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Altered Th1/Th2 cytokine profiles in the intestinal mucosa of patients with inflammatory bowel disease as assessed by quantitative reversed transcribed polymerase chain reaction (RT-PCR)

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SUMMARY

Cytokines serve a central function as key factors in the regulation of the intestinal immune response and mediation of tissue damage in inflammatory bowel disease (IBD). Abnormalities in the expression of immunoregulatory cytokines such as IL-2, IL-4, IL-10 and interferon-gamma (IFN-γ) may indicate a dysregulation of intestinal immunity probably associated with pathogenic events. Therefore, cytokine mRNA concentrations were determined in the mucosa of patients with IBD at sites of active ($n = 13$) and inactive ($n = 12$) ulcerative colitis (UC), active ($n = 11$) and inactive ($n = 11$) Crohn's disease (CD) and in control patients ($n = 14$) using quantitative RT-PCR. IL-10 mRNA concentrations were significantly increased in patients with both active UC ($P < 0.001$) and active CD ($P < 0.005$) compared with control patients. IFN-γ mRNA concentrations were also significantly increased both in patients with active UC ($P < 0.02$) and active CD ($P < 0.05$) compared with control patients, whereas IL-2 mRNA levels were significantly ($P < 0.02$) increased only in active CD. IL-4 mRNA expression in the intestinal mucosa was frequently below the detection limit. Our results demonstrate that chronic intestinal inflammation in patients with CD is characterized by an increase of Th1-like cytokines. Furthermore, the increased IL-10 mRNA expression at sites of active IBD suggests that IL-10 is an important regulatory component involved in the control of the inflammatory response in inflammatory bowel disease.

Keywords immunoregulatory cytokines competitive RT-PCR ulcerative colitis Crohn's disease

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are disorders characterized by chronic intestinal inflammation which leads to severe destruction of the mucosa. The underlying mechanisms responsible for initiation and perpetuation of the inflammatory processes are unclear, although there is ample evidence for an intense local mucosal immune response. As a consequence of the activation of immune cells, mediators such as cytokines are released that influence the activity, differentiation or proliferation of a variety of cells and mediate tissue injury. It is well established that proinflammatory cytokines such as IL-1β, IL-6, IL-8 and tumour necrosis factor-alpha (TNF-α) are released to a greater extent in the inflamed mucosa by macrophages/monocytes and are considered to be involved in secondary pathophysiologic mechanisms such as tissue damage observed in inflammatory bowel disease (IBD) [1–3]. Moreover, tissue concentrations of these cytokines have been suggested as indicators of disease activity [4]. In addition to these proinflammatory cytokines a compensatory anti-inflammatory response has been suggested in the inflamed mucosa, mainly due to observations showing an increased expression of IL-1 receptor antagonist and transforming growth factor-beta (TGF-β) [5,6]. So far, little is known about the concentration of the more immunoregulatory and/or anti-inflammatory effective cytokines, such as IL-2 and interferon-gamma (IFN-γ) (Th1-like cytokines) and IL-4 and IL-10 (Th2-like cytokines) in the intestinal mucosa of patients with IBD [7–9]. There is no doubt about the importance of the regulatory and reciprocal effects of these cytokines in controlling and/or enhancing the local immune response [10–13]. An increased release of one or more of these factors might have profound consequences for the strongly polarized Th1/Th2 responses resulting in either the protection or promotion of immunopathological reactions. Th1 cytokine profiles appear to be more involved in responses

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against bacterial infections and inflammation [14], whereas Th2 cytokine profiles may account for allergic disorders such as steroid-resistant asthma [15].

To quantify the amount and profile of the immunoregulatory cytokines in the mucosa of patients with IBD, we established a quantitative PCR by constructing synthetic mRNAs, which were used as competitors in an RT-PCR to assess the concentration of Th1 and Th2 cytokine mRNA transcripts in histologically active and inactive disease, and compared the results with concentrations present in normal intestinal mucosa of control patients.

