Activation of nuclear factor κB in colonic mucosa from patients with collagenous and ulcerative colitis


Background and aims: Expression of inducible nitric oxide synthase (iNOS) is greatly upregulated in the colonic mucosa of patients with collagenous and ulcerative colitis. As the transcription factor nuclear factor κB (NFκB) is a major inducer of iNOS gene expression, we compared activation and transcriptional activity of NFκB in colonic mucosal biopsies from these patients.

Patients: Eight patients with collagenous colitis, six with relapsing ulcerative colitis, and eight with uninflamed bowel were studied.

Methods: NFκB DNA binding activity was assessed by electrophoretic mobility shift assay and inhibitor of NFκB (IkB) kinase (IKK) activity by immunocomplex kinase assay. In vivo recruitment of NFκB to the iNOS promoter was determined by chromatin immunoprecipitation analysis and transcriptional activity by NFκB gene expression profiling arrays. Cells showing NFκB activation were identified by immunohistochemistry.

Results: In collagenous and ulcerative colitis, as opposed to uninflamed bowel, IKK activity and strong NFκB DNA binding gave rise to activation of identical NFκB subunits and recruitment of transcriptionally active p65 to the iNOS promoter. In collagenous colitis, activated NFκB was observed only in epithelial cells while up to 10% of lamina propria macrophages showed activation in ulcerative colitis.

Conclusions: In collagenous and ulcerative colitis, colonic mucosal NFκB is activated and recruited to the iNOS promoter in vivo via an IKK mediated pathway. As collagenous colitis is not associated with tissue injury, these data challenge the prevailing view that activation of NFκB per se mediates tissue injury. Our results suggest that downstream inflammatory reactions leading to tissue damage originate in lamina propria immune cells, as increased NFκB activity in collagenous colitis was localised solely in epithelial cells, but present also in macrophages in ulcerative colitis.

Collagenous colitis is an inflammatory bowel disease of unknown aetiology characterised by chronic watery diarrhea in the absence of mucosal injury. Endoscopic appearance is usually normal but colonic mucosal biopsies reveal infiltration of lymphocytes and plasma cells in the lamina propria, thickened subepithelial layer of collagen, and excess of intraepithelial lymphocytes. Recently, we have observed greatly increased production rates of nitric oxide (NO) into the colonic lumen of patients with collagenous colitis and provided evidence for the hypothesis that the enzyme, inducible NO synthase (iNOS), is the source of excess NO production. As surgical exclusion of the colon by split ileostomy induces clinical and histological remission and re-establishment of gut continuity causes a rapid relapse, one or more unknown luminal factor(s) may be responsible for induction of iNOS at the luminal border of the colonic epithelium.

One of the major transcriptional inducers of iNOS gene expression is the transcription factor nuclear factor κB (NFκB). Signal transduction through NFκB is initiated on binding of ligands to cell membrane receptors (for example, Toll-like receptor 2 and 4, tumour necrosis factor (TNF) receptor, and interleukin (IL)-1 receptor) and intracellular recognition receptors (for example, nucleotide oligomerisation domain (NOD)-1 and NOD-2) leading to activation of the IkB kinase (IKK) complex and subsequently phosphorylation of the inhibitor of NFκB (IkB) kinase. Phosphorylation targets the inhibitor to polyubiquitination and proteosomal degradation, thus activating NFκB. Active NFκB translocates into the nucleus where it binds to the promoter region of multiple genes with predominantly proinflammatory actions. In addition to stimulating expression of IL-1, TNF-α, IL-6, IL-8, major histocompatibility complex class II, and intercellular adhesion molecule 1, activated NFκB also stimulates expression of iNOS and its own inhibitor, IkBα. Thus a single central pathway mediates activation signals from multiple bacterial and cytokine stimuli to increase production of a characteristic profile of proinflammatory molecules.

In ulcerative colitis, which is characterised by relapsing inflammatory involvement of the colorectal mucosa, NFκB activation has been reported to occur both in macrophages and epithelial cells, resulting in high expression levels of iNOS4 and excess production of NO. As similarly high levels of iNOS are observed in the apparently normal colonic mucosa from patients with collagenous colitis, it seems a priori unlikely that upregulation of iNOS, and thus NFκB per se, should be responsible for the tissue injury observed in active ulcerative colitis. To test this hypothesis, we compared DNA binding and transcriptional activity of NFκB in colonic mucosal biopsies from patients with active collagenous colitis, active ulcerative colitis, and uninflamed bowel, in
addition to identifying the cell types responsible for NFkB activation in the named conditions.

