Epithelial-derived Fibronectin Expression, Signaling, and Function in Intestinal Inflammation*

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Vasanthi L. Kolachala‡, Rahul Bajaj¶, Lixin Wang†, Yutao Yan*, Jeff D. Ritzenthaler‡, Andrew T. Gewirtz‡, Jesse Roman‡, Didier Merlin‡, and Shanthi V. Sitaraman††

From the Divisions of ‡Digestive Diseases and ¶Pulmonary and Critical Care Medicine, Department of Medicine, and ††Department of Pathology Emory University, Atlanta, Georgia 30322

Fibronectin (FN) is a multifunctional extracellular matrix protein that plays an important role in cell proliferation, adhesion, and migration. FN expression or its role in colitis is not known. The goal of this study is to characterize FN expression, regulation, and role during intestinal inflammation. Wild-type and transgenic mice expressing luciferase under the control of the human FN promoter, given water or 3% dextran sodium sulfate, were used as animal models of colitis. The Caco2-BBE model intestinal epithelial cell line was used for in vitro studies. FN protein is abundantly expressed by surface epithelial cells in the normal colon. Immunohistochemistry and luciferase assay in mice expressing the FN promoter linked to luciferase demonstrated that FN synthesis was up-regulated during colitis, during both the acute phase and the healing phase. In vitro experiments demonstrated that FN increased the expression of the FN integrin receptor α5β1 in a dose- and time-dependent manner. FN also induced the expression and activation of NF-κB. Further, FN potentiated Caco2-BBE cell attachment and wound healing, which was inhibited by RGD peptide as well as NF-κB inhibitors MG-132 and 1-pyrrolidinocarbodiethioic acid, ammonium salt. In conclusion, FN is abundantly expressed and synthesized by colonic epithelial cells. FN is transcriptionally up-regulated in epithelial cells during both the dextran sodium sulfate-induced colitic and the recovery phase. FN enhances cell attachment and wound healing, which is dependent on binding to the integrin receptor and the NF-κB signaling. Together our data show that epithelial-derived FN potentiates cell attachment and wound healing through epithelial-matrix interactions and that FN expression may have important implications for maintaining normal epithelial integrity as well as regulating epithelial response to injury during colitis.

Mucosal tissue damage requiring efficient wound healing is a cardinal feature of inflammatory bowel disease as well as other intestinal inflammatory conditions, including radiation enteritis, chronic ischemic enteritis, and cystic fibrosis. Inappropriate or ineffective tissue repair underlies the persistence of disease symptoms and results in complications such as fibrosis or fistula formation. Epithelial response to injury is a complex process and cell-matrix interactions play important roles in the healing response. Extracellular matrix (ECM) proteins such as fibronectin (FN), collagen, and laminin have been demonstrated to play a critical role in the wound healing process. In this study, we examined the expression and role of fibronectin in intestinal inflammation.

FN is a high molecular weight adhesive glycoprotein that is found in basement membrane, lamina propria, and connective tissue matrices in the intestine (insoluble form) and in body fluids (soluble form). FN usually exists as a dimer composed of two nearly identical ~250-kDa subunits linked covalently near their C termini by a pair of disulfide bonds (1, 2). Each monomer is an extended and flexible molecule that is folded in a series of repeating protein domains known as type I, II, and III repeats. These domains are resistant to proteolysis and contain binding sites for other ECM proteins (e.g. collagen), cell-surface receptors (integrins), blood protein derivatives (fibrin), and glycosaminoglycans (heparin). There are more than 20 splice variants of FN. FN exists mainly in two forms, soluble plasma FN and less soluble cellular FN. Plasma FN is synthesized predominantly in the liver by hepatocytes and forms a soluble FN dimer as compared with tissue FN, which forms disulfide cross-linked fibrils and is deposited as a matrix.