PATIENTS AND METHODS

Patients

Thirteen patients with UC, six women and seven men, with a mean age of 40 years, ranging from 17 to 66 years, and 13 patients with CD, five women and eight men with a mean age of 35 years, ranging from 25 to 54 years, were entered in the study. Diagnosis was based on clinical, radiographic, endoscopic and histopathological criteria. A single biopsy specimen was taken from active and/or inactive intestinal areas of each patient with IBD. Biopsies were graded by an independent observer as active inflammation (presence of neutrophils) and chronic inflammation (presence of plasma cells, lymphocytes and macrophages) according to [16]. Fourteen patients, six women and eight men, with a mean age of 55 years, ranging from 43 to 68 years, undergoing colorectal endoscopy for suspected cancer, were investigated and included as normal control group. None had evidence for intestinal inflammation or evidence for cancer. All patients gave their informed consent according to the Helsinki rules.

RNA isolation and RT-PCR

Biopsy specimens were placed immediately in guanidine thiocyanate buffer and homogenized. Total RNA was isolated from tissue specimens by acid guanidium thiocyanate/phenol/chloroform extraction as described by Chomczynski & Sacchi [17]. Isolated RNA was stored at −70°C in diethylpyrocarbonate (DEPC)-treated water and quantity and quality of the RNA were determined by absorbance at 260/280 nm. Total RNA (1 μg) was reverse transcribed into complementary DNA (cDNA) using Moloney Murine Leukaemia Virus (M-MLV)-derived RNase H-reverse transcriptase (200 U/μl RNA; Superscript, Bethesda Research Laboratories, Eggenstein, Germany) and oligo(dT)$_{12-18}$ (2 μg/ml; Pharmacia, Freiburg, Germany) in a final reaction volume of 20 μl in the presence of 1 U/μl of RNasin (Promega, Heidelberg, Germany). The reaction mixture was incubated at 42°C for 60 min followed by heating for 5 min at 95°C to inactivate the enzyme and stored at −20°C until use. Aliquots of cDNA were subjected to PCR utilizing primers specific for CD3δ (5′ CTGGACCTGGGAAAACG-CATC 3′, 5′ GTACTGAGCATCATCTCGATC 3′), IL-2 (5′ ACTCACCAGGATGCTCACAT 3′, 5′ AGGTAATC-CATCTGTTCAGA 3′), IL-4 (5′ ATGGGTCTCACCTCC-CAACTGCT 3′, 5′ CGAACACTTTGAATATTTCTCTCT CAT 3′), IL-10 (5′ ATGCCCCAAGCTGAGAACCAA-GACCCA 3′, 5′ TCTCAAGGGGCTGGGTCAGCTAT-CCCA 3′) and IFN-γ (5′ ATGAAATATACAAGT TATATCTTGGCTTT 3′, 5′ GATGCTCTTCGACCTCGA-AACAGCAT 3′). The 20-μl reaction mixture consisted of: 1× PCR buffer (Promega) supplemented with 1 mM $MgCl_2$ for IL-4 and IL-10 and 2 mM $MgCl_2$ for IL-2 and IFN-γ, 200 μM dNTPs (Pharmacia), 0·3 μM of the respective sense and antisense primer and *Thermus aquaticus* (Taq) polymerase (1 U/50 μl; Promega). Amplification was performed by 30–35 cycles consisting of denaturation at 94°C for 30 s, primer annealing at 57°C for 45 s, and primer extension at 72°C for 60 s on a Programmable Thermal Controller PTC-100 (MJ Research, Watertown, MA).