MATERIALS AND METHODS

Patients

Permission for the study was obtained from the regional ethics committee and all participants gave informed written consent. Patients with an established diagnosis of collagenous colitis, based on typical histopathological features, were included if they had experienced diarrhoea (stool volume >300 ml) for at least three consecutive days during the week prior to endoscopy. All medication was discontinued at least two weeks prior to the study. Patients with relapsing ulcerative colitis were included if they had endoscopic disease activity at routine examination and had received no topical treatment or systemic corticosteroids within the past month or immunosuppressive drugs within the past three months. Oral 5-aminosalicylic acid at a daily dose of 2–4 g was allowed, if no change in medication had been made in the last two weeks prior to the investigation. Stool cultures and microscopy were performed in all patients with colitis to detect pathogens, including *Clostridium difficile*, and all were negative. Patients referred for endoscopy for symptoms of irritable bowel syndrome or haematochezia to exclude colorectal cancer served as controls if they had a normal colonoscopy and uninflamed mucosa at histopathological examination.

Preparation of fusion proteins

Four glutathione S-transferase (GST) fusion proteins were made: (a) GST-IkBz (1–54) WT containing the wild-type (WT) N terminal regulatory domain (residues 1–54) of the 1kBz protein; (b) GST-IkBz (1–54) MUT containing two phosphorylation sites, serine 32 and 36, replaced by alanine; (c) GST-p100 (754–900) WT containing the C terminal regulatory domain (residues 754–900) of the NfxB2 protein; and (d) GST-p100 (754–900) MUT containing two phosphorylation sites, serine 866 and 870, replaced by alanine. Constructs were made by cloning polymerase chain reaction (PCR) generated fragments corresponding to 1kBz or NfxB2 p100, respectively, into the pGEX-2T vector (Amersham Biosciences, Buckinghamshire, UK). Mutants were made by site directed mutagenesis with the Quickchange kit (Stratagene, La Jolla, California, USA) using the manufacturer’s recommendations. Mutations were verified by sequencing. Protein G beads were then pelleted by centrifugation and washed twice in wash buffer (20 mM HEPES-KOH (pH 7.6), 40 mM β-glycerophosphate, 20 mM NaF, 20 mM p-nitrophenyl phosphate (PNPP), 1 mM dithiothreitol (DTT) 1 mM Na3VO4, 0.1% NP-40, 1 mM phenylmethylsulfonyl fluoride (PMSF), and protease inhibitor cocktail (P8340; Sigma, St Louis, Missouri, USA) diluted 1:500), twice in wash buffer with 2 M urea, twice in kinase buffer (20 mM HEPES-KOH (pH 7.6), 20 mM β-glycerophosphate, 10 mM PNPP, 50 mM NaCl, 2 mM DTT, 0.1 mM Na3VO4, and protease inhibitor cocktail), and once in kinase buffer with 10 mM MgCl2. The assay was performed in 10 μl kinase buffer with MgCl2 and supplemented with 5 μCi [γ-32P]ATP (Amersham Biosciences) and 1 μg GST fusion protein at 30°C for one hour. The reactions were separated by electrophoresis and blotted onto a PVDF membrane. Activity was visualised on a phosphor-imager (FujiFilm, Stockholm, Sweden).

Western blot analysis

Western blotting was carried out as previously described. Antibodies used in this analysis were: anti IKKa (C-20) from Santa Cruz Biotechnology and anti-IKKβ from BD Pharmingen.

