenase, plasmin, trypsin, urokinase, or rat urinary elastase.

KBP, a major inhibitor of tissue kallikrein, was measured by an ELISA method (6). The functional levels of antithrombin activity were determined by the method of Scott (30).

Immunocytochemistry for tissue kallikrein. Rat cecal tissues injected with either PG-APS (control group) or HSA (control group) in the chronic phase of intestinal inflammation were used. The kallikrein in cecal tissue was detected using polyclonal sheep anti-RUK antibody as a primary
antibody. The specificity of antibody reactivity to ITK was analyzed by Western blot on rat cecal tissue homogenates. Western blotting was performed as previously described (26). Anti-sheep avidin-biotin complex was then added and detected using an indirect peroxidase technique (ABC kit; Vector Laboratories, Burlingame, CA).

Rat cecal tissues were embedded in paraffin, cut into sections 5 µm thick, and stained with hematoxylin and eosin for light microscopy examination. The tissue sections were then dewaxed, rehydrated in xylene and alcohol, and washed in PBS. The endogenous peroxidase was blocked by incubation for 30 min in 0.3% hydrogen peroxide in cold methanol. The sections were then incubated with rabbit serum (blocking serum) for 30 min to reduce nonspecific binding and with either anti-RUK antibodies (2, 0.75, and 0.2 µg/ml) or normal sheep serum (sheep immunoglobulin G, 2 µg/ml) as a control for 1 h. Antigen-antibody complexes were detected with an anti-sheep biotinylated secondary antibody and avidin-peroxidase complex according to the manufacturer’s instructions. Finally, the sections were stained with 3,3'-diaminobenzidine substrate and then counterstained with Mayer’s hematoxylin (Sigma Chemical, St. Louis, MO).

Immunocytochemistry for macrophages. On the same rat cecal tissue paraffin sections, we visualized macrophages with the use of a murine affinity-purified antibody, BMA-S32.2 (Accura Chemical Scientific, Westbury, NY). This monoclonal antibody detects the epitope MRP-14 expressed in the myelo-monocytic cell lineage of both human and rat cells and, specifically, by macrophages in granulomatous diseases. Rat inflamed cecal sections were incubated with BMA-S23.2 antibody (1:50 dilution) for 18 h at 4°C. Biotinylated anti-mouse IgG was added, followed by avidin peroxidase to detect antigen-antibody complexes. As a positive control, rat spleen sections containing macrophages were incubated with BMA-S32.2 antibodies, using the same procedure. Negative controls were performed with mouse ascites fluid containing unrelated antibody IgG.

Expression of ITK. Additional rats, which received either PG-APS or HSA, were killed in the acute experiment at 2 days (controls, n = 4; experimental, n = 5) and in the chronic phase at 26 days (controls, n = 5; experimental, n = 6). Intestinal inflammation was scored grossly as described above. Cecal tissue samples were snap frozen in liquid nitrogen and stored at −80°C for isolation of RNA. Total RNA was isolated from samples of cecal tissues, using guanidinium isothiocyanate as previously described (20, 38). RNA concentration and purity were quantified by absorbance (A) at 260 nm and A260/A280 ratios.

Evaluation of mRNA. The ITK mRNA was evaluated by RNase protection assay (from all cecal samples) and by RT-PCR (from cecal samples in the chronic phase only; controls, n = 3; experimental, n = 3).

RNase protection assay with rat tissue kallikrein antisense riboprobe. A partial rat salivary kallikrein cDNA 330 bp in length was cloned into the pSP73 cloning vector at BamH I sites. To generate an antisense riboprobe of rat tissue kallikrein, the BamH I-linearized plasmid DNA was used as a template for in vitro transcription (17). Both in vitro transcription and RNase protection assay (4) were performed with a commercial kit (Ambion, Austin, TX) according to the manufacturer’s instructions. The RNA samples were then analyzed on a 5% polyacrylamide, 8 M urea gel. Gel electrophoresis was run for 30 min at 220 V. The gel was exposed to Kodak X-Omat film for 16 h at −80°C. For normalization of RNA quantity, RT-PCR followed by Southern blot of β-actin was conducted for all RNA samples. The band density of β-actin and protected ITK mRNA of each sample was measured by a densitometer in a computer program (NIH Image version 1.47).