FN is widely expressed by multiple cell types and is critically important in vertebrate development, as demonstrated by the early embryonic lethality of mice with targeted inactivation of the FN gene (3). FN mediates a wide variety of cellular interactions with the ECM and plays important roles in cell adhesion, migration, growth, and differentiation (4, 5). Thus, not surprisingly, altered deposition of FN matrix has been correlated with several pathological states. For example, increased deposition of an FN-rich matrix has been associated with atherosclerosis and fibrosis, while restoration of FN matrix assembly in transformed cells has been correlated with a reduction in tumorigenicity (6). FN mRNA and protein levels are altered in patients with Crohn disease or ulcerative colitis, (7, 8, 9). FN has also been shown to play an important role in myofibroblast and

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† To whom correspondence should be addressed: Division of Digestive Diseases, Rm. 201-F, 615, Michael St., Whitehead Research Bldg., Emory University, Atlanta, GA 30322. Tel.: 404-727-2430; Fax: 404-727-5767; E-mail: ssitar2@emory.edu.

2 The abbreviations used are: ECM, extracellular matrix; FN, fibronectin; DSS, dextran sodium sulfate; PBS, phosphate-buffered saline; ECIS, electric cell-substrate impedance sensing; ADTC, 1-pyrrolidinocarbodiethioic acid, ammonium salt.
epithelial cell migration and proliferation (10) during wound healing. Surprisingly, despite the important roles of this matrix protein in cell-ECM interactions, not much is known about the cellular source, the signaling mechanism, or the role of FN in the context of intestinal inflammation. In this study, we explored the cellular source, the signaling, and the potential role of FN using a well established animal model of inflammatory bowel disease.

MATERIALS AND METHODS

Reagents—Reagents for SDS-PAGE and nitrocellulose membranes (0.45-μm pores) were from Bio-Rad. FN antibody, luciferase antibody was from Sigma Aldrich (1:1000 dilutions), α5 integrin receptor antibody (1:500) was from Abcam, NF-κB p65 (1:500) was from BD Transduction Laboratories, β-tubulin antibody (1:1000) was from Santa Cruz Biotechnology (Santa Cruz, CA), FN from bovine plasma was obtained from Sigma, and horseradish peroxidase-conjugated IgG was from Jackson ImmunoResearch Laboratory (West Grove, PA). Vectastain ABC kit was from Vector Laboratories, Inc. (Burlingame, CA), and 8W1E culture plates were from Applied BioPhysics (Troy, NY). MG-132 and ADTC were from Acros Organics.

DSS-induced Colitis in Mice—6–8-week-old C57/B6 mice were separated into control (water, n = 5) and dextran sodium sulfate (DSS)-treated groups (n = 5) and recovery groups. Colitis was induced in mice by the addition of DSS (3% weight-to-volume ratio dissolved in distilled water; molecular mass 40 kDa) to their drinking water for 6 days after which the mice were sacrificed or allowed to recover. Following the induction of colitis, the recovery group was switched back to water (day 6) to assess the effects during the recovery phase. Daily body weights were recorded, and stool samples were collected to determine occult blood loss. The severity of colitis was assessed by using a standard protocol that includes clinical score (0–12) based on loss of body weight, presence of diarrhea, and occult blood loss (11). Colonic inflammation and damage were assessed by histological scoring (0–11) that includes severity of immune cell infiltration, crypt damage, and ulcers (12).

Generation of Mice Transgenic for the Human Fibronectin Promoter Connected to a Luciferase Reporter Gene—Mice transgenic for the human fibronectin promoter connected to a luciferase reporter gene were created by the Emory University Winship Cancer Center Transgenic Mouse Core Facility. The transgenic mice were created by direct microinjection of a transgenic mice were created by direct microinjection of a 1.2-kb human fibronectin promoter fragment connected to a 1.9-kb luciferase gene fragment (pFN[1.2kb]LUC) into 200 fertilized one-cell (C57BL/6 × SJL) F2 hybrid mouse eggs. All surviving eggs were reimplanted into pseudo pregnant recipient female mice to generate the pFN[1.2 kb]LUC founder transgenic mice. The offspring were backcrossed to C57B/6 mice for at least seven generations. These mice do not show phenotypic abnormalities. In these mice, immunohistochemical studies and luciferase detection revealed a pattern of luciferase expression in lung reminiscent of that shown by the endogenous fibronectin gene at baseline and after stimulation with nicotine.3 We have previously reported that nicotine stimulates fibronectin expression in lung and in cultured primary lung fibroblasts (13). As expected, primary lung fibroblasts isolated from the transgenic animals showed increased expression of luciferase driven by the human fibronectin promoter when exposed to nicotine (14).

