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SPECIAL REPORT

Effects of steroid treatment on activation of nuclear factor κB in patients with inflammatory bowel disease

[3]E. Ardite, [1,4]J. Panés, [3]M. Miranda, [1]A. Salas, [1]J.I. Elizalde, [1]M. Sans, [2]Y. Arce, [1]J.M. Bordas, [3]J.C. Fernández-Checa & [1]J.M. Piqué

[1]Gastroenterology and [2]Pathology Departments, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, and [3]Consejo Superior Investigaciones Científicas, Barcelona, Spain

Nuclear factor κB (NFκB) is a transcription factor that controls several genes important for immunity and inflammation. The aim of this study was to assess if activation of NFκB plays a role in the pathogenesis of inflammatory bowel disease (IBD), and whether steroid treatment affects NFκB activation. Activation of NFκB was analysed in colon biopsy samples of 13 patients with active IBD (8 Crohn's colitis, 5 ulcerative colitis) by electrophoretic mobility-shift assays, under basal conditions and 3 weeks after treatment with 0.75 mg kg^{-1} day^{-1} prednisolone. The presence of interleukin-8 mRNA in biopsies was assessed by RT–PCR. A specific NFκB band was present in all nuclear extracts from inflamed mucosa, whereas the band was barely detectable in uninflamed colonic mucosa. NFκB bands were super-shifted by antibodies against p50 subunit, whereas antibodies against p65, p52, c-Rel, or Rel B did not modify the mobility of the band. Increased interleukin-8 mRNA was detected at the same sites of NFκB activation. Steroid-induced healing of colonic inflammation was associated with disappearance of NFκB from nuclear extracts. These results support the notion that NFκB plays an important role in the pathogenesis of IBD, and that blockade of NFκB activation is one of the mechanisms by which steroids suppress the inflammatory cascade in IBD.

Keywords: Inflammation; inflammatory bowel disease; Crohn's disease; ulcerative colitis; nuclear factor κB; interleukin-8; steroids

Introduction Studies aimed at clarifying the pathogenesis of inflammatory bowel disease (IBD) have documented activation of many different gene products which have κB elements in their promoter region. Compounds which block nuclear factor (NF) κB activation (Conner *et al*., 1996), or an antisense oligonucleotide against the translation site of NFκB (Neurath *et al*., 1996), have protective effects in animal model of colitis, suggesting that blockade of this transcription factor may represent a valuable therapeutic strategy for human IBD. Indeed, glucocorticoids, which have proved highly effective in treatment of active IBD, can prevent migration of activated NFκB into the cell nucleus and binding to DNA (Auphan *et al*., 1995; Scheinmann *et al*., 1995).

The aims of the present study were: (1) to assess whether NFκB activation correlates with the site of inflammatory activity in patients with IBD, (2) to characterize which members of the NFκB family are activated, and (3) to assess if steroid treatment modifies NFκB activation in IBD, and whether this modification correlates with the clinical response.

Methods Thirteen adult patients with active IBD (8 Crohn's colitis and 5 ulcerative colitis) under no medication were studied. All patients gave their informed consent, after approval of the project by the local ethical committee. At diagnostic colonoscopy twelve biopsy samples were obtained from inflamed areas, and from endoscopically normal mucosa that were used to assess histology, NFκB activation by electrophoretic mobility-shift assay (EMSA) following a previously described method (Schreiber *et al*., 1989). Expression of interleukin-8 (IL-8) mRNA was determined by RT–PCR (Mukaida *et al*., 1994) using 500 ng of total RNA. Cycling conditions were 45 min at 48°C, 27 cycles of 94°C 30 s, 55°C 1 min, and 68°C 1 min, followed by 68°C 7 min. Coamplification reactions contained 13 pmol each of primers for IL-8 and S14 mRNA designed according to the human IL-8 cDNA and human S14 ribosomal protein as a control (Foley *et al*., 1993). The amplified products were subjected to electrophoresis on 3% agarose. Autoradiographic signals were quantified with a densitometer. Specimens for NFκB and RT–PCR analysis were snap-frozen and stored in liquid nitrogen until use. Colonoscopy was repeated after 3 weeks treatment with prednisolone 0.75 mg kg^{-1} day^{-1} and a new set of biopsies was taken from the same locations.