RT-PCR dot blot analysis. RT-PCR analysis was performed using three oligonucleotides specific for rat tissue kallikrein, essentially as previously described (11). Briefly, the tissue kallikrein cDNA fragment was synthesized from the total intestine RNA using a specific 3′ primer. After reverse transcription, the RT product was amplified for 25, 30, and 35 cycles with an annealing temperature of 55°C. The RT-PCR products (20, 10, 5, and 2.5 µl) were dotted on a nitrocellulose membrane and hybridized at 42°C with a tissue kallikrein-specific oligonucleotide probe. The ITK mRNA levels were quantitated by densitometric scanning.

Statistical analysis. The unpaired two-tailed Student’s t-test (36) was used to evaluate differences between control and experimental groups. P values ≤0.05 were considered significant.

RESULTS

Assessment of intestinal inflammation. To confirm induction of intestinal inflammation, the gross gut score for enterocolitis was determined. PG-APS-injected Lewis rats displayed marked intestinal inflammation in both acute and chronic phases. HSA-injected control rats had only minimal changes. Gross gut score was significantly higher in the acute phase at day 2 (4.8 ± 0.5 in the experimental group vs. 0.6 ± 0.2 in the control group, P < 0.0001) and in the chronic phase at day 19 (13.9 ± 1.3 in the experimental group vs. 0.1 ± 0.1 in the control group, P < 0.0001). Similarly, in Lewis rats evaluated for RNA isolation, the gross gut score was significantly higher in rats injected with PG-APS in both the acute phase at day 2 and in the chronic phase at day 26 of inflammation compared with control rats injected with HSA.

Immunohistochemical localization of tissue kallikrein. To examine the specificity of the anti-RUK antibodies, we used two normal HSA-injected Lewis rats. Representative intestinal tissue samples were evaluated for the relative molecular mass (M r) distribution of ITK by Western blotting. The anti-RUK antibodies visualized two bands of ITK (M r = 36 and 33 kDa) (data not shown), as previously reported (21).

Tissue kallikrein was visualized in normal as well as inflamed rat cecal tissues. Figure 1A and B, depicts the localization of immunoreactive kallikrein in goblet cells of the epithelial crypts of the inflamed rat colon 26 days after PG-APS injection. The lumen of the crypts often contained stained secretion, indicating the discharge of the enzyme into the intestinal lumen. Specific staining for kallikrein was also clearly seen in goblet cells of normal, HSA-injected rat cecal sections (not shown). In both normal and inflamed cecum, positive staining for tissue kallikrein was shown in all concentrations of anti-RUK antibodies used (0.25–2 µg/ml).

Figure 2A shows positive immunostaining localized within a cecal granuloma. A similar immunostaining pattern was observed within mesenteric granulomas (not shown). At higher magnification (×1,000) of the center of this granuloma, tissue kallikrein is localized in macrophages, as shown in Fig. 2B. Cecal sections treated with nonimmune serum (sheep IgG) instead of
anti-RUK antibodies were always unstained (not shown).

To confirm that the cells containing tissue kallikrein at the center of the granulomas were macrophages, we used mouse monoclonal antibodies (BMA-S32.2) that detect rat macrophages. Figure 3 shows positive immunostaining within the intestinal submucosal granulomas (Fig. 3A) as well as within a mesenteric granuloma (Fig. 3B). At a higher magnification (×400), stained macrophages within granulomas were visualized (Fig. 3C). Macrophages were also stained in rat spleen (×400, Fig. 3D) as a positive control section. The specimens treated with unrelated mouse IgG are unstained (not shown).

Concentration of ITK. The ITK level measured by radioimmunoassay in each intestinal extract sample was determined in relation to the total protein value in each sample. The specific antigenic ITK level was significantly lower in the acute phase (61% of control, 122 ± 11 ng/mg protein, n = 16, P < 0.05) and even more dramatically decreased in the chronic phase (39% of the control, 102 ± 20 ng/mg protein, n = 7, P < 0.01) of intestinal inflammation compared with controls (198 ± 18 ng/mg protein, n = 8, and 259 ± 20 ng/mg protein, n = 5, respectively), as shown in Fig. 4A. Although the acute and chronic controls are significantly different (P < 0.05), the differences are less dramatic (acute = 78% of chronic). The changes in the ITK level are more striking in the chronic phase.