Luciferase Assay—Transgenic mice expressing the human FN promoter linked to a luciferase reporter gene were grouped into control (n = 5), DSS-treated (n = 5), and recovery groups (n = 5). Mucosal scrapings were homogenized in a minimum volume of luciferase lysis buffer, and luciferase activity was determined using a Promega Luciferase Reporter Assay kit according to the manufacturer’s instructions.

Cell Culture—Caco2-BBE cells were grown and maintained in Dulbecco’s modified Eagle’s medium supplemented with penicillin (40 mg/liter), streptomycin (90 mg/liter), and 10% fetal bovine serum. Confluent stock monolayers were subcultured by trypsinization (15, 16). Experiments were performed on cells plated for 15 to 7–8 days on permeable supports of 0.33-cm², 4.5-cm² inserts (Costar, Cambridge, MA). Cell culture plates were coated with various concentrations of FN (0.1–20 μg/ml) overnight at 4°C. The plates were rinsed with PBS, and then Caco2-BBE cells were plated after blocking with bovine serum albumin blocking buffer (10 mg of bovine serum albumin/PBS). Dulbecco’s modified Eagle’s medium without serum was used in all experiments.

Cell Adhesion Assay—24-Well plates were coated with FN (0–20 μg/ml) dissolved in 0.1 M sodium bicarbonate. The plates were incubated overnight at 4°C, rinsed with PBS twice, and then blocked with 100 μl of bovine serum albumin blocking buffer for 2 h at 37°C. Caco2-BBE cells were trypsinized and plated on FN-coated plates at a density of 3 × 10⁵ cells/ml in the presence of Dulbecco’s modified Eagle’s medium (without serum). After 1 h, medium was removed carefully, and the plates were rinsed twice with PBS. Adherent cells were incubated with hexosaminidase substrate (100 μl/well) at 37°C overnight. Quenching buffer was then added to each well, and cell attachment was quantified using a plate reader at 405 nm (17).

Wound Healing Assays—Wound healing assays were performed with electric cell-substrate impedance sensing (ECIS) (Applied BioPhysics) technology. The ideal frequency was determined as described previously (18). For resistance measurements performed on Caco2-BBE cells, the frequency used was 500 Hz and amplitude was set at 1 V. For the wound healing assays, confluent Caco2-BBE monolayers were plated with or without GLY-ARG-GLY-ASP-SER-PRO-LYS (GRGDSPK) (C₉H₉N₄O₁₁, Sigma Aldrich), an FN analog that binds to integrins, and cultured on ECIS 8W1E plates. Cells were then submitted to an elevated voltage pulse of 40-kHz frequency, 4.5-V amplitude, and 30-s duration, which led to death and detachment of cells present on the small active electrode, resulting in a wound in the cell monolayers. This wound is normally healed by cells surrounding the small active electrode that have not been submitted to the elevated voltage pulse. Wound healing was then assessed by continuous resistance measurements for ~24 h.

Western Blotting—Colon was flushed with PBS and everted with the help of a glass rod. Then the colonic mucosa was
scraped gently into PBS on ice, homogenized in minimum volume of lysis buffer, and centrifuged. The supernatant was subjected to Western blot or luciferase assay. Equal amounts of protein (40 μg) were separated on SDS-PAGE and transferred onto nitrocellulose paper. The proteins were probed with anti-FN (1:1000 dilutions), anti-integrin α5 (1:500), or anti-NF-κB p65 (1:500) overnight and then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (1:4000 dilution) for 1 h. Band detection was performed by chemiluminescence.

