Basic and clinical aspects of mucosal inflammation and healing in Crohn's disease

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Chapter 3

basic Fibroblast Growth Factor (bFGF) as a response parameter to infliximab in fistulizing Crohn's disease

Short title: bFGF in fistulizing CD

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Basic fibroblast growth factor as a response parameter to infliximab in fistulizing Crohn's disease

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SUMMARY

Background: Fibroblast growth factors play an important role in (patho)physiological processes such as wound healing and tissue repair. We previously showed that basic fibroblast growth factor is actively involved in inflammatory bowel disease processes. In the present retrospective study, we assessed whether serum basic fibroblast growth factor levels in Crohn's disease patients reflect the response to anti-tumour necrosis factor-α antibody infliximab treatment.

Aim and methods: Serum samples, biopsies and patient data from a subgroup of patients included in two placebo-controlled trials were used. Fistulizing Crohn's disease patients ($n = 42$) were administered placebo or infliximab intravenously three times and evaluated for response up to 18 weeks. Biopsies from a subgroup of patients were stained for basic fibroblast growth factor using indirect immunohistochemistry. In the active Crohn's disease trial, patients ($n = 24$) received either placebo or infliximab once, and disease activity and serum basic fibroblast growth factor were assessed at weeks 0 and 4.

Results: Basic fibroblast growth factor levels at inclusion were comparable in the fistulizing Crohn's disease patients regardless of whether the fistulas did or did not respond or completely heal (median range: 9.3–10.6 pg/mL). At the end of follow-up basic fibroblast growth factor levels were lower in patients who responded (9.2 pg/mL, $P = 0.06$) or who were completely healed (8.9 pg/mL, $P = 0.009$) when compared with patients did not respond/heal (14.5 pg/mL), the latter not significantly increased from baseline. Decreases in the perianal disease activity index and open fistula scores at the end of the follow-up were significantly correlated with the decrease in basic fibroblast growth factor ($R = 0.41$; $P = 0.012$ and $R = 0.35$; $P = 0.027$, respectively). Immunohistological evaluation also showed a trend towards decreased basic fibroblast growth factor expression in intestinal biopsies of these patients. Patients with active disease, i.e. a Crohn's disease activity index ≥220 combined from the two studies, were found to have significantly ($P = 0.0046$) lower baseline serum basic fibroblast growth factors levels than those with inactive disease (5.3 vs. 10.3 pg/mL, respectively). Treatment of the active disease patients did not affect the serum basic fibroblast growth factor level, although a general decrease in disease activity was observed with infliximab treatment.

Conclusions: Healing of fistulizing/perianal Crohn's disease seems to be reflected by a decrease in high serum basic fibroblast growth factor. Basic fibroblast growth factor levels do not relate with response in active Crohn's disease patients.

INTRODUCTION

Crohn's disease (CD) is characterized by granuloma formation and inflammation, transmural ulcers and intestinal fistulization.[1] The formation of fistulas is the result of the extension of burrowing intestinal ulcers in

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CD; this complication develops in about one-third of CD patients. Surgery is frequently required since fistulas rarely heal spontaneously or close in response to routine therapy such as 5-aminosalicylates or corticosteroids.[2]

Although the precise cause of CD remains unknown, increasingly data suggest that genetic susceptibility, immunological mechanism, microbial agents and environmental factors are involved in the aetiology of inflammatory bowel disease (IBD). A vast number of inflammatory mediators, including cytokines and growth factors, participate in the pathogenic process of intestinal injury and healing in CD.[3–5] The multifunctional proinflammatory cytokine tumour necrosis factor-alpha (TNF-α) plays a pivotal role in the development of CD. TNF-α has been demonstrated to induce or up-regulate the production of not only other inflammatory mediators, e.g. interleukin (IL)-1, -6, -8 and interferon (IFN)-γ, but also adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1. In addition, growth factors, like the epidermal growth factor family (EGF), transforming growth factor-alpha and -beta (TGFα and TGFβ), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGFs), have been shown to play an important role in the pathogenesis of CD.[6]