ITK levels in in vitro cecal culture. As previously observed at day 2 and day 19 (see above), the antigenic ITK level was significantly lower in cecal homogenates 24 h after PG-APS injection (n = 5, 55 ± 6 ng/mg protein, P < 0.01) compared with control HSA-injected rats (n = 4, 211 ± 8 ng/mg protein). To compare the concentration of ITK released from inflamed intestinal tissue and normal intestinal tissue, we performed an in vitro study. We cultured midcecal samples from both acutely inflamed and normal rats for 6 h. The specific ITK level was significantly lower (P < 0.05) in the supernatant from cultured inflamed cecal tissues compared with control HSA-injected rats.
pared with that from cultured control cecal tissues, as shown in Table 1.

ITK expression. ITK mRNA was evaluated by RNase protection assay, and values were quantitated by densitometric scanning and presented in densitometric units. The control, β-actin as assessed in the RT-PCR assay followed by Southern blot, shows equal loading. Thus the ITK values were calculated. ITK mRNA levels were not significantly different in rats treated with PG-APS (183 ± 16) compared with control rats injected with HSA (107 ± 32) during the acute phase and were nearly equal in PG-APS-injected rats (146 ± 13) and in HSA-injected rats (154 ± 38) in the chronic phase (Fig. 4B). Similarly, there were no significant differences in densitometric values between PG-APS-injected rats (158 ± 1) and HSA-injected rats (175 ± 3) as measured in the chronic phase by RT-PCR assay (data not shown). Thus there is no significant difference in ITK mRNA expression in inflamed and normal control Lewis rat colon.

Immunoreactive level of KBP in rat plasma. The antigenic level of the rat endogenous tissue kallikrein inhibitor, KBP, was evaluated in rat plasma by a highly sensitive and specific ELISA. The antigenic KBP levels were significantly decreased (80% of control) (P < 0.01) in PG-APS-treated rats (231 ± 9 µg/ml) compared with controls (288 ± 12 µg/ml) in the acute phase of inflammation. The decrease of KBP was more pronounced in PG-APS-treated rats (50% of control) during the chronic relapsing phase of enterocolitis (114 ± 16 vs. 225 ± 3 µg/ml, P < 0.001) (Fig. 5). The chronic control is 78% of the acute control (P < 0.01). The changes in KBP are therefore much more striking in the chronic phase. Because the decrease of KBP plasma concentration may be due in part to liver injury (7, 9), we evaluated the activity of glutamic pyruvic transaminase (GPT) in rat plasma according to the method of Bergmeyer et al. (1), as previously described (33). GPT values in all control and PG-APS-treated rats in the acute phase (50 ± 4 vs. 42 ± 2 U/ml, means ± SE) and in the chronic phase (50 ± 1 vs. 38 ± 1 U/ml) were within the normal range (normal upper limit = 80 U/ml). Plasma antithrombin concentration is a sensitive marker of hepatic synthetic activity (26). There were no significant differences between the functional levels of antithrombin in acute and chronic phases of inflammation in PG-APS-injected rats or in HSA-injected controls (94 ± 1% vs. 96 ± 1% and 106 ± 2% vs. 105 ± 1%, respectively), indicating that liver injury did not account for the observed changes in KBP plasma levels.