Preparation of cytosolic and nuclear extracts

Six fresh biopsies were gently homogenised in ice cold 500 μl buffer H (10 mM HEPES-KOH (pH 7.9), 10 mM KCl, 0.1 mM ethylenediaminetetra-acetic acid (EDTA), 0.1 mM ethylene glycol-bis-(2-aminoethylether) tetra-acetic acid (EGTA), 0.75 mM spermidine, 0.15 mM spermine, 1 mM DTT, 1 mM PMSF, and protease inhibitor cocktail diluted 1:500) in a Dounce homogeniser with a B-type pestle. Then, 5 μl 10% NP-40 was added and the homogenate was allowed to stand for 10 minutes on ice followed by two gentle strokes with the B-type pestle. The homogenate was transferred to a microfuge tube, underlaid with 400 μl sucrose, and centrifuged at 1200 g at 4°C for 10 minutes in a swinging bucket rotor. After centrifugation, the upper (cytosolic) phase was transferred to a new tube, frozen in liquid nitrogen in small aliquots, and stored at -80°C until required for analysis. The nuclear pellet was resuspended in 400 μl buffer B (20 mM HEPES-KOH (pH 7.6), 20% (v/v) glycerol, 10% (v/v) sucrose, 420 mM KCl, 5 mM MgCl2, 0.1 mM EDTA, 1 mM DTT, 1 mM PMSF, and protease inhibitor cocktail 1:500) and incubated for 24 hours on an end over end rotor in a cold room followed by centrifugation for 30 minutes at 20 000 g. The nuclear extract was then dialysed overnight against buffer D (20 mM HEPES-KOH (pH 7.9), 20% (v/v) glycerol, 0.2 mM EDTA, 0.1 M KCl, 0.5 mM PMSF, and 1 mM DTT). The formed precipitate was removed by centrifugation and the extract was concentrated by centrifugation through an Ultrafree-0.5 centriﬁfuge tube (Millipore, Bedford, Massachusetts, USA) with a 5K cut off membrane until the sample volume was reduced to approximately 50 μl. Extracts were frozen in small aliquots in liquid nitrogen and stored at -80°C until required. Protein concentration was determined by the Bradford method (Bio-Rad, Hercules, California, USA) using bovine serum albumin (BSA) as a reference.

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was performed by incubating 5 μg nuclear extract with 1 μg sonicated poly (dI:dC) (Amersham Bioscience), 1.5 μl mobility shift buffer
(100 mM HEPES-KOH, pH 7.9, 600 mM KCl, 40 mM MgCl₂, 1 mM EDTA, 1 mg/ml BSA, and 2.5 mM DTT), 1.5 μl glycerol, and water to a final volume of 14 μl, followed by addition of 5 fmol ³²P labelled double stranded oligonucleotide (specific activity 15 000 cpm/fmol) corresponding to the Ig-kB promoter (5'-AGC TTC AGG GGG TCT CCG AGA GGT CGA-3'). Samples were incubated at room temperature for 30 minutes and loaded on a pre-run 5% acrylamide gel containing 45 mM Tris borate and 1 mM EDTA (0.5×TBE), and run for 25 minutes at 200 V in 0.5×TBE. Then the gel was fixed in 10% acetic acid/20% methanol/70% water for 15 minutes, dried under vacuum on a piece of Whatman 3 MM paper (Whatman International, Kent, UK), exposed to a phosphor-imaging plate for at least 12 hours, and analysed on a phosphor-imager (FujiFilm). For supershift experiments, extracts were incubated with 2 μg antibody overnight at 4°C prior to addition of labelled probe.