Results EMSAs revealed a strong single retarded band bound to κB oligonucleotide in nuclear extracts from all samples derived from endoscopically and histologically affected mucosa but not in nuclear extracts from normal mucosa (Figure 1a). Densitometry measurements of intensity of NFκB activation expressed as fold activation of NFκB in inflamed areas relative to the activity in histologically normal mucosa was 4.33 ± 0.46 in ulcerative colitis and 5.0 ± 0.50 in Crohn's disease. Incubation of nuclear extracts with antibody to p50 completely shifted the NFκB-DNA complex to a slower mobility antibody-NFκB-DNA complex. Antibodies against the rest of Rel-family of polypeptides tested (p52, p65, c-Rel and Rel B) did not change the mobility of the band (Figure 1b). A strong IL-8 mRNA band was consistently detected in all areas showing activation of NFκB. In patients with Crohn's disease the densitometry of IL-8 bands relative to S14D was 1.89 ± 0.09 for affected zones and 0.63 ± 0.07 for normal mucosa ($P < 0.01$), and in patients with ulcerative colitis it was 1.43 ± 0.18 for affected zones and 0.32 ± 0.06 for normal mucosa ($P < 0.01$). The binding of

[4]Author for correspondence at: Gastroenterology Department, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain.

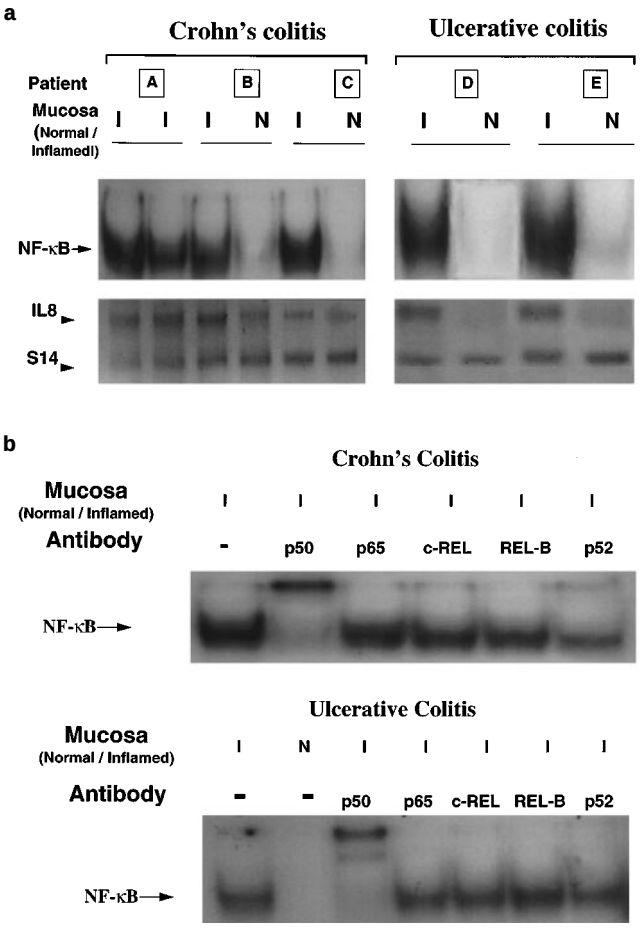

Figure 1 (a) Electromobility shift assays of biopsy samples from three patients with Crohn's colitis (A–C), and two patients with ulcerative colitis (D–E), revealed absence of NF-kB band in endoscopically and histologically normal areas (N), whereas an activated band was uniformly present in areas of inflamed mucosa (I). In patient A, a NF-kB band was also detected in an area of endoscopically normal mucosa in which histological analysis revealed the presence of a moderate inflammatory infiltrate (lane 2). IL-8 messenger RNA was detected in areas of inflamed intestinal mucosa in which NF-kB was activated. (b) NF-kB bands were super-shfited by antibodies against the p50 subunit, but not by antibodies against other subunits.

