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Keratinocyte Growth Factor in Inflammatory Bowel Disease

Increased mRNA Transcripts in Ulcerative Colitis Compared with Crohn's Disease in Biopsies and Isolated Mucosal Myofibroblasts

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Inflammation in the gastrointestinal tract is associated with increased epithelial cell proliferation. Keratinocyte growth factor (KGF) is an epithelial cell mitogen widely expressed by mesenchymal cells subjacent to the epithelial cells. In this study, we have investigated the expression and distribution of KGF in normal and diseased (Crohn's disease and ulcerative colitis(UC)) intestine by quantitative competitive reverse-transcriptase polymerase chain reaction in whole biopsies and purified lamina propria myofibroblasts and by *in situ* hybridization. Analysis of whole mucosal biopsies reveals significantly higher numbers of KGF mRNA transcripts in UC compared with Crohn's colitis and control colon ($P < 0.001$). KGF transcripts were also elevated in Crohn's ileitis compared with normal ileum. *In situ* hybridization showed a marked increase in cells expressing KGF mRNA throughout the lamina propria in both UC and Crohn's tissue. In Crohn's disease, positively hybridizing cells were only rarely seen in the submucosa but were abundant around the bases of the crypts and were not associated with lymphoid aggregates. In purified mucosal myofibroblasts, increased (15- to 20-fold) KGF mRNA expression was seen in UC compared with control and Crohn's tissue. These results confirm and extend earlier studies showing that KGF transcripts are elevated in inflammatory bowel disease, but they show for the first time that transcripts are higher in UC than Crohn's disease because of increased production by mucosal mesenchymal cells. ***(Am J Pathol 1997, 151:1469–1476)***

Inflammatory bowel disease (IBD) encompasses two major entities: Crohn's disease (CD) and ulcerative colitis (UC). Both are of unknown etiology and are characterized by chronic inflammation with periods of clinical remission and exacerbation.[1] In both diseases there is a marked infiltration of the mucosa with inflammatory cells, penetrating into the submucosa in Crohn's disease. There are well documented differences between Crohn's disease and ulcerative colitis, because in Crohn's disease there are more features of a Th1-type cell-mediated immune response,[2–4] whereas in UC, the lesion is predominantly neutrophilic and shows more features of antibody-mediated hypersensitivity.[5–7] In either case however, accompanying the chronic inflammation, there is an increase in epithelial cell proliferation, which may remain high even in clinical remission in ulcerative colitis.[8,9] It is considered that the increased epithelial proliferation is a major determinant in the increased incidence of malignancy in long-standing UC,[10] but the relative risk in patients with Crohn's colitis has not been established with certainty.[11–13] However, in general, the relationship between intestinal inflammation and increased epithelial proliferation has not been well characterized at the molecular level.

Keratinocyte growth factor (KGF) is a member of the heparin-binding fibroblast-derived growth factor family.[14] It is predominantly expressed by mesenchymal cells, the KGF receptor is present on epithelial cells,[15,16] and it acts in a paracrine fashion to enhance the proliferation and differentiation of epithelial cells in a wide variety of tissues.[17–19] KGF is markedly up-regulated in the skin during wound healing[20,21] and used therapeutically to accelerate wound healing in porcine skin.[22] Infusion of KGF into rats increases intestinal epithelial cell proliferation.[19] In addition, KGF production is markedly increased

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in fibroblasts by cytokines, such as interleukin-1 and tumor necrosis factor-α,[23,24] which are increased in the mucosa in IBD.[25–27] Therefore, KGF is a highly attractive candidate molecule to explain elevated epithelial cell proliferation in IBD. Previous studies have shown increased KGF mRNA expression in IBD by RNase protection assays and *in situ* hybridization, and it was suggested that the KGF was being made by T cells.[28] Elevated KGF transcripts in IBD have been confirmed in a second study, and KGF protein has also been visualized by immunohistochemistry in cells with stromal cell morphology.[29] No differences have been observed in KGF production between Crohn's disease and ulcerative colitis.

To resolve the issue of whether KGF is made by T cells or stromal cells in IBD and to determine whether Crohn's disease and ulcerative colitis have equivalently increased KGF mRNA production, we have produced a synthetic mRNA standard for KGF to allow competitive quantitative reverse transcriptase polymerase chain reaction (RT-PCR) for KGF transcripts. In addition, we have isolated mucosal myofibroblasts to a high degree of purity from intestinal biopsies and measured KGF transcripts in these cells. A consistent observation in our studies is that KGF transcripts are markedly elevated in UC compared with Crohn's disease and that the increase is also seen in freshly isolated stromal cells.