Basic fibroblast growth factor (bFGF or FGF-2) is a heparin-binding regulatory peptide and a member of a family comprising at least 22 factors with pleiotropic functions.[7] FGFs affect the embryonic development and formation of tissues and organs. In the adult body, FGFs regulate fundamental cellular activities, such as morphogenic events involving epithelium cell survival, apoptosis, interaction of cell–matrix and cell–cell, and cell motility and differentiation. The bFGF is also a potent angiogenic factor in (patho)physiological processes that include wound healing and tissue repair.[8–11] The bFGF has been shown to promote proliferation of endothelial cells, to increase the number of fibroblasts and myofibroblasts, and to activate these fibroblasts.[12, 13] The induction of collagen secretion from IBD fibroblasts by bFGF may be one of the mechanisms relevant to the stromal processes of the disease, including transmural fibrosis and stricture formation, as well as tissue repair and healing.[14] Elevated levels of bFGF in serum and intestinal perfusion fluid have been reported in paediatric and adult patients with IBD.[15–18] Although the significance of the increase of bFGF is not completely understood, the increase of bFGF in endothelial cells seems to be related to stimulation by TNF-α.[19, 20] The application of infliximab, a human-murine chimaeric immunoglobulin (Ig)G1 anti-TNFα antibody, was previously demonstrated to be an effective and important biological therapeutic option for patients with active, refractory CD and for patients with fistulas.[21–23] In a previous study, we found indications that bFGF was actively involved in the processes of IBD inflammation and tissue healing.[24] In the present study, based on the observed reduction of serum bFGF levels that paralleled the closure of fistulas and a decrease in the Perianal Disease Activity Index (PDAI), we describe the role that bFGF may have as a response parameter in the healing of fistulas and perianal disease during the treatment with infliximab.

MATERIALS AND METHODS

In the present study, we included a subgroup of patients from three centres that participated in a multicentre, placebo-controlled trial of infliximab either for the treatment of fistulas in patients with CD or for the treatment of active CD.[21, 23]

Patients

Patients with confirmed Crohn's disease were required to be between 18 and 65 years of age. Assessment of disease severity was made according to the Crohn's Disease Activity Index (CDAI) and the PDAI. For inclusion in the fistula trial, patients had to have single or multiple draining abdominal or perianal fistulas of at least 3 months' duration. Patients with CDAI scores between 220 and 400, were eligible for the study of infliximab in the treatment of active CD. Acceptable concomitant medication and exclusion criteria for the studies were previously described.[21, 23]

Protocol 1: Fistulas

Eligible patients were randomly assigned to receive one of three treatments at weeks 0, 2 and 6: placebo ($n = 14$) or 5 or 10 mg/kg of infliximab intravenously (total $n = 28$). After the first infusion of study medication, patients returned for clinical and laboratory assessments at weeks 2, 6, 10, 14 and 18. Blood samples were drawn at each study visit through week 18.

Efficacy evaluations included the number of patients with a response (defined as a reduction of 50% or more

in the number of draining fistulas from baseline) or with complete healing (defined as the absence of any draining fistulas) at two consecutive visits. Changes in the CDAI and the PDAI were also evaluated.

Protocol 2: Active disease

Patients were randomly assigned to receive a single dose of either placebo ($n = 7$) or 5, 10 or 20 mg/kg of infliximab (total $n = 17$). Disease activity according to the CDAI was assessed and serum samples were collected at weeks 0 and 4.

The demographic and baseline clinical characteristics of both patient groups are summarized in Table 1. No significant differences were observed between placebo and infliximab treatment groups and, in addition, these characteristics were also found to have a comparable distribution with the original studies.[21, 23]

Enzyme-linked immunosorbent assay for bFGF

Serum bFGF levels were determined with the high sensitivity Quantikine enzyme-linked immunosorbent assay (ELISA) kit (HSFB75, R&D System, Inc., Minneapolis, MN, USA). Analyses were performed according to the instructions of the manufacturer. At appropriate serum dilutions the sensitivity of this ELISA was <0.23 pg/mL. The intra- and interassay coefficients of variation were 3.9% and 11.6%, respectively.