DISCUSSION

The kallikrein-kinin system is a part of the humoral defense system that participates in the inflammatory response. In mild, acute insults, kallikrein and kinins play a salutary role, recruiting proteases, acute phase proteins, cytokines, and neutrophils to the extravascular milieu. In severe inflammation, however, the same system amplifies the inflammatory cascades and contributes to tissue destruction and chronic inflammation. Our recent studies indicated that the plasma kallikrein-kinin system is integrally involved in intestinal inflammation in genetically susceptible Lewis rats (33, 35). However, previous investigations concentrated mainly on the role of tissue kallikrein in normal intestine. Only Zeitlin and Smith (41) reported the presence of tissue kallikrein in normal human colon and a higher concentration in the inflamed colon of patients with ulcerative colitis. In the present study we have demonstrated tissue kallikrein in both normal and experimental inflamed colon, using four different methods. Morivaki et al. (21) demonstrated two tissue kallikreins in the small intes-
tine in rats (Mr = 36 and 33 kDa), both of which are recognized by the antibody used. The immunovisualization of tissue kallikrein in goblet cells of normal intestine has been previously demonstrated by Schachter et al. (27, 28). Our present data extend those investigations to demonstrate tissue kallikrein localization in both normal and inflamed rat colon. We have demonstrated tissue kallikrein for the first time in macrophages, which form granulomas in inflamed intestine. Previously, Figueroa et al. (13) demonstrated tissue kallikrein on human blood neutrophils but did not detect the enzyme in other blood leukocytes, including monocytes. It is possible that tissue kallikrein is only expressed in stimulated monocytes or macrophages, as is the case for tissue factor (16).

If tissue kallikrein in the intestine is important, it must depend on the secretion of active enzyme in the presence of kininogens, especially LK, with subsequent kinin generation. The multiple actions of kinins in the intestine may include stimulation of the release of soluble mediators from endothelial, epithelial, and white blood cells, such as eicosanoids, nitric oxide, cytokines, platelet-activating factor, and substance P (2). Increased production of all of these mediators has been described in human inflammatory bowel disease (IBD) (24). Kinins act by direct binding to and stimulation of constitutive B2 receptors. Des-Arg⁹-BK stimulates B1 receptors induced during inflammation by endotoxins or inflammatory cytokines (22). Zeitlin and Smith (41) indicated the presence of kininogen in both normal and inflamed human colon. More recently, investigators have indicated the presence of B2 receptors in intestinal epithelium and B1 receptors in ileal muscle of rat (12, 45). Thus all components necessary for kinin action are present in the intestine. In addition, kallistatin, a major tissue kallikrein inhibitor, and kininase II, a kinin inactivator, have been demonstrated in intestinal tissue (2, 9). Kinins are involved in regulation of intestinal glucose and electrolyte transport and local blood flow under normal conditions (23).

**Fig. 4.** Intestinal tissue kallikrein (ITK) concentration and mRNA expression. A: levels of ITK are calculated as nanograms per milligram protein in the total intestinal extract by RIA. B: ITK mRNA levels as determined by RNase protection assay, expressed as densitometry units. Data are means ± SE. *P < 0.05, **P < 0.01. Solid bars, control groups; hatched bars, PG-APS-injected groups.

**Fig. 5.** Plasma level of kallikrein-binding protein (KBP). Levels of KBP in rat plasma are expressed as micrograms per milliliter (means ± SE). *P < 0.01, **P < 0.001. Solid bars, control groups; hatched bars, PG-APS-injected groups.

| Table 1. Tissue kallikrein level in cultured intestinal samples in vitro |
|-------------------------------|------------------|
| **Compound Administered to Lewis Rats** | **Specific ITK, ng/mg protein** |
| PG-APS                     | 82.8 ± 10*       |
| HSA                        | 247 ± 60         |

Data are means ± SE. PG-APS, peptidoglycan-polysaccharide from group A streptococci. HSA, human serum albumin. Cecal tissue from inflamed (PG-APS) and control (HSA) rats was cultured in medium that did not contain serum. After 6 h of incubation, the intestinal tissue kallikrein (ITK) levels were measured in the supernatant by RIA as described in MATERIALS AND METHODS.* P < 0.05.
However, in the intestine, kinins may also be important pathophysiological mediators. Zipser et al. (43) showed that bradykinin produces a two- to fourfold greater concentration of prostanooids in animals with experimental colitis than in normal control animals, which may contribute to the increased intestinal secretion of chloride. However, bradykinin may also act through a direct effect on secretion by epithelial cells (19). Of particular interest is the ability of kinins to stimulate macrophage release of interleukin-1 and tumor necrosis factor (37), key cytokines in human and experimental IBD (24), an effect mediated by stimulation of the B1 receptor (37). Kinins also cause intestinal muscle contraction, which would explain the shortening of the colon and altered motility observed in IBD (24).