Materials and Methods

Patients and Tissues

This study received ethical approval from City and Hackney Health Authority. Mucosal biopsies were obtained from 12 patients with histologically proven active CD, 11 patients with active UC, and 11 control patients with no significant histological abnormality. Biopsies were immediately snap-frozen and stored at −70°C. The control patients (six female and five males) ranged in age from 12 to 27 years (median 17 years). The CD patients (seven female and five male) ranged in age from 11.9 to 20 years (median 13.8 years), and the UC patients (four female and seven male) ranged in age from 2.5 to 40.5 years (median 12 years). Of the CD patients, five were on no medication, four were being treated with prednisolone, two with salazyopyrin, and one with metronidazole. For the UC patients, two were untreated, seven were treated with prednisolone, one with sulfasalazine, and one with azathioprine. Control patients were not receiving any medication.

In a second part of the study, biopsies were obtained from six control patients (four female and two male), six UC patients (four male and two female), and six CD patients (3 male and 3 female) for positive selection of mucosal myofibroblasts. The ages of controls ranged between 5.5 and 15.6 years (median 15.6 years), the UC patients between 3 and 53.1 years (median 13 years), and the CD patients between 11.8 and 26 years (median 18 years). Only one of the CD patients and two of the UC patients were being treated with prednisolone.

Finally, *in situ* hybridization was carried out in archival paraffin-blocked full-thickness samples of resection tissue and biopsies from patients with Crohn's disease or UC. Control tissues studied were biopsies taken from two girls and one boy aged 3.8, 12.8, and 13.6 years. For UC, tissue studied was from three males, aged 14.4, 15.4, and 30.9 years. For CD, tissue was studied from two females and a male, aged 14, 26.2, and 23.7 years.

RNA Standard for Quantitative RT-PCR

To facilitate quantitation of KGF mRNA by PCR, we constructed a plasmid that encodes a standard KGF RNA molecule. KGF specific primer-sequences, $5'$-^{654} TCTGTCGAACACAGTGGTACCT^{675}−$3'$ and $5'$-^{919} GTGTGTCCATTTAGCTGATGCAT-^{897} $3'$ were cloned into plasmid pHCQ2,[30] kindly provided by Dr. M.F. Kagnoff, (Department of Medicine, University of California, San Diego). The sequence of the new construct was confirmed by dideoxy sequencing (Pharmacia, St. Albans, UK). To generate standard RNA, the plasmid was linearized with *Hin*dIII and transcribed *in vitro* using T7 RNA polymerase under conditions recommended by the supplier (Promega, Southhampton, UK). Using the same primer set, RT-PCR of the standard molecule produces a PCR product of 440 bp, whereas the target tissue yields a 266 bp fragment. The difference in size allows quantitation of the KGF mRNA transcripts.

RNA Isolation and PCR Amplification

Total RNA isolation from whole mucosal biopsies and isolated mesenchymal cells was performed using a monophasic solution of phenol and guanidine thiocyanate (Life Technologies, Paisley, UK), as recommended by the suppliers. Serial 10-fold dilutions of the standard (10 pg to 0.01 fg) were cotranscribed with total cellular RNA (1 to 5 μg) at 42°C for 50 minutes in 20 μl of reaction containing 50 mmol/L Tris, pH 8.3, 75 mmol/L KCl, 3 mmol/L $MgCl_2$, and 3 mmol/L DTT, using 200 units of reverse-transcriptase (Superscript II RNaseH -ve RT, Life Technologies), 500 μmol/L dNTP mix and 0.5 μg oligo dT (Pharmacia). The reaction was stopped by heat inactivation. PCR amplification was routinely carried out in 50 μl of reaction volume (10 mmol/L Tris, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 20 pmol of $5'$ and $3'$ primers, and 0.5 U of *Taq* polymerase (Pharmacia). The amplification cycle was as follows: 45 seconds of denaturation at 94°C, 45 seconds of annealing at 58°C, and 75 seconds of extension at 72°C, for 37 cycles. PCR products were analyzed on 1% agarose gels and the bands visualized by ethidium bromide staining. Band intensities were quantitated by densitometry (Seescan 1D gel analysis package v 1.00, Seescan, Cambridge, UK). The ratios of the band intensities of the PCR products from the standard RNA and the target RNA were plotted against the starting number of standard RNA molecules on a double logarithmic scale. In this way, the point at which the starting number of standard RNA transcripts is equal to the starting amount of cellular target RNA

transcripts can be determined. This technique allows us to quantify the number of KGF transcripts in a tissue sample down to as low as 1000 transcripts per μg total RNA.