Immunohistochemistry

Intestinal biopsy samples from macroscopically non-inflamed areas of the intestine were available from six fistulizing CD patients. Samples were obtained prior to treatment and at 8–12 weeks of following the start of infliximab treatment. Immunohistochemistry for bFGF was performed as follows. The primary antibody was a polyclonal antibody directed against bFGF (code no. sc-79-G; Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). This rabbit affinity-purified antibody to bFGF was raised against the amino-terminal domain of human bFGF. Secondary biotinylated antibodies (code no. E 0432), i.e. goat antirabbit IgG, were purchased from Dako, Denmark. The colour reaction was developed by 3-amino-9-ethylcarbazole in acetate buffer containing H_2O_2. Specificity of the immunohistochemical staining was assessed by neutralization incubation of the primary antibodies with a five- to 10-fold (by weight) excess of blocking peptide (code no. sc-79-P) also obtained from Santa Cruz Biotechnology, Inc., and incubation of preimmune serum or buffer instead of the primary antibody, all showing negative staining.

Table 1. Patient demographic and baseline clinical characteristics

	Fistula study		Active disease study	
	Placebo	Infliximab	Placebo	Infliximab
n	14	28	7	18
Male/female	8/6	9/19	2/5	5/13
Disease duration (years)				
Median	10.7	7.6	6.6	5.2
IQR	4.7–15.5	4.0–13.6	3.5–10.6	2.8–11.4
Location				
Ileum	0	7	3	2
Colon	5	10	2	9
Both	9	11	2	7

IQR, interquartile range.

Statistical analysis

Descriptive statistics such as median and interquartile range are used to summarize study results. Analysis of variance on the van der Waerden normal scores was used to compare groups. Correlations were evaluated by the Spearman rank test for non-parametric data. All *P*-values are two-sided.

RESULTS

Relation between disease activity and serum bFGF

At inclusion patients with active disease, i.e. a CDAI ≥ 220 combined from the two studies, were found to have significantly ($P = 0.0046$) lower baseline serum bFGF levels than patients with inactive disease: 5.3 (3.2–10.0, $n = 31$) vs. 10.3 (5.3–18.8, $n = 26$) pg/mL, respectively. Treatment of active disease patients with infliximab, according to protocol 2, did not affect the serum bFGF levels at week 4, although a general decrease in disease activity was observed (Table 2).

Healing of fistulizing/perianal disease reflected by serum bFGF

On average, the serum bFGF level in fistula patients who received placebo was slightly, but not significantly, increased after 18 weeks follow-up, from 11.4

Parameter:	bFGF*		CDAI	
Treatment:	Pre-treatment	Week 4	Pre-treatment	Week 4
Placebo ($n = 7$)	3.2 (2.7–6.9)†	3.6 (2.4–5.2)	257 (233–348)†	288 (281–298)
Infliximab ($n = 17$)‡	5.2 (3.5–9.0)	4.8 (3.1–10.1)	335 (286–363)	143 (69–222)

* Basic FGF expression in pg/mL.
† Median (interquartile range).
‡ 5–10–20 mg/kg combined.

Table 2. Basic fibroblast growth factor (bFGF) levels and Crohn's Disease Activity Index (CDAI) prior to and following treatment of patients with active Crohn's disease

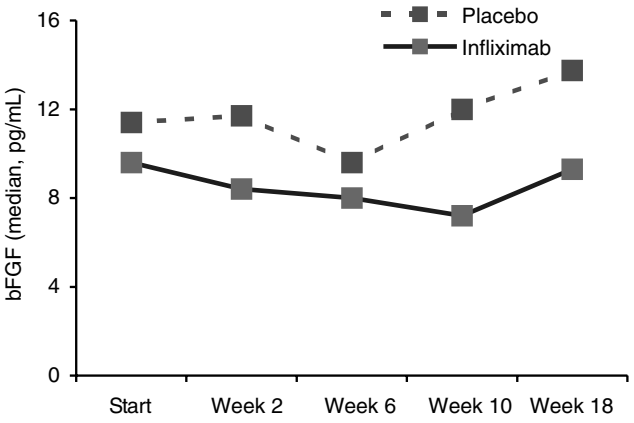

Figure 1. Change in serum basic fibroblast growth factor (bFGF) in patients with fistulas during treatment. Twenty-eight patients were treated with infliximab and 14 patients received placebo.