Construction of synthetic cytokine mRNAs

Preparation of synthetic cytokine mRNAs was performed as described by Kozbor *et al.* [18] with some modifications. Briefly, total cellular RNA was extracted from mitogen-stimulated peripheral blood mononuclear cells (PBMC) from healthy volunteers and reverse transcribed RT-PCR was performed with primer pairs specific for the respective cytokine. Each RT-PCR product was digested with two distinct restriction endonucleases whose restriction sites were separated by a fragment of approximately 30–70 basepairs (Table 1). After digestion, overhanging cohesive ends were blunt ended with Mung Bean nuclease (New England Biolabs, Schwalbach, Germany) and the resulting fragments were separated on a 2·5% agarose gel. The two longer restriction fragments from each digested RT-PCR product were eluted using DEAE cellulose (Schleicher & Schüll, Dassel, Germany), purified, ligated with T4 DNA ligase (BRL, Eggenstein, Germany) and reamplified with the original primer pairs. The appropriate PCR products were subcloned into the pSP64 (poly A) RNA expression vector (Promega). *In vitro* transcription with SP6 RNA polymerase (Promega) was performed after linearization of the plasmid and the DNA template was removed by digestion with RNase-free DNase 1 (RQ1; Promega), purified and the amount of synthetic RNA (sRNA) was determined. This approach was used to construct longer sRNA templates for IL-2, IL-4, IL-10 and IFN-γ in comparison with cellular mRNA, thus allowing electrophoretic separation of the co-amplified PCR products.

Quantification of cytokine mRNAs by competitive RT-PCR

For quantification of cytokine mRNAs total RNA was extracted from the mucosal biopsy specimens. Unknown total

Table 1. Construction of synthetic cytokine mRNAs

Cytokine	Restriction endonuclease (5′ restriction site, bp)	Size of the cytokine mRNAs (bp)*	
		Native	Synthetic
IL-2	MvaI (140)		
	XbaI (70)	266	331
IL-4	PstI (211)		
	SnoI (141)	456	518
IL-10	ClaI (99)		
	HaeIII (131)	351	381
IFN-γ	BfrI (101)		
	DdeI (156)	501	549

*The length of the sRNA is shorter than the sum of the ligated fragments due to the digestion of a few nucleotides by the enzyme Mung Bean nuclease.

RNA (1 μg) was reverse transcribed into cDNA together with serial dilutions of the synthetic mRNA competitors in the same reaction mixture and co-amplified by PCR in a total volume of 20 μl. After amplification, the amount of PCR products generated by competitor and target mRNA was separated electrophoretically and compared by visualization on ethidium bromide-stained 2·5% agarose gels. This approach using an internal control that contains the same primer template sequences as the target provides a strategy for reproducible quantification of absolute amounts of target mRNA by allowing known amounts of synthetic RNA to compete with the target sequence for primer binding and amplification.

Estimation of the amount of CD3$^+$ T cells in mucosal biopsy specimens

In order to avoid having to account for different numbers of T cells in intestinal biopsy specimens derived from sites with histologically active and inactive disease of patients with IBD and normal mucosa of control patients, lymphokine mRNA transcripts were normalized to the amount of T cell-specific transcripts in each sample. Therefore, semiquantitative estimation of the amount of RNA encoding for the CD3/T cell receptor complex in intestinal biopsies was performed using a control PCR with a primer pair specific for the δ-chain of the T cell marker CD3. For each sample the same amount of cDNA used for competitive RT-PCR was amplified with the CD3δ-specific primer pairs. The resulting PCR products were visualized by ethidium bromide-stained gel electrophoresis and band intensities were compared.

Statistical analysis

For calculating cytokine mRNA concentrations in each group the median value was determined. For unpaired data statistical comparison was made by the Mann–Whitney test. Paired data were analysed during the Wilcoxon test (non-parametric distribution) and the *t*-test (parametric distribution).

RESULTS

Quantification of IL-2 mRNA in intestinal mucosa

In order to account for differences due to the numbers of T cells in tissue specimens derived from histologically active and inactive disease, semiquantitative estimation of T cell-specific mRNA was performed using a CD3δ chain-specific PCR. All samples used for cytokine measurements showed approximately equal amounts of those T cell-specific transcripts both in active and inactive mucosal tissue biopsies (Fig. 1). Thus, differences in cytokine mRNA concentrations observed in active and inactive mucosa are not due to an altered number of T cells.