Semiquantitative RT-PCR was also carried out in some samples. cDNA was prepared from the samples as described above and the product was divided into 2 aliquots. One sample underwent RT-PCR for KGF, as described above. The other underwent PCR for the housekeeping gene GAPDH using the following primers, $5'^{630}$ CTACTGGCGCTGGCAAGGCTGT^{651} 3′ and $5'^{989}$ GCCATGAGGTCCACCACCCTGCTG^{966} 3′. Both reactions underwent 35 cycles of amplification after which the products were analyzed on agarose gels using ethidium bromide.

In Situ *Hybridization*

A 110-bp *Eco*RI/*Pst*I KGF fragment was PCR-amplified from 16-week-old fetal gut tissue. This *Eco*RI/*Pst*I fragment was additionally subcloned into pGEM-3Zf(-) plasmid (Promega) at the appropriate restriction sites, and the sequence of the probe was confirmed by dideoxy sequencing (Pharmacia). After linearization of the plasmid with *Eco*RI or *Hin*dIII enzymes, single-stranded antisense and sense-KGF riboprobes (specific activity 0.8 to 1.7×10^9 disintegrations/minute/μg RNA) were obtained using SP6 or T7 polymerase, using [^{35}S]UTP (800 Ci/mmol; Amersham, Bucks, UK). Histological 4-μm paraffin wax sections were treated as described by Senior et al.[31] Briefly, 1×10^6 cpm of unhydrolyzed probe was hybridized overnight at 55°C to dewaxed sections permeabilized with proteinase K. Posthybridization steps included several large volume washes in a 50% formamide buffer at 55°C to remove unhybridized probe, followed by RNase A treatment to digest imperfectly hybridized molecules and with extensive washing to remove small fragments. The final washes were in 0.5× SSC (1× SSC is 0.15 mol/L NaCl, 0.015 mol/L sodium citrate) at 65°C for 30 minutes, twice. Slides were dehydrated and processed for autoradiography using Ilford K5 emulsion. The slides were processed at 10 and 20 days after exposure at 4°C.

As controls, parallel sections were hybridized to equal-radioactivity amounts of other riboprobes, including the sense-KGF riboprobe (negative control) and β-actin antisense probe (positive control), and exposed for the same time.

Isolation of Myofibroblasts from Biopsies

Single cell suspensions of the biopsies were obtained by initial incubation in Hanks'/EDTA medium to remove the epithelium and subsequent collagenase digestion to isolate lamina propria cells.[32] The resultant cell suspension was incubated with 2D3 monoclonal antibody[33] (culture supernatant diluted 1 in 5) for 30 minutes on ice. The 2D3 antibody is of the IgG_1 isotype and recognizes 140 kd surface antigen on the cells of the pericryptal sheath and stromal cells of the lamina propria but does not react with cardiac or skeletal muscle or fibroblasts. In frozen sections of normal and IBD bowel, there are no $2D3^-$ cells that are α-smooth muscle cell actin$^+$, and no $2D3^+$ cells that are α-smooth muscle cell actin negative (not shown). Therefore, we feel that in the gut mucosa the $2D3^+$ cells are all α-smooth muscle cell actin$^+$. After incubation with the primary antibody at 4°C, the cells were washed with cold phosphate-buffered saline supplemented with 2% fetal bovine serum and then incubated with sheep anti-mouse IgG-coated magnetic beads (Dynabeads, Dynal, Oslo, Norway) by gentle mixing at 4°C for 30 minutes. 2D3 cells were positively selected using a magnetic particle concentrator (Dynal). Positive fractions were snap-frozen in liquid nitrogen and stored at −70°C until later use. Dynabeads were prepared according to manufacturers instructions. To determine the purity of the positively selected cells, aliquots were incubated overnight in tissue culture medium (RPMI 1640 medium and 10% heat-inactivated fetal calf serum) to allow the magnetic beads to detach. The mixture of cells and beads was then resuspended in tissue culture medium and the beads removed on the magnetic particle concentrator. Cytocentrifuge preparations were made of the remaining cells, and these were stained with 2D3 and α-smooth muscle cell actin (Dako, High Wycombe, Bucks, UK) by the immunoperoxidase method as described.[34]

In addition, small aliquots of the initial unselected cell suspensions were used to make cytocentrifuge preparations and stained for 2D3 to determine the frequency of myofibroblasts in cell suspensions from controls and patients with IBD.