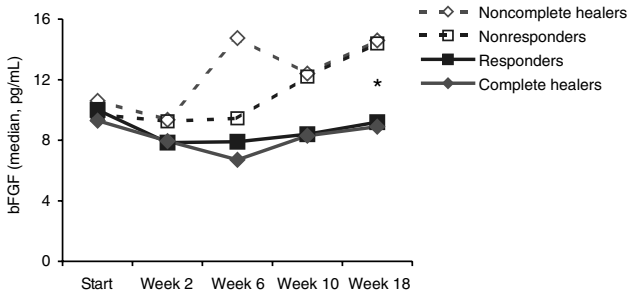

Figure 2. Serum basic fibroblast growth factor (bFGF) levels in patients with fistulas in relation to treatment response. *$P = 0.06$ for responders ($n = 23$) vs. non-responders ($n = 17$); *$P = 0.009$ for complete healers ($n = 19$) vs. non-complete healers ($n = 21$).

(7.0–29.3) pg/mL at inclusion to 13.8 (7.7–17.9) pg/mL at week 18. The patients who received infliximab showed relatively stable levels of bFGF: 9.6 (5.4–13.6) pg/mL at inclusion and 9.3 (5.4–15.5) pg/mL at the end of follow-up. The differences in serum bFGF levels at inclusion and at all follow-up time-points between placebo and infliximab-treated patients were not statistically significant (Figure 1).

Fistulizing CD patients who responded or completely healed had baseline bFGF levels comparable with those observed in non-responders/non-healers at inclusion [median range: 9.3 (5.0–12.8) to 10.6 (8.6–18.8) pg/mL]. At the end of follow-up these levels were found to be lower in the responders/healers [9.2 (4.9–12.6), $P = 0.06$ and 8.9 (4.8–12.4) pg/mL, $P = 0.009$, respectively) compared with the non-responders/non-healers, both with bFGF levels of 14.5 pg/mL (8.6–23.0 and 9.4–23.0; Figure 2). The non-responders/non-healers were found to have increased bFGF levels, although not significantly, compared with their baseline values.

The PDAI in fistulizing patients was found to decrease during infliximab treatment, from 9 at baseline to 5 at week 18. At the same time, the PDAI in patients receiving placebo decreased from 8.5 to 7. At the end of follow-up (week 18), open fistula scores changed by 1.5 to 1 in infliximab-treated patients and 2 to 1 in the placebo group (Table 3). The decrease in PDAI and open fistulas scores was significantly correlated with the decrease in bFGF ($R = 0.41$; $P = 0.012$ and $R = 0.35$; $P = 0.027$, respectively).

Immunohistological results

In colonic biopsies of patients included in the fistula study, immunoreactivity for bFGF was observed in epithelial cells, (myo)fibroblasts and endothelial cells. In some cases monocyte/macrophages and neutrophils were found to be positive as well. In areas with signs of inflammation, the bFGF reaction in the extracellular matrix was relatively intense. A trend towards decreased bFGF expression in intestinal biopsies was observed at the end of follow-up, i.e. 4 sets with a lower, one with the same and one set with a higher overall staining intensity compared with pre-treatment biopsies (Figure 3a–d).

Table 3. Outcome of treatment in Crohn's disease patients with fistulas

	Pre-treatment	Week 18
PDAI, median (IQR)		
Placebo ($n = 14$)	8.5 (7–10)	7 (4–9)
Infliximab ($n = 24$)*	9 (7–10.5)	5 (2.5–9.5)
Fistulas score, median (IQR)		
Placebo ($n = 14$)	2 (1–4)	1 (0–2)
Infliximab ($n = 28$)*	1.5 (1–3)	1 (0–1)

PDAI, Perianal Disease Activity Index; IQR, inter quartile range.
* 5 and 10 mg/kg doses combined.