Using competitive RT-PCR, quantification of cytokine mRNA levels was performed in intestinal biopsies derived from histologically proven active ($n = 13$) and inactive ($n = 12$) sites of patients with UC, from active ($n = 11$) and inactive ($n = 11$) sites of patients with CD and from normal mucosa of control patients ($n = 14$). All normal colonic tissue samples of control patients demonstrated measurable IL-2 mRNA levels within a range of 0·25–8 pg IL-2 mRNA/μg total RNA (median, 1·4 pg/μg total RNA). IL-2 mRNA transcripts in biopsies obtained from sites with histologically

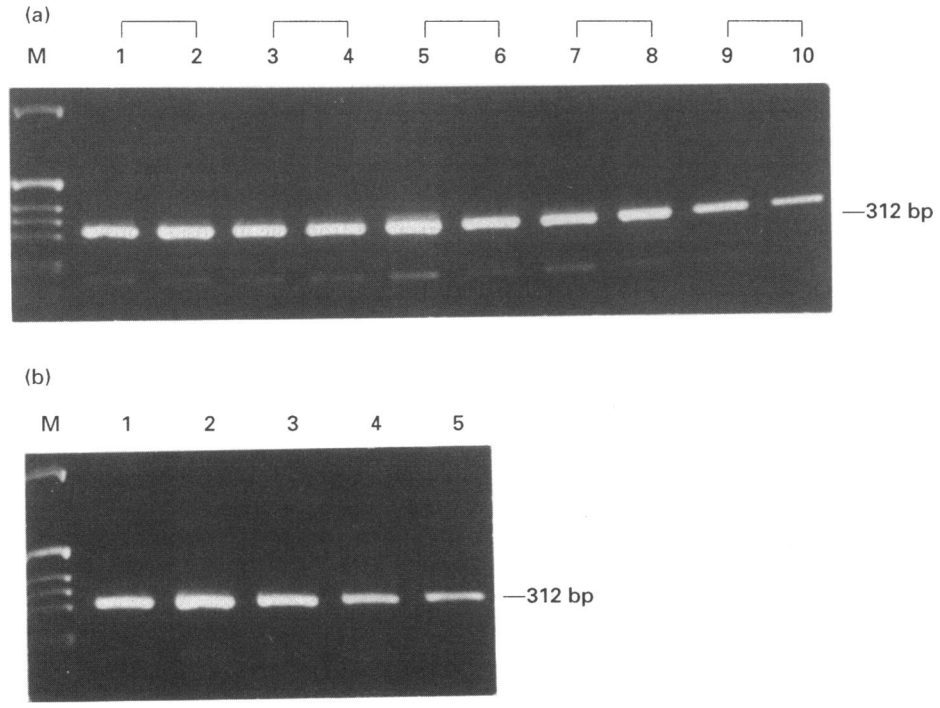

Fig. 1. (a) Ethidium bromide-stained agarose gel of PCR products of the CD3δ chain of the T cell receptor amplified from cDNA made by reverse transcription of total RNA extracted from mucosal tissue with histologically active (paired numbers) and inactive (unpaired numbers) disease from five patients with inflammatory bowel disease (IBD). (b) CD3δ PCR products in normal tissue of five control patients (lanes 1–5). M, Molecular weight marker.

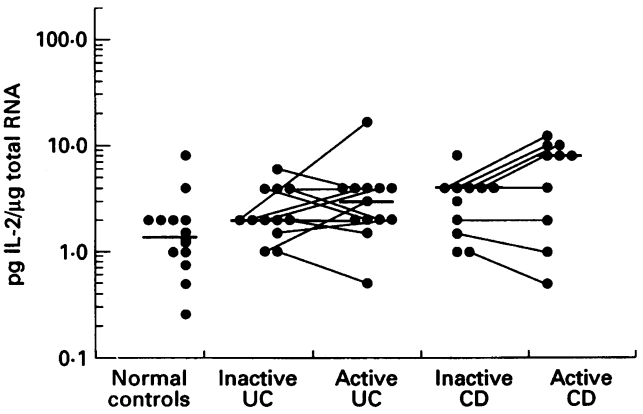

Fig. 2. IL-2 mRNA levels in mucosal tissue from normal control patients ($n = 14$), patients with histologically active ($n = 13$) and inactive ($n = 12$) ulcerative colitis (UC) and active ($n = 10$) and inactive ($n = 11$) Crohn's disease (CD). The bar indicates median values of the IL-2 mRNA levels in each group. Values obtained from samples derived from histologically active and inactive disease of the same patients are connected by thin lines.