Caco2-BBE cells were lysed with PBS containing 1% Triton X-100 and 1% Nonidet P-40 (v/v), protease inhibitor mixture (Roche Applied Science), EDTA, SDS, sodium orthovanadate, and sodium fluoride. Separation on SDS-PAGE was performed according to the Laemmli procedure. Proteins were electrotransferred onto nitrocellulose membranes and probed with respective antibodies, NF-κB p65 (1:500), integrin α5β1 (1:500), or β-tubulin (1:500). Membranes were incubated with corresponding peroxidase-linked secondary antibody, washed, and incubated with ECL-plus (Amersham Biosciences) according to the manufacturer’s directions before exposure to high performance chemiluminescence films (Denville Scientific, Inc., Metuchen, NJ). For molecular mass determination, polyacrylamide gels were calibrated using standard proteins (Bio-Rad) with molecular mass markers within the range of 10 to 250 kDa. The band intensity of the Western blots was quantified by the use of a gel documentation system (Alpha Innotech Co., San Leandro, CA).

**Immunohistochemistry**—Immunohistochemical staining of FN and luciferase was carried out using the Vectastain ABC kit (Vector Laboratories) according to the manufacturer’s protocol. To perform the standard staining procedure, tissue sections were deparaffinized and rehydrated before the application of primary FN antibody (1:500 dilution), luciferase antibody (1:1000 dilution, incubated 4°C overnight). Enzyme-conjugated secondary antibodies were applied, and the specific staining was visualized after the addition of the enzyme-specific substrate. These tissues were counterstained by hematoxylin.

**Electrophoretic Mobility Shift Assay**—Nuclear protein extracts from Caco2-BBE cells plated on FN-coated plates for various time intervals were prepared for electrophoretic mobility shift assay (15). The protein content of the nuclear extract was determined using the Bradford protein assay. Electrophoretic mobility shift assay was performed using the Lightshift chemiluminescent electrophoretic mobility shift assay kit (Pierce) according to the manufacturer’s instructions. Briefly, nuclear protein was assayed for DNA binding to biotin-labeled, double-stranded oligonucleotides corresponding to the NF-κB binding site (Promega). The oligonucleotides were end-labeled using the biotin 3’-end DNA-labeling kit (Pierce) according to the manufacturer’s instructions. Biotin-labeled oligonucleotides were then incubated with nuclear proteins for 20 min at room temperature in binding buffer provided with the kit. Binding was competed by 200-fold excess of unlabeled NF-κB oligonucleotides (cold). Specificity of the binding was confirmed using NF-κB p50 antibody in supershift assay. NF-κB binding complexes were resolved by electrophoresis using 5% Tris borate-EDTA Criterion gels (Bio-Rad), transferred to Bio-dyne B pre-cut nylon membranes (Pierce), UV-cross-linked, and visualized using the chemiluminescent nucleic acid detection system (Pierce).

**RESULTS**

**FN Is Up-regulated in Murine Colitis**—To investigate the effect of colonic inflammation on FN expression in intestinal epithelia in vivo, we used an established model of murine colitis induced by DSS. Mice were given 3% DSS in drinking water for a total of 6 days. Age- and sex-matched C57/B6 mice receiving plain drinking water served as controls. One group of DSS-treated mice was sacrificed at this time to evaluate the effect of active colitis on FN expression, while a second group of DSS-treated mice was switched back to plain drinking water for an additional 6 days to evaluate FN expression during the recovery phase. The mice were evaluated for clinical signs of disease (weight changes, stool consistency, and occult blood), and histological assessment of inflammation (crypt destruction, mucosal damage, epithelial erosions, and infiltration of inflammatory cells into the mucosal tissue) was compared according to the grading system previously described (19–21). All mice developed bloody diarrhea and weight loss consistent with severe colitis 6 days after DSS treatment (clinical score: control 1 (range, 0–2), DSS 11 (range, 7–12) $p < 0.001$). The histological examination of the colonic mucosa confirmed severe colitis in these mice (histological scores: control 1.2 (range, 0–2.2) DSS 8.4 (range, 7–10.9), $p < 0.001$. Recovery from DSS was reflected by resolution of bloody diarrhea and recovery of body weight at the end of recovery phase. Histological examination revealed healing of mucosal ulcerations. Western blot analysis performed on the colonic mucosa obtained from mice treated with DSS showed up-regulation of FN compared with control mice given water (Fig. 1a) $(n = 5)$. Densitometric values demonstrated a 2.2-fold increase in FN in DSS-treated mice compared with control mice. Western blot analysis performed on colonic mucosa obtained from mice during the recovery phase (clinical and histological scores were normal) demonstrated a 6.5-fold increase in FN expression compared with controls (Fig. 1a).