DISCUSSION

The severe intestinal ulcers of CD can result in the formation of fistulas, one of the major complications of this disease. The healing of gastrointestinal tract ulcers is related to cell migration, proliferation, angiogenesis and re-epithelialization. In the present study, we show that healing of fistulas/perianal disease in CD is correlated with a reduction of serum bFGF concentrations and suggest that the latter may be a potential serological marker for fistula healing. Basic FGF is one of the growth factors that plays a role in the diverse consecutive stages of inflammatory response, fibrosis and stricture formation, and tissue healing.[8, 9, 14] As an important inducer of angiogenesis, bFGF promotes the proliferation, differentiation and migration of endothelial cells, and enhances the quality of ulcer healing by affecting muscle regeneration and proliferation in healing.[25, 26] The bFGF and VEGF can stimulate the formation of granulation connective tissue within ulcers.[27] Basic FGF also affects the proliferation of fibroblasts and epithelial cells and the migration of epithelial cells.[28] All of these bFGF-related processes might contribute to mucosal remodelling and healing.

The source of bFGF in serum has not yet been defined. Basic FGF is expressed in various cell types such as fibroblasts, epithelial cells, endothelial cells, smooth muscle cells and some activated monocytes/macrophages in tissue or in the circulation.[18, 25, 29] Serum bFGF is possibly derived from activated leucocytes in the circulation because the condition of leucocytes is essentially changed in IBD.[30] However, it is more likely that the elevated serum bFGF in fistulizing CD is derived from local inflammatory sites. Thörn *et al.*[16] found that the high concentrations of bFGF in the intestinal perfusion fluid of patients with ulcerative colitis reflected disease activity; however, they did not simultaneously determine serum bFGF levels. In a study of collagenous colitis, Taha *et al.*[31] concluded that the increased colorectal mucosal secretion of bFGF was of significance in the pathology of this colitis but was not reflected in the serum bFGF levels. The results from these studies showed that the high expression of bFGF at local sites was directly related to the intestinal disease process.

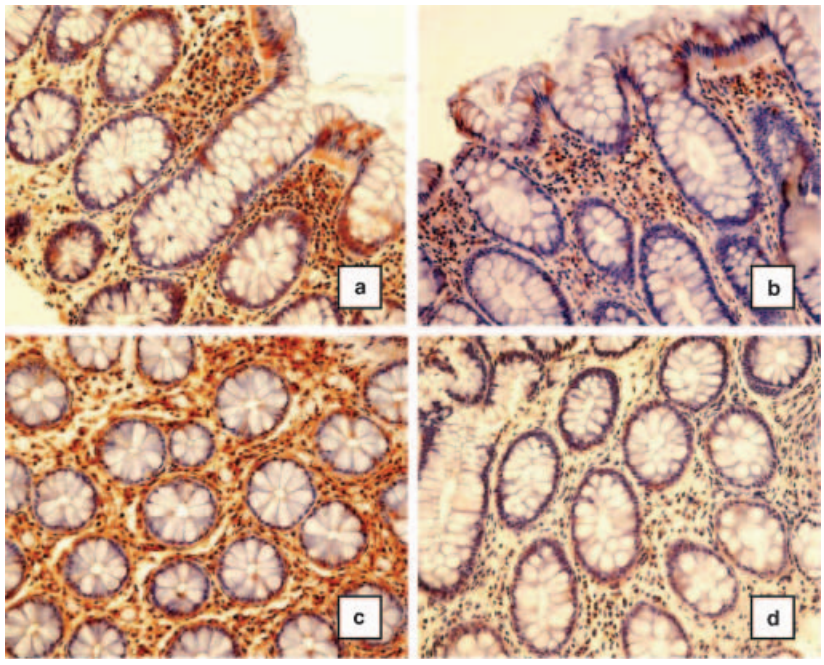

Figure 3. Immunohistochemical staining showed that basic fibroblast growth factor (bFGF) was present in epithelial cells, (myo)fibroblasts, endothelial cells and extracellular matrix. The expression of bFGF in the intestinal mucosa from Crohn's disease (CD) patients with fistulas (a, c) was decreased after 10 (b) or 7 (d) weeks of treatment (magnification: ×200).