active disease in patients with UC ranged from 0·5 to 16 pg/μg total RNA (median, 3 pg/μg total RNA) compared with 1 to 6 pg/μg total RNA (median, 2 pg/μg total RNA) in inactive disease. Inconsistent results were found with respect to IL-2 mRNA transcripts in biopsies derived from active and inactive sites of the same patient with UC as outlined by connected data points (Fig. 2). In biopsy specimens with active CD, concentrations of IL-2 mRNA ranged from 0·5 to 12 pg/μg total RNA (median, 8 pg/μg total RNA) and were significantly ($P < 0·02$) higher compared with normal control patients (median, 1·4 pg/μg total RNA). At sites with inactive disease of patients with CD, IL-2 mRNA concentrations ranged from 1 to 8 pg/μg total RNA (median, 4 pg/μg total RNA).

Quantification of IL-4 mRNA in intestinal mucosa

In contrast, IL-4 mRNA transcripts were detectable only in a minority of samples analysed, including patients and controls. As illustrated in Fig. 3, six out of 14 histologically normal biopsies of control patients (43%), three out of 13 biopsies derived from active (23%) and two out of 12 biopsies from inactive UC (17%) showed measurable IL-4 mRNA levels. In CD, one out of 11 biopsies from active (9%) and two out of 11 biopsies from inactive (18%) disease showed detectable levels of IL-4 mRNA. Thus, the percentage of samples with measurable amounts of IL-4 mRNA transcripts was smaller in patients with UC and CD than in control patients. However, the failure to detect measurable IL-4 mRNA transcripts in most biopsies prevented the statistical comparison of IL-4 mRNA transcripts in different groups.

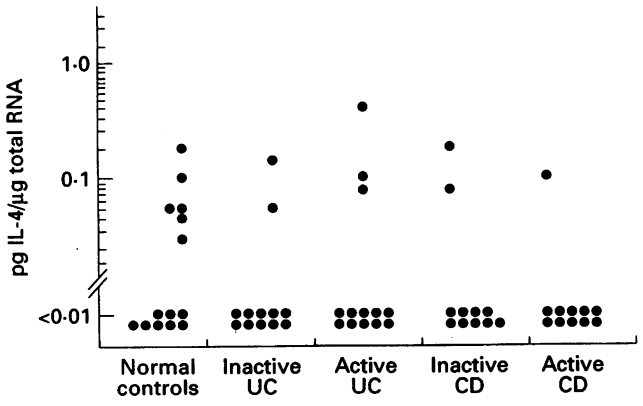

Fig. 3. IL-4 mRNA levels in mucosal tissue from normal control patients, patients with histologically active and inactive ulcerative colitis (UC) and Crohn's disease (CD).