**FN Localizes to Epithelial Cells and Extracellular Matrix**—We next determined the localization of FN expression using immunohistochemical analysis in control, DSS-treated, and recovered mice. In normal colon, FN staining was readily evident in the superficial colonic epithelial cells at basal as well as apical surface (Fig. 1b). FN expression was also evident in the lamina propria surrounding the epithelial cells. As expected, FN expression was also seen in the muscularis propria. FN, however, was up-regulated during DSS-induced colitis, and FN deposition was seen throughout the crypt epithelial cells. In addition, there was increased FN staining in the lamina propria and at the ulcer base. During the recovery phase, FN staining returned to its normal distribution, i.e. superficial epithelial cells and surrounding lamina propria. However, there was clear up-regulation of the intensity of staining, suggesting an increase in its expression (Fig. 1b).

**Statistical Analysis**—The data are presented as mean ± S.E. *, $p$ values <0.05 by $t$ test were considered statistically significant.
FN Is Transcriptionally Activated—To determine whether FN expression is transcriptionally activated during colitis and to determine the cellular source of FN, we used transgenic mice expressing luciferase under the control of the FN promoter as described under “Materials and Methods.” We administered 3% DSS in drinking water \((n = 10)\) or plain water \((n = 10)\) to age- \((6–8\) weeks) and sex-matched C57/B6 FN-luciferase transgenic mice. The mice were evaluated for the clinical signs of disease, including weight changes, stool consistency, and occult blood, and sacrificed at the end of 6 days. A second group of mice were allowed to recover for an additional 6 days (recovery phase). Clinical and histological assessments were performed according to the established grading system described above. The expression of FN promoter-luciferase did not affect the development of colitis or recovery. These mice developed colitis similar to wild-type mice (clinical score, 10 \((7–12)\), histological score, 9 \((7–10)\). Luciferase assay was performed on colonic mucosal strippings from control and DSS-fed transgenic mice. Luciferase activity was 1.7-fold higher \((*, p < 0.02)\) during the colitic phase and 4.1-fold higher \((*, p < 0.001)\) during the recovery phase compared with water-treated controls (Fig. 2a). Consistent with the biochemical assay, immunohistochemistry with luciferase antibody demonstrated up-regulation of luciferase expression in DSS-treated mice during the colitic and the recovery phases. Importantly, luciferase expression was seen predominantly in epithelial cells (Fig. 2b) during both active colitis and during recovery. In the next series of experiments, we addressed FN-epithelial interaction and its role on cell attachment and wound healing using the intestinal epithelial cell line Caco2-BBE plated on FN-coated transwells.