In adult patients with IBD, increased serum bFGF levels and endothelial bFGF expression were found; however, whether these elevated bFGF levels reflected the activity of CD disease was not conclusive.[17, 18] In contrast, Bousvaros *et al.*[15] studied children with CD and found a correlation between the serum bFGF and disease activity, although the overall bFGF level was not increased. In this study, no relation was found between bFGF levels and the presence of fistulas. In addition, they did not include follow-up of serum bFGF levels during treatment with an effective therapy.

In the present study, we found significantly higher baseline serum bFGF levels in patients with fistulas/perianal disease than in patients with active CD, and an apparent decrease in serum bFGF after 18 weeks of follow-up in healers and responders. Very recently, Di Sabatino *et al.*[32] reported an almost identical observation to our study, i.e. down-regulation of bFGF levels in CD patients who responded to treatment with infliximab. Furthermore, evaluation of bFGF levels and efficacy of infliximab in the 'Expanded Access Program for Anti-TNF Chimeric Monoclonal Antibody in the Treatment of Crohn's Disease'[33] we found again a decrease in serum bFGF of responding patients with fistulizing disease (unpublished observations). These independent studies indicate that a decrease in serum bFGF upon treatment with infliximab strongly relates to therapy response, whereas absence of a decrease, or even an increase, in serum bFGF indicates non-reponse. The immunohistochemical results in the present study showed that bFGF seems to be reduced in gut tissue of CD patients with fistulas at the end of the treatment period. Apparently, at the local intestinal sites fibroblasts, endothelial cells and leucocytes in patients with fistulas/perianal disease express more bFGF, probably under the stimulation of inflammatory mediators, the activation of the stromal component of the disease process, and the demand of local tissue wound repair. Accompanying the closure of fistulas and alleviation of inflammation, the concentration of bFGF in the intestinal tissue cells was reduced. In addition, serum bFGF levels seem to reflect this change of bFGF in intestinal tissue. In a time course study, Vincze *et al.*[34] revealed that the levels of bFGF in local tissues returned to normal as the experimental upper gastrointestinal tract ulcers healed.

During follow-up in the present study, we did not find a correlation between changes in serum bFGF levels and the CDAI score, i.e. the successful treatment of the active disease patients with infliximab did not affect the serum bFGF levels. The reason for stability of the relatively low serum bFGF level in patients with active disease might be that the bFGF is possibly not primarily derived from the inflammatory cell infiltrate. Further, the lack of association between serum bFGF and CDAI might reflect the inclusion of several subjective parameters in the overall CDAI score. By contrast, the patients with fistulas may have a stronger stromal component in the disease process that coincides with the immunohistological expression of bFGF in fibroblasts, endothelia, epithelial cells and muscle cells, and this might be a further illustration of a pathophysiological difference between fistulizing and active CD.[35] In this context, it is interesting that the down-regulation of bFGF by infliximab was speculated to be relevant for the process of intestinal fibrinogenesis and strictures/stenosis formation in CD, which was reported to be prevented by infliximab treatment.[32, 36] Previous studies already indicated that bFGF in these cells might be a marker for tissue damage in IBD.[9, 16, 18, 31] Basic FGF is a potent mitogen for fibroblasts and endothelial cells. These two kinds of cells are essential and fundamental elements in the formation of granulation and angiogenesis in tissue repair, and are also the important producers of bFGF. Like other regulatory peptides, the activity of the bFGF gene is cell-type specific, and developmentally or conditionally regulated. TNF-α can enhance the expression of bFGF mRNA in endothelial cells and stromal cells.[19, 20] Results reported by Yoshida *et al.*[20] showed that TNF-α stimulates the expression of bFGF and VEGF mRNA in endothelial cells to mediate angiogenesis. This might be a partial explanation for the decreased levels of bFGF following TNF-α inhibition by infliximab, since this latter inhibition was also observed with local intestinal cells obtained from similar macroscopically non-inflamed biopsies of CD patients as used in our study, which showed reduction of the intrinsically enhanced TNF-α and IFN-γ production at 2–3 months after infliximab infusion.[37] This reduced cytokine production by infliximab occurred in parallel with the decrease in bFGF expression in our biopsies and in the serum.