Flow Cytometry

Single cell suspensions of lamina propria mononuclear cells (5×10^5) were incubated at 4°C for 30 minutes in 100 μl 2D3 or 100 μl anti-CD3 culture supernatant (UCHT1, Dako). The cells were then washed in cold phosphate-buffered sailine/0.1% azide and incubated in a 1:50 dilution of fluorescein isothiocyanate-conjugated rabbit-anti-mouse IgG (Dako) for an additional 30 minutes. For control cells, the primary antibody staining was omitted. After an additional wash, surface fluorescence was analyzed on a Becton Dickinson FACStar Plus flow cytometer.

Statistical Analysis

All medians and 95% confidence intervals were calculated using the Wilcoxon Method. Comparison of KGF mRNA expression among various groups was made using the Student's *t*-test for parametric data.

Results

KGF Transcripts in Whole Biopsies from Patients with IBD

KGF mRNA transcripts in the control biopsies were generally low in control colon (5 to 10×10^3 transcripts/μg of RNA) and in control ileum (15 to 30×10^3 transcripts/μg

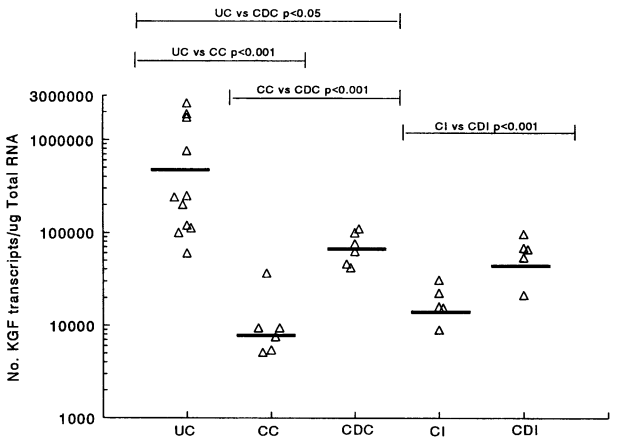

Figure 1. KGF mRNA transcripts in whole biopsies from control colon (**CC**), control ileum (**CI**), ulcerative colitis (**UC**), Crohn's disease of the colon (**CDC**), or Crohn's disease of the ileum (**CDI**). The **bar** represents the median and all values fall within 95% confidence limits.

of RNA). In Crohn's colitis and Crohn's ileitis the number of KGF mRNA transcripts was significantly higher than the control colon and ileum ($P < 0.0001$, Figure 1). In the UC patients, KGF mRNA transcripts were more strikingly up-regulated ($P < 0.0001$, median 4.6×10^5 transcripts/μg of total RNA; 95% confidence intervals, 1×10^5 to 1.3×10^6 transcripts/μg of total RNA) compared with the control group and were also increased compared with Crohn's colitis ($P < 0.03$). Most patients in this study were children with a recent onset of disease and so it was not possible to determine whether KGF transcripts were related to disease duration. However, in the few specimens from older IBD patients we investigated, there was no obvious increase in KGF transcripts compared with the younger patients.