Despite an additional source for tissue kallikrein from macrophages, which could contribute to the total antigenic tissue kallikrein level in the affected gastrointestinal tract, we found that tissue kallikrein concentrations were markedly reduced in the inflamed cecum compared with the normal cecum: by 40% in the acute phase and by 60% in the chronic phase. Administration of PG-APS causes marked changes in host metabolism and activates many inflammatory cells with release of cytokines, proteolytic enzymes, and inflammatory mediators such as eicosanoids and nitric oxide (20, 26, 29, 33, 40). It is now well recognized that exposure to endotoxin, a similar biological modifier, may cause, in vitro and in vivo, a refractory state to synthesis of cytokines as well as nitric oxide production (5, 44). Thus, we investigated whether the decreased ITK level in inflamed colon is related to its lower synthesis or reflects its release during PG-APS-induced inflammation. In the acute phase of inflammation (day 2), we observed a modest increase of ITK mRNA, but it did not reach significance. Moreover, in the chronic phase the ITK mRNA level was nearly equal in both inflamed and normal control rat cecum as measured by two different methods. To our knowledge, this is the first report of ITK mRNA determinations in inflamed intestinal tissue. Because the decrease of ITK protein concentrations is associated with unchanged ITK mRNA levels, ITK reduction is not due to suppression of its gene expression.

We then tested the hypothesis that inflamed intestinal tissue cells had secreted ITK in vivo to a greater extent than normal. Because the mRNA levels were not different, we postulated that each cell initially synthesized equivalent amounts of ITK and that enhanced secretion by the inflamed cells would deplete the stores of the protease. The ITK level was significantly lower in inflamed cecum than in normal cecal tissue 24 h after intramural PG-APS injection. Our data from in vitro culture studies show that the amount of ITK secreted into the medium during a 6-h period of culture of inflamed tissues was only one-third that secreted from normal tissues. Taken together, our results suggest, but do not prove, that increased in vivo release of ITK in the course of inflammation is induced by PG-APS. Moreover, we cannot rule out a posttranscription block in synthesis.

The factors that determine ITK secretion and activation are not defined. Interestingly, our data indicate that secreting macrophages could contribute significantly to levels of tissue kallikrein. We postulate that the proinflammatory effects of ITK in the intestine are due to macrophage production and secretion rather than goblet cell release into the lumen. Further studies are required to settle this point.

Recent studies (6, 9) indicate that tissue kallikrein bioavailability and catabolism are normally regulated by the endogenous inhibitor, KBP (the rat equivalent of human kallistatin). Chao et al. (9) demonstrated in humans a ~100- to 15,000-fold higher concentration of kallistatin in the circulation than in various tissues. Under normal conditions, kallistatin concentrations in plasma and in colonic tissue are 20 µg/ml and 34 ng/mg protein, respectively (9), indicating that the primary role of kallistatin is in plasma. In addition, Chao et al. (9) demonstrated a marked decrease in plasma kallistatin levels in patients with sepsis. Our present data show a decrease of KBP in plasma of inflamed rats, presumably due to formation and clearance of enzyme-inhibitor complexes.

Most recently, we have shown decreased plasma levels of kallistatin in IBD patients, whereas plasma levels of tissue kallikrein were barely detectable (Stadnicki and Colman, unpublished observations). Taken together, the data suggest that tissue kallikrein may be secreted into the plasma in sufficient quantities to partially deplete the levels of KBP in intestinal inflammation. Although our current data suggest participation of ITK during PG-APS-induced intestinal inflammation, the role of ITK in the pathogenesis of experimental IBD can only be definitively confirmed if a selective inhibitor modulates the intestinal pathology of this model.

We thank Rita Stewart for expert manuscript preparation. We are also indebted to Dr. C. Katsetos, Dept. of Microbiology and Immunology, Temple University, for helpful advice on the immunohistochemistry study.

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-43535 (R. W. Colman) and DK-40249 and DK-34987 (R. B. Sartor).

Address for reprint requests: R. W. Colman, Sol Sherry Thrombos Research Center, Temple Univ. School of Medicine, 3400 N. Broad St., Philadelphia, PA 19140.

Received 17 April 1998; accepted in final form 25 June 1998.

REFERENCES