In conclusion, bFGF actively participates in the processes of inflammation and tissue repair in CD, particularly in the formation and healing of fistulas. Therefore, bFGF might be a useful serological marker for treatment response in fistulizing Crohn's disease.

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REFERENCES

1 Kirsner JB. The historical basis of the idiopathic inflammatory bowel diseases. Inflamm Bowel Dis 1995; 1: 2–26.
2 Lashner BA. Clinical features, laboratory findings, and course of Crohn's disease. In: Kirsner, JB, ed. Inflammatory Bowel Disease. Philadelphia: W.B. Saunders, 2000: 305–14.
3 Papadakis KA, Targan SR. Role of cytokines in the pathogenesis of inflammatory bowel disease. Annu Rev Med 2000; 51: 289–98.
4 Lichtenstein GR. Chemokines and cytokines in inflammatory bowel disease and their application to disease treatment. Curr Opin Gastroenterol 2000; 16: 83–88.
5 Houchen CW. FGF-20: FGFs – they keep growing and growing and growing! Inflamm Bowel Dis 2003; 9: 133.
6 Beck PL, Podolsky DK. Growth factors in inflammatory bowel disease. Inflamm Bowel Dis 1999; 5: 44–60.
7 Ornitz DM, Itoh N. Fibroblast growth factors. Genome Biol 2001; 2: 1–12.
8 Fernig DG, Gallagher JT. Fibroblast growth factors and their receptors: an information network controlling tissue growth, morphogenesis and repair. Prog Growth Factor Res 1994; 5: 353–77.
9 Nugent MA, Iozzo RV. Fibroblast growth factor-2. Int J Biochem Cell Biol 2000; 32: 115–20.
10 Szebenyi G, Fallon JF. Fibroblast growth factors as multifunctional signaling factors. Int Rev Cytol 1999; 185: 45–106.
11 Galzie Z, Kinsella AR, Smith JA. Fibroblast growth factors and their receptors. Biochem Cell Biol 1997; 75: 669–85.
12 Gonzalez AM, Buscaglia M, Fox R, *et al.* Basic fibroblast growth factor in Dupuytren's contracture. Am J Pathol 1992; 141: 661–71.
13 Buntrock P, Jentzsch KD, Heder G. Stimulation of wound healing, using brain extract with fibroblast growth factor (FGF) activity: I. Quantitative and biochemical studies into formation of granulation tissue. Exp Pathol 1982; 21: 46–53.
14 Lawrance IC, Maxwell L, Doe W. Altered response of intestinal mucosal fibroblasts to profibrogenic cytokines in inflammatory bowel disease. Inflamm Bowel Dis 2001; 7: 226–36.
15 Bousvaros A, Zurakowski D, Fishman SJ, *et al.* Serum basic fibroblast growth factor in pediatric Crohn's disease. Implications for wound healing. Dig Dis Sci 1997; 42: 378–86.
16 Thörn M, Raab Y, Larsson A, Gerdin B, Hällgren R. Intestinal mucosal secretion of basic fibroblast growth factor in patients with ulcerative colitis. Scand J Gastroenterol 2000; 35: 408–12.
17 Day R, Forbes A. Serum basic fibroblast growth factor in inflammatory bowel disease (IBD). Gut 1999; 44: 296–7.
18 Kanazawa S, Tsunoda T, Onuma E, Majima T, Kagiyama M, Kikuchi K. VEGF, basic-FGF, and TGF-beta in Crohn's disease and ulcerative colitis: a novel mechanism of chronic intestinal inflammation. Am J Gastroenterol 2001; 96: 822–8.
19 Okamura K, Sato Y, Matsuda T, *et al.* Endogenous basic fibroblast growth factor-dependent induction of collagenase and interleukin-6 in tumor necrosis factor-treated human microvascular endothelial cells. J Biol Chem 1991; 266: 19162–5.
20 Yoshida S, Ono M, Shono T, *et al.* Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. Mol Cell Biol 1997; 17: 4015–23.
21 Targan SR, Hanauer SB, Van Deventer SJ, *et al.* A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. N Engl J Med 1997; 337: 1029–35.
22 Keating GM, Perry CM. Infliximab: an updated review of its use in Crohn's disease and rheumatoid arthritis. BioDrugs 2002; 16: 111–48.
23 Present DH, Rutgeerts P, Targan S, *et al.* Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999; 340: 1398–405.
24 Gao Q, Kruidenier L, van Hogezand RA, Lamers CBWH, Verspaget HW. Immunohistochemical evaluation of bFGF, FGFR-1 and syndecan-1 in inflammatory bowel disease (IBD). Eur J Gastroenterol Hepatol 1999; 11: A100.
25 Bikfalvi A, Klein S, Pintucci G, Rifkin DB. Biological roles of fibroblast growth factor-2. Endocrinol Rev 1997; 18: 26–45.
26 Szabo S, Vincze A. Growth factors in ulcer healing: lessons from recent studies. J Physiol Paris 2000; 94: 77–81.
27 Jones MK, Tomikawa M, Mohajer B, Tarnawski AS. Gastrointestinal mucosal regeneration: role of growth factors. Front Biosci 1999; 4: D303–9.
28 Dignass AU, Tsunekawa S, Podolsky DK. Fibroblast growth factors modulate intestinal epithelial cell growth and migration. Gastroenterology 1994; 106: 1254–62.
29 el Hariry I, Pignatelli M, Lemoine N. Fibroblast growth factor 1 and fibroblast growth factor 2 immunoreactivity in gastrointestinal tumours. J Pathol 1997; 181: 39–45.
30 Lügering N, Kucharzik T, Stoll R, Domschke W. Current concept of the role of monocytes/macrophages in inflammatory bowel disease – balance of proinflammatory and immunosuppressive mediators. Ital J Gastroenterol Hepatol 1998; 30: 338–44.
31 Taha Y, Raab Y, Larsson A, *et al.* Mucosal secretion and expression of basic fibroblast growth factor in patients with collagenous colitis. Am J Gastroenterol 2003; 98: 2011–7.