**Chromatin immunoprecipitation**

This method was based on a protocol from Peggy Farnham’s Laboratory[1] (http://genomcenter.ucdavis.edu/farnham/farnham). Two colonic mucosal biopsies were collected in PBS with 1% formaldehyde and allowed to stand at room temperature for 15 minutes before cross linking was stopped by addition of glycine to a final concentration of 0.125 M. Then the biopsies were washed in ice cold PBS with 1 mM PMSF and inhibitor cocktail (diluted 1:500) and transferred to a small Dounce homogeniser. Homogenisation was done with a B-type pestle and cells were swelled in lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP40, 1 mM PMSF, and inhibitor cocktail) for 10–15 minutes followed by two strokes with the pestle. The nucleus was isolated by centrifugation, resuspended in nuclear lysis buffer (5 mM PIPES pH 8.0, 10 mM EDTA, 1% sodium dodecyl sulphate (SDS), 1 mM PMSF, and inhibitor cocktail) and left in ice for 30 minutes. Chromatin was sheared by sonication to an average length of 1000–500 bp and centrifuged at 15 000 rpm for 10 minutes. The chromatin solution was precleared with addition of Staphylococcus aureus Cowan 1 (SAC) for 15 minutes at 4°C. Before use, SAC was blocked with 1 μg/ml sheared herring sperm DNA and 1 μg/ml BSA and incubated overnight at 4°C. The precleared chromatin sample was split into two, diluted 10 times with IP dilution buffer (16.7 mM Tris-Cl pH 8.0, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton×100, and 0.01% SDS) and incubated overnight at 4°C with 2 μl anti-p65 (#SA-238; Biomol, Plymouth Meeting, Pennsylvania, USA), or with no antibody, respectively. A third sample containing only IP dilution buffer and antibody was also included. The next day, antibody was precipitated by addition of SAC for one hour followed by centrifugation. The supernatant from the sample with no antibody was set aside for total input assessment. Samples were then washed twice in cold dialysis buffer (50 mM Tris-Cl pH 8.0, 2 mM EDTA, and 0.2% Sarkosyl) and five times in cold IP wash buffer (100 mM Tris-Cl pH 9.0, 500 mM LiCl, 1% NP-40, and 1% deoxycholic acid). Thereafter, the samples were eluted from the SAC by incubation twice in 150 μl elution buffer (50 mM NaHCO₃, 1% SDS) for 15 minutes. The eluates were collected into a 1% agarose gel containing 2.2 M formaldehyde in 3-Morpholino-propane-sulfonic acid (MOPS) electrophoresis buffer (20 mM MOPS (pH 7.0), 2 mM Na-acetate, and 1 mM EDTA (pH 8.0)). Gene expression profiling was done as described by the manufacturer using the GEArray Q Series Human NFκB Signaling Pathway Gene Array (SuperArray Bioscience Corp, www.superarray.com) and 1.5 μg total RNA. Images were recorded with FujiFilm LAS-1000 equipment and quantification of spots was done using FujiFilm ImageGauge v 4.0 software. Data were normalised to the mean of all housekeeping genes provided on the array.

**Double labelled immunohistochemistry**

A phospho specific antibody, phospho-NFkB p65(Ser276) (#3037; Cell Signalling Technology, Beverly, Massachusetts, USA) was employed to distinguish active from inactive NFkB. Antibodies against CD68 (a macrophage marker) and vimentin (a fibroblast marker) were purchased from DakoCytomation (Glostrup, Denmark). Parallel sections of formalin fixed and paraffin embedded tissue samples (5 μm) were dewaxed in Tissue-Clear (Sakura Finetek Europe, The Netherlands). DNA binding activity in nuclear extracts of colonic mucosal biopsies from patients with uninflamed bowel, collagenous colitis, or ulcerative colitis. Nuclear extracts were incubated with a ³²P labelled kB oligonucleotide probe and subjected to electrophoretic mobility shift assay (EMSA). Lanes 1, 2: patients with uninflamed bowel; lanes 3–5: patients with collagenous colitis; lanes 6, 7: patients with mild and severe ulcerative colitis, respectively. A representative result of two experiments is shown. (B) Nuclear extracts of colonic mucosal biopsies from patients with active ulcerative colitis or collagenous colitis were incubated with antibodies prior to EMSA analysis. An asterisk (*) denotes supershifted bands. A representative result of two experiments is shown.

![Image 1](http://gut.bmj.com/)

Figure 1 (A) Nuclear factor kB (NFkB) DNA binding activity in nuclear extracts of colonic mucosal biopsies from patients with uninflamed bowel, collagenous colitis, or ulcerative colitis. Nuclear extracts were incubated with a ³²P labelled kB oligonucleotide probe and subjected to electrophoretic mobility shift assay (EMSA). Lanes 1, 2: patients with uninflamed bowel; lanes 3–5: patients with collagenous colitis; lanes 6, 7: patients with mild and severe ulcerative colitis, respectively. A representative result of two experiments is shown. (B) Nuclear extracts of colonic mucosal biopsies from patients with active ulcerative colitis or collagenous colitis were incubated with antibodies prior to EMSA analysis. An asterisk (*) denotes supershifted bands. A representative result of two experiments is shown.
The specificity of phosphorylation was determined by comparing reactions with wild-type (WT) or mutant (MUT) substrates, in which serine 866 and 870 (A) or serine 32 and 36 (B) were replaced by alanine. The reaction mixture was resolved on an acrylamide gel, transferred to a polyvinylidene difluoride membrane and subjected to phosphorimager analysis. The membrane was subsequently probed with antibody against IKKα or IKKβ as a loading control using western blot analysis (WB). A representative result of six assays is shown.