FN Up-regulates \(\alpha 5\)-Integrin Levels—Integrins are structurally and functionally related cell surface heterodimeric receptors that link the ECM with the intracellular cytoskeleton. Many of the biological effects of FN are mediated via \(\alpha 5\)-\(\beta 1\) integrin, which belongs to the integrin family of transmembrane receptors involved in cell-matrix interactions, cell-cell adhesion, differentiation, and wound healing \((22–24)\). However, the effect of FN on the expression of its receptor in epithelial cells is unknown. We set out to examine the effect of FN on \(\alpha 5\beta 1\) integrin levels in Caco2-BBE cells. As shown in Fig. 3a, Caco2-BBE cells exposed to FN demonstrated increased \(\alpha 5\) integrin receptor expression at a dose of 0.1 \(\mu g/ml\) and plateaued at 1 \(\mu g/ml\). Caco2-BBE cells plated on 1 \(\mu g/ml\) FN demonstrated increased \(\alpha 5\) expression with time that was maximal between 30 min and 2 h (Fig. 3b).

FN Activates NF-kB Signaling Pathway—Little is known about FN signaling in intestinal epithelial cells. In view of the importance of the NF-\(\kappa B\) signaling pathway in inflammation \((25, 26)\) and wound healing \((27, 28)\), we explored the activation of NF-\(\kappa B\) signaling pathway in Caco2-BBE cells plated on FN-coated plates. Caco2-BBE cells were exposed to FN \((1 \mu g/ml)\) for various time points. Cell lysates were subjected to Western blotting with anti-NF-\(\kappa B p65\) antibody. As shown in Fig. 4a, FN-induced NF-\(\kappa B p65\) activation was detected at 15 min, peaked at 30 min, and subsequently returned to baseline at 2 h after exposure to FN. We next examined the effect of FN on
NF-$\kappa$B DNA binding activity in Caco2-BBE cells exposed to FN for various time intervals. As shown in Fig. 4b, FN increased nuclear protein binding to DNA, which was maximal at 30 min after FN treatment. Binding is competed by unlabeled NF-$\kappa$B oligonucleotides (cold). In the presence of FN, the addition of NF-$\kappa$B p50 antibody to the reaction resulted in supershift of this complex. Together, these data demonstrate that FN is a potent activator of NF-$\kappa$B signaling pathway.

To investigate the effect of colonic inflammation on NF-$\kappa$Bp65 activation and $\alpha$5 integrin receptor expression in vivo, we used established model of murine colitis as described in Fig. 1. Western blot analysis performed on the colonic mucosa showed up-regulation of NF-$\kappa$Bp65 and $\alpha$5 integrin receptor both in the colitic phase and the recovery phase compared with control mice given water. (Fig. 5). The NF-$\kappa$B activation and $\alpha$5 integrin level paralleled the increase in FN level seen during colitic and recovery phase.

**FN Potentiates Caco-2-BBE Cell Attachment**—Given that FN is an extracellular matrix protein synthesized and secreted by the epithelia and enriched in wounded areas, we examined the effect of FN on epithelial cell attachment. Caco2-BBE cells were plated on various concentrations of FN as described under “Materials and Methods.” Cell attachment was determined by quantifying adherent cells using hexosaminidase-based calorimetric assay. As shown in Fig. 6a, FN enhanced Caco2-BBE cell attachment in a dose-dependent fashion. Cell attachment was increased by 46 and 56% in the presence of 1.0 and 10.0 $\mu$g/ml FN, respectively.

**FN Mediates Cell Attachment through Activation of NF-$\kappa$B Signaling Pathway**—To determine the role of FN-induced NF-$\kappa$B in cell attachment, Caco2-BBE cells were exposed to FN in the presence or absence of NF-$\kappa$B inhibitors MG-132 (29) or ADTC (30). As shown in Fig. 6b, MG-132 and ADTC inhibited FN-mediated cell attachment by $\sim50\%$ (FN+MG-132, $*, p < 0.006$) and FN+ ADTC (*, $p < 0.002$). MG-132 and ADTC alone had no effect on cell attachment in the absence of FN.