In Situ *Hybridization*

Under the conditions used in this study, KGF mRNA transcripts were barely detectable in control tissue by *in situ* hybridization (Figure 2, A and B). In UC, positive cells were seen scattered throughout the lamina propria, with no obvious association with the subepithelial myofibroblasts (Figure 2, C and D). In CD tissue, positively hybridizing cells were again seen throughout the lamina propria (Figure 2, E and F). The high number of positive cells in the CD sample shown probably reflects the very intense inflammation in that part of the section.[29] There was a tendency for increased numbers of positive cells to be present around the crypt bases. KGF expressing cells were rarely seen in the deeper layers of the gut in CD despite extensive submucosal inflammation. Shown well in the UC specimen, reactivity was often abundant in the lamina propria above the muscularis mucosa but the smooth muscle cells of the muscularis mucosa themselves were negative (Figure 2, C and D). There was no obvious association of KGF mRNA with the frequent loose lymphoid aggregates seen in IBD tissue, although occasionally positive cells were seen at these sites. Likewise, although diseased tissue, especially Crohn's disease, contained abundant intraepithelial lymphocytes, these cells never showed a positive hybridization signal. *In situ* hybridization is a semiquantitative technique and there was no obvious difference in the intensity and number of the positively hybridizing cells between UC and CD. Sections incubated with anti-sense to β-actin showed abundant positive cells throughout the tissue. Sense probes for KGF were invariably negative in both control and IBD patients.

KGF Transcripts in Purified Intestinal Myofibroblasts

Because it is controversial whether the increased KGF transcripts in IBD are T cell derived or mesenchymal cell derived, we decided to investigate KGF transcripts in purified mucosal myofibroblasts. In single cell suspensions of lamina propria cells, flow cytometry revealed that myofibroblasts are as abundant as T cells (Figure 3). In control patients, 27% of the lamina propria cells were $2D3^+$ ($n = 6$); in CD patients, 28% were $2D3^+$ ($n = 4$); and in UC patients, 25% were $2D3^+$ ($n = 6$). Positive selection of $2D3^+$ cells using Dynabeads enriched the percentage of $2D3^+$ cells to 90 to 99% ($n = 9$), and there was an equivalent enrichment for α-smooth muscle $actin^+$ cells.

Quantitative RT-PCR on the purified myofibroblasts demonstrated relatively low numbers of transcripts in control patients (Figure 4). In CD patients, there was an approximate fourfold increase in KGF transcripts ($P < 0.001$; 95% confidence intervals, 2×10^4 to 4.2×10^4 transcripts/μg of total RNA), whereas in UC patients, there was more than a 10-fold increase ($P < 0.001$; 95% confidence intervals, 7.3×10^4 to 1.2×10^5 transcripts/μg of total RNA).

Insufficient cells could be isolated from the biopsies to divide the sample in two and positively select $CD3^+$ cells and $2D3^+$ cells for direct comparison. But to get some notion of how many KGF transcripts were in the residual $2D3^-$ cells (presumably containing the T cells), semiquantitative RT-PCR was used to compare GAPDH and KGF transcripts. In the nine patients (three controls, three CD, and three UC) with whom this was done, no KGF transcripts were seen after 35 cycles, although a strong GAPDH signal was obtained (negative data, not shown). We therefore conclude that any contribution of the $2D3^-$ cells to the elevated KGF transcripts seen in IBD must be minimal.

Discussion

Chronic inflammation and increased epithelial cell proliferation are characteristic features of IBD, suggesting the presence of factors in the inflamed mucosa that promote epithelial cell proliferation. In this study, we have investigated the possible role of KGF in these processes. We have confirmed the up-regulation of KGF mRNA in inflammatory bowel disease as reported by Finch et al[28] and Brauchle et al.[29] The novel findings of this study are that