**Statistics**

Categorical data were analysed by χ² test and continuous data by one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Prior to ANOVA, Bartlett’s test was performed to test for equal variances. If positive, data were log transformed and if unequal variances persisted the final analysis was done by Kruskal-Wallis test. A p value of <0.05 (two tailed) was considered significant.

**RESULTS**

**DNA binding activity of NFκB is enhanced in collagenous and ulcerative colitis**

DNA binding activity in nuclear extracts of colonic mucosal biopsies from patients with uninflamed bowel showed weak binding activity (fig 1A, lanes 1 and 2) while strong binding activity was observed in all patients with collagenous colitis (fig 1A, lanes 3–5) and relapsing ulcerative colitis (fig 1A, lanes 6 and 7).

**Patterns of activated NFκB subunits are similar in collagenous and ulcerative colitis**

Supershift analysis using antibodies specific for NFκB revealed activation of a similar subset of NFκB subunits in collagenous and ulcerative colitis, including p65, p50, and p52 (fig 1B).

**IKKβ is activated both in collagenous and ulcerative colitis in the absence of IKKα activation**

As illustrated in fig 2A, the intensity of bands in wild-type substrates and mutated substrates, respectively, showed no specific IKKβ activity. In contrast, IKKβ was equally activated in patients with collagenous colitis and relapsing ulcerative colitis (p<0.05 compared with uninflamed bowel; fig 2B).

**NFκB binds to the iNOS promoter in vivo in collagenous and ulcerative colitis**

In biopsies from all patients with collagenous and ulcerative colitis, chromatin immunoprecipitation analysis showed that NFκB p65 was recruited to an NFκB regulatory element 5808 bp upstream of the transcriptional start site of the iNOS gene but not to an irrelevant region (fig 3). No recruitment was observed in uninflamed bowel.

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**Figure 2** In vitro IκB kinase (IKK) activities in cytosolic extracts of colonic mucosal biopsies from patients with uninflamed bowel (C), active collagenous colitis (COC), or ulcerative colitis (UC). [A, B] IKKα and IKKβ activities determined by immune complex kinase assay (KA) using cytosolic extracts of colonic mucosal biopsies incubated with glutathione S-transferase (GST)-p100 (754–900) (A) or GST-IκBα (1–54) (B) substrate and radioactive ATP. The specificity of phosphorylation was determined by comparing reactions with wild-type (WT) or mutant (MUT) substrates, in which serine 866 and 870 (A) or serine 32 and 36 (B) were replaced by alanine. The reaction mixture was resolved on an acrylamide gel, transferred to a polyvinylidene difluoride membrane and subjected to phosphorimager analysis. The membrane was subsequently probed with antibody against IKKα or IKKβ as a loading control using western blot analysis (WB). A representative result of six assays is shown.

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**Figure 3** Chromatin immunoprecipitation assay of p65 binding to a nuclear factor κB (NFκB) regulatory region within the inducible nitric oxide synthase (iNOS) promoter in nuclear extracts of colonic mucosal biopsies from patients with uninflamed bowel (C; n=2), collagenous colitis (COC; n=2), or active ulcerative colitis (UC; n=2). Soluble chromatin was prepared from formaldehyde cross linked sonicated biopsies. Specific antibody against p65 was used to precipitate protein bound DNA fragments which were subsequently amplified by polymerase chain reaction using primers flanking an NFκB regulatory region (–5979 to –5779; primer pair A) or a non-regulatory region (–2741 to –2534; primer pair B). Total input refers to amplification of 1% of the total amount of DNA prior to immunoprecipitation.
Transcriptional activity of NFκB occurs both in collagenous and ulcerative colitis

The transcriptional status of 96 selected NFκB dependent genes was assessed using a microarray expression profiling array which showed statistically significant differences in mRNA expression between biopsies from patients with collagenous and ulcerative colitis compared with patients with uninflamed bowel. As illustrated in table 1, six genes were significantly upregulated or downregulated in collagenous colitis while 10 genes were upregulated or downregulated in ulcerative colitis compared with uninflamed bowel. Three of these genes (iNOS, complement factor B, and IL-1α) were upregulated in both groups of colitis patients, as illustrated in fig 4 using iNOS as an example.