**FN Mediates Cell Attachment and Wound Healing through $\alpha$5 Integrin Receptor**—Extensive analyses have narrowed down the regions involved in cell adhesion along the lengthy FN mol-
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**FIGURE 4.** FN activates the NF-κB signaling pathway. *a*, Caco2-BBE cells plated on 1 μg/ml FN for the indicated time points were assessed for NF-κBp65 expression by Western blotting with anti-NF-κBp65 antibody. β-tubulin served as loading control. *b*, electrophoretic mobility shift assay showing binding of nuclear proteins from Caco2-BBE cells treated with FN for various time intervals. Oligonucleotide containing the NF-κB sites were end-labeled with biotin and incubated with nuclear extract (5 μg/ml) from the Caco2-BBE cells pretreated with or without FN for 30 min, 1 h, or 2 h. For competition assay a molar excess (200-fold) of consensus NF-κB oligonucleotide was added to the binding reaction (Cold). Lanes 1, 4, 7, and 10 show nuclear proteins binding to NF-κB oligonucleotides; lanes 2, 5, 8, and 11 show supershift of NF-κB nuclear binding with anti-NF-κB p50 antibody with time. Lanes 3, 6, 9, and 12 show cold competition.

**FIGURE 5.** FN-κBp65 and α5 integrin receptor expression are up-regulated during colitis. C57/B6 mice were weighed and randomized into three groups as described in Fig. 1 to evaluate FN-κBp65 activation and α5 integrin receptor expression during colitis and recovery phase. *a*, colonic tissues were processed for Western blot analysis as described under “Materials and Methods.” Equal amount of protein (30 μg) was loaded on 4–20% SDS-PAGE and then transferred to nitrocellulose membrane and probed with FN-κBp65 (b) and α5 integrin receptor (a) antibody. Lane 1 represents the control group, lane 2 DSS-induced colitis, and lane 3 the recovery group.

Taken together, these data demonstrate that FN plays an important role in cell attachment and wound healing through integrin receptor and NF-κB signaling.

**DISCUSSION**

FN is an important ECM protein that is found in the basement membrane and interstitial matrix of the intestinal mucosa. In this study we determined the localization, regulation, and potential role of FN during experimental colitis. We demonstrate that (i) in normal colon, FN is synthesized and expressed abundantly by the surface epithelial cells, (ii) FN synthesis is transcriptionally up-regulated in epithelial cells during colitis, during the acute phase as well as the recovery phase, (iii) FN activates the NF-κB signaling pathway and induces the expression of its receptor, α5 integrin, in Caco2-BBE cells and during colitis in animal model, and (iv) FN mediates cell attachment and wound healing, which is dependent on its binding to the integrin receptor as well as NF-κB signaling. To our knowledge, the findings reported here on localization, signaling, and expression of FN in normal colon or during colitis have not been reported before.

A striking finding of our study, based on the FN promoter luciferase assay as well as immunohistochemical localization in vivo, is the synthesis and expression of FN by the surface epithelial cells in the normal colon. Such expression of FN suggests an active role for FN in the mucosal epithelial protection of surface epithelial cells that are constantly exposed to luminal aggressors such as bacterial products and toxins and thus are subjected to injury (31). Prompt repair of injury is vital to the restoration of the mucosal barrier and the prevention of an immune response to luminal contents. Such a physiologic repair system is required for recovery from pathologic mucosal injury as seen in chronic ulceration in ulcerative colitis and Crohn disease. Our data suggest that a potential role for FN is to restore such barrier breaches that occur under physiological conditions as well as to facilitate the repair of ulcers associated with chronic inflammation. This function of FN is consistent with the important role of FN in cell attachment and wound healing (32, 33). Second, the localization of FN in surface epi-
The secretion of FN by epithelial cells suggests that FN is also secreted apically into the lumen. Indeed, we have previously shown that FN is secreted apically in response to adenosine and mediates epithelial-bacterial interactions (34). Our earlier in vitro studies using cultured intestinal epithelial cell lines showed that apically secreted FN mediates attachment, invasion, and interleukin 8 secretion by *Salmonella typhimurium* (34). Subsequent works by others have characterized bacterial proteins that bind to FN, and mutation of bacterial FN-binding proteins (e.g., autotransporter protein encoded by *Salmonella* pathogenicity island-3) (35) leads to impaired attachment by bacteria.