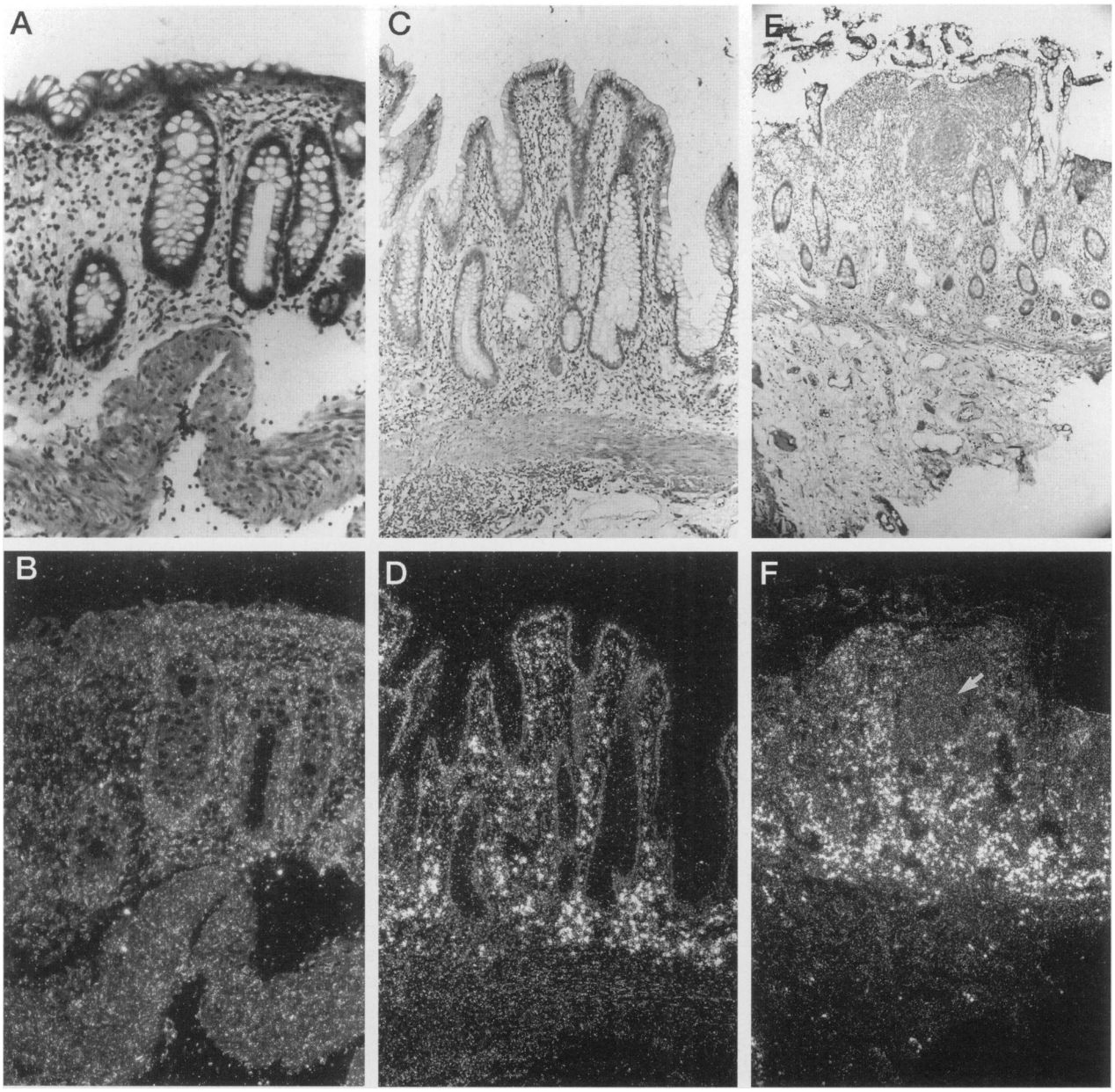

Figure 2. Detection of KGF mRNA by *in situ* hybridization. **A** and **B** show the light and dark field images of a sample of control colon (magnification, ×250). **C** and **D** show light and dark field images of a sample from a patient with ulcerative colitis (magnification, ×100). There is an accumulation of lymphocytes below the muscularis mucosa, but no KGF hybridization signal. **E** and **F** show light and dark field images of a sample from a patient with active Crohn's disease (magnification, ×100). Note the absence of KGF staining around the lymphoid aggregate in the lamina propria (**arrow**) and the relative sparsity of positive cells in the submucosa. All specimens shown were exposed for 20 days.

by using quantitative competitive RT-PCR, we have shown that KGF transcripts are markedly increased in UC compared with Crohn's disease and that this is because of increased numbers of transcripts in mucosal myofibroblasts. The less sensitive technique of *in situ* hybridization revealed no differences between the two diseases.

The first and most important point to be addressed is whether the differences we have observed between Crohn's disease and UC are real or artificial. It is known that KGF transcripts are down-regulated by corticosteroids,[35] so differential steroid use among the groups could explain the differences. However, this was not the case. In the part of the study in which we evaluated KGF transcripts in whole biopsies, steroid use was actually greater in the UC patients (7 of 11) compared with the CD patients (4 of 12), and there was no difference in the dosage. Alternatively, the mucosal lesion in UC is typically continuous, whereas in Crohn's disease, it is often described as patchy. Thus, there is the possibility that, in Crohn's disease, only a part of the tissue was inflamed despite biopsies being taken from endoscopically inflamed areas in both conditions, whereas in UC, it was uniformly inflamed. We consider that this is unlikely for several reasons. First, many of the patients with Crohn's