NFκB translocation occurs both in collagenous and ulcerative colitis but is limited to the epithelium in collagenous colitis

In biopsies from uninflamed bowel, NFκB staining (red) was negative (0) or showed weak but distinct staining in less than 10% of epithelial cells (+) (fig 5A, B; table 2). No stromal cells

Figure 4  Expression of inducible nitric oxide synthase (iNOS) mRNA in biopsies from colonic mucosa of patients with uninflamed bowel, collagenous colitis, or active ulcerative colitis determined by a microarray system and quantified by densitometry. Data were normalised by dividing individual densitometric values with the mean value of all housekeeping genes provided with the array. The table shows mRNA expression from normalised data for each gene.

<table>
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<th>Gene name</th>
<th>Median Uninflamed bowel (n = 7)</th>
<th>Median Collagenous colitis (n = 8)</th>
<th>Median Ulcerative colitis (n = 6)</th>
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<td>91.5† (62–145)</td>
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*p<0.05 by one way ANOVA and Tukey’s test compared with uninflamed bowel.

Data were normalised by dividing individual densitometric values with the mean value of all housekeeping genes provided with the array. The table shows mRNA expression from normalised data for each gene. Data were normalised by dividing individual densitometric values with the mean value of all housekeeping genes provided with the array. The table shows mRNA expression from normalised data for each gene. **Table 1** Multiple mRNA expression of nuclear factor κB dependent genes in biopsies of colonic mucosa from patients with collagenous colitis, active ulcerative colitis, or uninflamed bowel.

**Figure 5** Double immunohistochemical detection of activated nuclear factor κB (NFκB) and cellular markers for macrophages (CD68) in paraffin embedded formalin fixed biopsies from patients with uninflamed bowel (A, B), collagenous colitis (C, D), or ulcerative colitis (E, F). Negative NFκB staining (A) or weak but distinct focal staining of epithelial cells (B; arrows; red staining) was seen in uninflamed bowel. In collagenous colitis, NFκB nuclear staining was predominantly seen in the epithelium (C, D; arrows) while CD68 positive macrophages (C, D; arrowheads; blue staining) and CD68 negative stromal cells were left unstained. In ulcerative colitis, epithelial cells (E, F; arrows), CD68 positive macrophages (E, F; arrowheads), and CD68 negative stromal cells (E; broken arrows) showed intense nuclear expression of NFκB. Magnification ×100 (A–E) and ×150 (F).
showed positive NFκB staining. In biopsies from patients with collagenous colitis, diffuse NFκB staining was observed in superficial epithelial cells (+++) while no, or sporadic (<1%), macrophages were observed (0) (fig 5, table 2). In biopsies from patients with ulcerative colitis, NFκB staining was observed both in epithelial cells (+) and macrophages (++) (as well as CD68 negative stromal cells ++) (fig 5E, F; table 2). Fibroblasts (vimentin positive stromal cells) were negatively stained for NFκB activation (0) in uninfamed bowel, collagenous colitis, and ulcerative colitis (table 2).

**DISCUSSION**

NFκB activation in the colonic mucosa from patients with ulcerative colitis has previously been reported. The results of the present study demonstrate that NFκB is activated both in collagenous and ulcerative colitis compared with uninfamed bowel, as judged by EMSA, chromatin immunoprecipitation, and immunohistochemistry. The results of 1b kinase assays revealed that IKKβ, but not IKKα, was activated, while gene expression profiling showed markedly increased expression of iNOS and several other NFκB dependent genes. We could not demonstrate any differences between collagenous and ulcerative colitis as regards IKK activity, activation of NFκB subunits, recruitment of NFκB to the iNOS promoter, or activation of NFκB in epithelial cells. In contrast, macrophages with activated NFκB were observed only in colonic mucosa from patients with ulcerative colitis.