Another significant finding of our study is that epithelial cells are an important source of FN during active inflammation and recovery from inflammatory damage. Using transgenic mice expressing luciferase under the control of the FN promoter, we show that FN is actively synthesized by epithelial cells during the inflammatory phase as well as the healing phase. These data are consistent with the increase in FN mRNA and protein in the intestinal mucosa of patients with active ulcerative colitis and in patients with Crohn disease with fibrosis (9). During colitis, FN is present throughout the mucosa, but its distribution to surface epithelial cells is restored upon healing at significantly increased levels.

The literature suggests that the extracellular matrix plays a vital role in regulating cell attachment, migration, and wound healing (36, 37). In this context, a recent study (38) has elegantly shown that FN promotes epithelial cell migration/restitution in response to strain in Caco2 cells by inducing phosphorylation...
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and activation of extracellular signal-regulated kinase signaling pathway and myosin light kinase. Our data demonstrate that FN induces NF-κB activation that is required for attachment of cells to FN matrix. NF-κB is a vital component in the signaling pathway that plays indispensable roles in the inflammatory and immune responses, as well as cell survival (39). The FN-mediated NF-κB activation demonstrated in our study is consistent with the role of NF-κB as a transcriptional regulator of epithelial restitution and migration (27, 40). Egan et al. (27) demonstrated NF-κB activation at the wound edge in a scrape wound model using an intestinal epithelial monolayer. In both these studies, blocking NF-κB signaling resulted in inhibition of epithelial restitution. The mechanism by which FN-mediated NF-κB activation modulates cell attachment or wound healing is not known. One possibility is that NF-κB may induce integrin expression. Consistent with this, NF-κB-induced α5 integrin expression has been demonstrated to mediate cell attachment to matrix in some tissues (41–43). The induction of integrin by FN in Caco2-BBE cells clearly plays a role in cell attachment since it was inhibited by RGD peptides.

As mentioned above, epithelial cell attachment to matrix is mediated by the specific interactions of cell surface receptors with ECM proteins. The best characterized cell adhesion receptors are the integrins. Integrins comprise a family of more than 23 noncovalent, heterodimeric complexes consisting of an α and a β subunit. Although many integrins can bind FN, α5β1 is the major FN receptor on cells, including the intestinal epithelia (44). This integrin is responsible for cellular responses to FN such as adhesion, migration, assembly of extracellular matrix, and signal transduction. FN has at least two independent cell-adhesive regions; one region located near the center of the polypeptide chain in the ninth and tenth type III modules binds to the α5β1 integrin. The biological function of the central cell adhesive region requires two critical amino acid sequences, an Arg-Gly-Asp (RGD) sequence and a Pro-His-Ser-Arg-Asn (PHSRN) sequence, which function in synergy for optimal binding to the α5β1 integrin. Furthermore, the spacing between the crucial RGD and PHSRN sequences is also important for activity, suggesting the sequences themselves are necessary, but not sufficient, to account for the cell-adhesive activity of FN (45). As predicted, our data show that FN mediates cell attachment through its binding to α5β1 integrin based on the inhibition of FN-mediated cell attachment in the presence of the RGD sequence. These data are consistent with the data demonstrating that FN enhanced restitution while RGD peptides inhibited restitution in intestinal epithelial cells (46). However, our data show that FN increased the expression of α5β1 integrin in a dose- and time-dependent manner. The increase in α5β1 integrin protein expression was rapid and occurred within 30 min of exposure to FN. Whether the increase in α5 integrin is dependent on transcription/translation and whether such increase is necessary for FN to mediate cell attachment is not known.

In summary, we show that epithelial cells are an important source of FN in normal colon during intestinal inflammation and repair. FN modulates cell attachment through NF-κB signaling and binding to integrin receptors. Our data suggest that increased FN expression by epithelial cells in the setting of chronic injury or inflammation may play a role in cell attachment and wound healing through direct matrix-epithelial interactions.

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