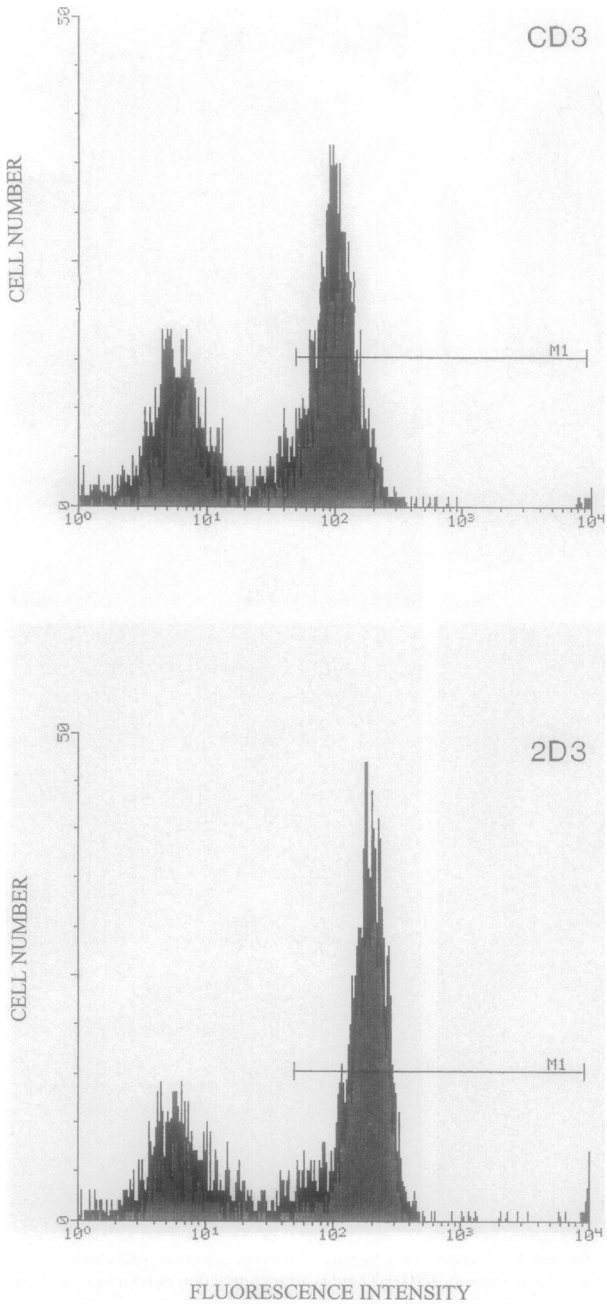

Figure 3. Flow cytometric analysis of isolated lamina propria mononuclear cells from a normal patient stained for CD3 (T cells) or 2D3 (myofibroblasts). 10,000 events were counted. **Horizontal bars** indicate the zones where cells were considered to show positive fluorescence.

disease had severe inflammation, and the other biopsies taken for histology showed inflammation throughout the tissues. Second, although the Crohn's lesion is often described as patchy, this does not mean that intervening areas are normal. In fact, there is very good evidence that in Crohn's disease there is panenteric inflammation.[36,37] Third, the segregation of the groups was clear cut and, whether patchiness was the reason for the low KGF transcripts in Crohn's disease, it is highly unlikely that in a small pinch biopsy all of the samples contained patchy inflammation.

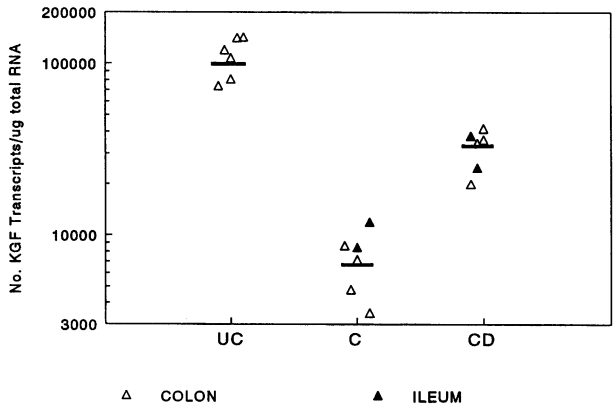

Figure 4. Measurement of KGF mRNA transcripts in purified myofibroblasts from control biopsies and biopsies from patients with active ulcerative colitis or Crohn's disease. The UC samples were all from colonic biopsies, the CD patients and controls each comprised four colonic biopsies and two ileal biopsies. The **bar** represents the median and all values fall within 95% confidence limits.