Supershift analysis showed DNA binding activity of three different NFκB subunits both in collagenous and ulcerative colitis—namely, p65, NFκB1 p50, and NFκB2 p52. The presence of p65 and p50 acords with our findings of IKKβ activity in both inflammatory conditions whereas the presence of NFκB2 p52 would imply activity of IKKα also. Although IKKα was present in the mucosa, as judged by immunoblotting, there was no detectable activity in our extracts (fig 2A). Recently, it was reported that IKKα translocates into the nucleus on activation and phosphorylates histone H3 at serine 10, which is a requirement for induction of immediate-early genes. If only a small fraction of active IKKα is present in the cytosolic compartment, the observed discrepancy might be explained by the absence of nuclear proteins in our IKK assays, which were performed on cytosolic extracts. Experiments on whole cell extracts should therefore be performed to clarify this issue.

Colonic mucosal biopsies consist of a variety of cell types, including epithelial cells and immune cells. Although iNOS is localised primarily to the epithelium in collagenous colitis, the observed NFκB DNA binding activity is not necessarily associated with iNOS expression. Thus NFκB might be active in one cell type while another cell type is the source of iNOS upregulation. At any rate, our demonstration of NFκB p65 binding to the iNOS promoter in vivo indicates that NFκB is the transcriptional activator of iNOS in the epithelium of patients with collagenous colitis.

The observation of NFκB binding to DNA is insufficient for demonstration of transcriptional activity. Numerous layers of control mechanisms, such as phosphorylation and acetylation of p65 and histones, determine the transcriptional activity of NFκB. To exclude the possibility that NFκB is transcriptionally silent in collagenous colitis, we performed gene expression profiling on a subset of NFκB dependent genes, including iNOS, using a microarray system. Of 96 genes tested, only a few were significantly upregulated in patients with colitis but minor differences in expression pattern between collagenous and ulcerative colitis were observed, most likely reflecting the fact that NFκB was activated in different cell types. As the genes upregulated are NFκB dependent genes, it seems likely that NFκB is indeed transcriptionally active both in collagenous and ulcerative colitis.

As demonstration of in vivo p65 binding to the iNOS promoter in biopsy specimens from collagenous colitis provides no direct evidence of the cell types involved, we used double labelled immunohistochemical detection of activated NFκB and cellular markers for macrophages and fibroblasts (fig 5, table 2). These data showed activation of NFκB both in collagenous and ulcerative colitis compared with uninfamed bowel. In collagenous colitis, NFκB activation was located in epithelial cells. In contrast, activation of NFκB in ulcerative colitis was demonstrated both in macrophages and CD68 negative stromal cells which were not fibroblasts, in addition to epithelial cells. This discrepancy suggests that activation of NFκB in epithelial cells does not lead to mucosal damage in itself. Instead, activation of NFκB in macrophages may be a prerequisite of injury in colonic inflammation. Clearly, further studies are needed to draw safe conclusions.

In summary, our results demonstrate activation of NFκB via an IKKβ mediated pathway, recruitment of NFκB to the iNOS promoter in vivo, and expression of NFκB dependent genes in colonic mucosal biopsies from patients with collagenous and ulcerative colitis. In collagenous colitis, activated NFκB was observed only in epithelial cells while lamina propria macrophages showed activated NFκB in ulcerative colitis. As collagenous colitis is never associated with tissue injury, our findings challenge the prevailing view that activation of NFκB per se mediates mucosal damage in colonic inflammation. Instead, the downstream inflammatory reactions are more likely to be determined by the cellular origin of NFκB activation.

**ACKNOWLEDGEMENTS**

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**References**


EDITOR’S QUIZ: GI SNAPSHOT

Answer
From question on page 468

Figure 1 reveals a chronic, giant, high lesser curve ulcer with clots, and a rosette-like mucosal island. This gastrocolic fistula “stoma” was intubated at initial gastroscopy (fig 2A, 2B). Biopsy excluded malignancy. Figure 1B shows the ulcer but the fistula is incompletely visualised.

The patient was given acid suppression and nasojejunal feeding, prior to limited resectional surgery. Histology revealed no malignancy. He was discharged on day 7 and remains well three months later.

Gastrocolic fistula is an unusual complication of peptic ulceration. It must be suspected, especially in young females on ulcerogenic agents, with halitosis, feculent vomiting, postprandial diarrhoea, with or without dyspepsia or weight loss. The ulcer is usually situated on the greater curve. We postulate that this ulcer was caused by its location on the lesser curve. Further study of its aetiology may reveal the mechanism of this unusual complication.