32 Di Sabatino A, Ciccocioppo R, Benazzato L, Sturniolo GC, Corazza GR. Infliximab downregulates basic fibroblast growth factor and vascular endothelial growth factor in Crohn's disease patients. Aliment Pharmacol Ther 2004; 19: 1019–24.
33 van Balkom BP, Schoon EJ, Stockbrügger RW, *et al.* Effects of anti-tumour necrosis factor-alpha therapy on the quality of life in Crohn's disease. Aliment Pharmacol Ther 2002; 16: 1101–7.
34 Vincze A, Sandor Z, Nagata A, Tarnawski A, Szabo S. The role of endogenous basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) in experimental duodenal ulcer development and healing. Gastroenterology 1996; 110: A287.
35 Ohtani H, Nakamura S, Watanabe Y, Mizoi T, Saku T, Nagura H. Immunocytochemical localization of basic fibroblast growth factor in carcinomas and inflammatory lesions of the human digestive tract. Lab Invest 1993; 68: 520–7.
36 Lichtenstein GR, Olson A, Bao W, Travers S, Diamond RH. Infliximab treatment does not result in an increased risk of intestinal strictures or obstruction in Crohn's disease patients: ACCENT I study results. Am J Gastroenterol 2002; 97: S254–5.
37 Cornillie F, Shealy D, D'Haens G, *et al.* Infliximab induces potent anti-inflammatory and local immunomodulatory activity but no systemic immune suppression in patients with Crohn's disease. Aliment Pharmacol Ther 2001; 15: 463–73.