It is difficult to explain these differences between CD and UC mechanistically, given the reasonable assumption that the increased transcripts seen in both UC and CD reflect local stimulation by cytokines such as IL-1β and tumor necrosis factor-α. In Crohn's disease and UC, there is no evidence that there are major differences in the local concentrations of those molecules. Cytokine stimulation of KGF mRNA in fibroblasts is because of increased production of transcripts[23] and not because of their increased stability. Thus, one would have to postulate an inherent difference between CD and UC patients in their ability to initiate KGF transcription in response to inflammatory cytokines. Although, because we still know very little about KGF gene regulation, in Crohn's disease, there is the possibility that there is excess production of an unidentified molecule that down-regulates KGF production. Nonetheless, the findings of increased KGF transcripts in UC compared with Crohn's disease, if also reflected in protein secretion, may be extremely relevant to the chronically increased epithelial cell proliferation seen in UC and the known risk of malignancy. It has been reported that expression of the proliferating cell nuclear antigen is significantly greater in the bottom half of intestinal crypts in ulcerative colitis compared with Crohn's disease,[38] although there was considerable overlap among the groups.

The source of the increased KGF transcripts in IBD is somewhat controversial. Finch et al[28] suggested that KGF was T-cell derived on the basis of colocalization of KGF transcripts in the lamina propria and CD3 staining in serial sections. Cells expressing KGF mRNA were also described as having a lymphoid appearance. Brauchle et al,[29] using immunohistochemistry, suggested that KGF in inflamed gut was made by stromal cells. It has been suggested that intraepithelial $\gamma\delta$ T cells make KGF in mice.[39] In the studies reported here, we were not persuaded that the *in situ* hybridization signals were associated with mononuclear cells. Loose lymphoid aggregates in the lamina propria showed no bias in the numbers of positively hybridizing cells. Likewise, in Crohn's disease,

in which there is a transmural T-cell infiltrate with dividing T cells in the submucosa,[40] positively hybridizing cells were almost completely in the mucosa and virtually absent from the submucosa.

Therefore, we developed methodologies to purify mucosal myofibroblasts from intestinal biopsies to answer this question. When this was done, the results seen in the whole biopsies were recapitulated in that markedly increased KGF transcripts were seen in UC compared with Crohn's disease. We were unable to do a direct comparison of purified stromal cells and T cells in the same samples because insufficient cells were available. However, when the $2D3^-$ cells were analyzed by semiquantitative PCR with KGF transcripts being compared with GAPDH transcripts, no KGF transcripts were detectable, although all samples contained GAPDH. This makes it unlikely that the T cells contribute to the increased KGF transcripts in IBD, although there is the possibility that they make KGF at lower levels than stromal cells. Therefore, we consider that these data show that most of the KGF transcripts seen in IBD come from mesenchymal cells and that any contribution from T cells must be quite minor.

The ability to up-regulate KGF production by cytokines in the mesenchymal cells of the gut can be seen as an important part of the effector immune response to intestinal pathogens. KGF-mediated increased proliferation would lead to increased shedding of epithelial cells and pathogens either within or attached to their surface. Likewise, increased proliferation will lead to younger cells on the villus surface with reduced numbers of the cell surface receptors that pathogens use to bind to and enter cells. Once the pathogen is eliminated, the antigenic drive for cytokine production is reduced, and KGF production can return to normal. In IBD, the problem is the chronic nature of the inflammation, although the antigenic stimuli in both CD and UC remains to be identified. Even in clinical remission there is ongoing mucosal inflammation. The mitogenic capacity of KGF, however, can be exploited therapeutically as has been described in promoting wound healing in the skin, inhibiting radiation and bleomycin-induced lung injury,[41] and more recently in reducing the extent of intestinal injury in trinitrobenzenesulfonic acid/ethanol colitis in rats.[42] The recent development of a KGF knockout mouse[43] together with mouse models of inflammatory bowel disease[44] means that the tools are now available to study the role of KGF in gut inflammation *in vivo*.

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