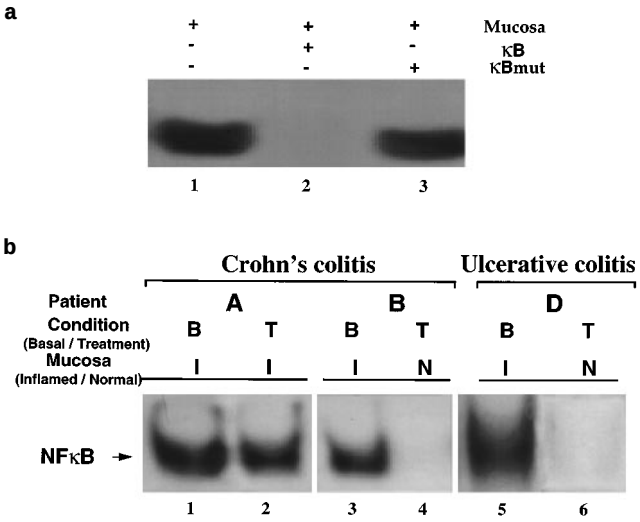

Figure 2 (a) The binding of nuclear proteins to the labelled NF-kB was abolished by an excess of unlabelled oligonucleotide, but not by an excess of unlabelled mutant oligonucleotide. (b) Nuclear extracts from inflamed zones uniformly revealed the presence of NF-kB before treatment (lanes 1, 3, 5). The bands disappeared when lesions healed in response to steroid treatment (lanes 4, 6), but NF-kB remained activated in one case in which inflammatory lesions persisted (lane 2).

nuclear proteins to the labelled NFκB oligonucleotide is sequence specific, since an excess of unlabelled oligonucleotide virtually abolished binding of the labelled probe, whereas a similar molar excess of unlabelled mutant oligonucleotide did not (Figure 2a).

Three weeks after treatment with 0.75 mg kg^{-1} day^{-1} prednisolone, the NFκB band disappeared (1.1 ± 0.2 times normal tissue) in all cases in which intestinal mucosa had healed, judged by endoscopy and histological criteria, whereas an activated band was still present (2.8 times normal tissue) in one patient in which Crohn's lesions persisted (Figure 2b). In this patient a strong IL-8 mRNA band was detected by RT–PCR, whereas it was very weak in the rest of the samples in which NFκB was absent.

Discussion In this paper we present evidence than NFκB is activated in active IBD, this activation was restricted to intestinal areas with inflammatory phenomena, and was associated with activation of IL-8 gene expression. Activation of NFκB is necessary for IL-8 gene activation in all types of cells examined (Mukaida *et al*., 1994), including intestinal epithelial cells in which blockade of NFκB activation inhibits IL-8 gene transcription in response to IL-1β stimulation (Jobin *et al*., 1997). Several members of the NFκB family, including p65, p50, p52, and c-Rel, can bind to the IL-8 promoter region (Stein & Baldwin, 1993). The observation of increased IL-8 mRNA in the same inflammatory sites in which NFκB p50 homodimers were detected strongly suggests that they possess transactivating activity in cells of intestinal mucosa, and is in keeping with results obtained in cultured intestinal epithelial cells infected with enteropathogenic *Escherichia coli* (Hecht & Savkovic, 1997).

In vitro studies suggest that glucocorticoids can inhibit NFκB-DNA binding by complexing with NFκB subunits (Brostjan *et al*., 1996; Unlap & Jope, 1997), or by increasing the rate of IκBα protein synthesis, resulting in sequestration of activated NFκB in inactive cytoplasmic complexes (Auphan *et al*., 1995). In the present study we provide evidence that in the human intestine cessation of the inflammatory activity in response to steroid treatment is associated with the disappearance of NFκB from nuclear extracts of intestinal mucosa and that failure to abrogate NFκB activation results in the persistence of gut inflammation, suggesting that NFκB has a key role in the pathogenesis of inflammation in IBD, and may be a major molecular target for the anti-inflammatory action of glucocorticoids. Steroids may break the cycle in which NFκB activates transcription of proinflammatory cytokines (e.g. tumour necrosis factor-α, IL-1) which in turn further activate NFκB in a variety of intestinal cell types.

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