Quantification of IL-10 mRNA in intestinal mucosa

An example for the quantification of IL-10 by competitive PCR with synthetic (sIL-10) and native IL-10 mRNA in a single control patient is shown in Fig. 4. Quantification of IL-10 mRNA showed that detectable levels were found in most tissue specimens examined. In normal mucosa of control patients, IL-10 mRNA transcripts ranged from 0·02 to 0·4 pg/μg total RNA (median, 0·06 pg/μg total RNA) (Fig. 5), whereas IL-10 mRNA transcripts in biopsies obtained from sites with histologically active disease in patients with UC were significantly higher ($P < 0·001$), ranging from 0·1 to 2·4 pg/μg total RNA (median, 0·5 pg/μg total RNA). In histologically inactive UC, IL-10 mRNA concentrations ranged from 0·075 to 1·6 pg/μg total RNA (median, 0·2 pg/μg total RNA), and were also significantly higher ($P < 0·02$) than in normal control subjects. In patients with active CD, concentrations of IL-10 mRNA ranged from 0·05 to 4 pg/μg total RNA (median, 0·5 pg/μg total RNA), and were significantly increased ($P < 0·005$) compared with controls. In biopsy specimens derived from inactive CD, IL-10 mRNA concentrations were comparable to normal control mucosa, ranging from 0·025 to 1·6 pg/μg total RNA (median, 0·1 pg/μg total RNA). Furthermore, the comparison of IL-10 mRNA in paired biopsy specimens from histologically active and inactive disease of the same patient with UC ($n = 12$) or with CD ($n = 9$) revealed that in more than 90% of the patients analysed, significantly higher (UC, $P < 0·02$; CD, $P < 0·025$) IL-10 mRNA levels were expressed in tissue specimens derived from histologically active sites of the disease, as indicated in the scattergram by connected data points (Fig. 5). Thus, sites of active inflammation in both diseases were characterized by significantly higher IL-10 mRNA levels.

Quantification of IFN-γ mRNA in intestinal mucosa

The results of the IFN-γ mRNA quantification are summarized in Fig. 6. Most of the samples derived from patients and controls expressed detectable levels of IFN-γ mRNA transcripts. The concentration of IFN-γ mRNA in histologically normal mucosa of control patients was within a range of 0·025–0·5 pg/μg total RNA (median, 0·05 pg/μg of total RNA). Samples derived from patients with active UC showed IFN-γ concentrations within a range of 0·025–0·8 pg/μg total RNA (median, 0·25 pg/μg total RNA), and samples derived from inactive UC expressed IFN-γ mRNA ranging from 0·025 to 0·6 pg/μg total RNA (median, 0·15 pg/μg total RNA). Thus, IFN-γ mRNA transcripts were significantly ($P < 0·02$) increased in biopsy specimens derived from mucosa with active UC compared with control subjects. In patients with active CD, significantly higher ($P < 0·05$) IFN-γ mRNA levels

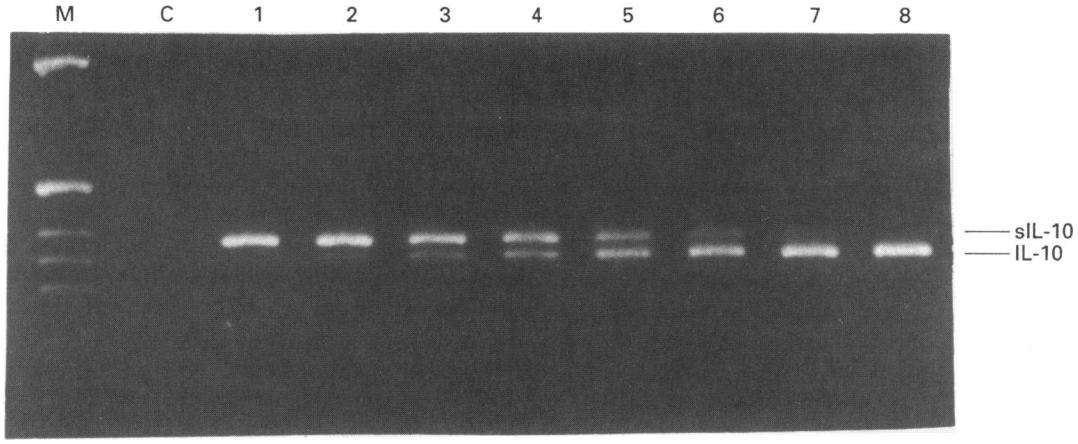

Fig. 4. Agarose gel showing PCR products of synthetic IL-10 mRNA (sIL-10) and native IL-10 mRNA stained with ethidium bromide. PCR products of serial dilutions (from 1·6, 0·8, 0·4, 0·2, 0·1, 0·05, 0·025 to 0 pg; lanes 1–8) of sIL-10 (upper bands) and native IL-10 mRNA (lower bands) after RT-PCR.

compared with normal control mucosa were detected, ranging from 0·05–0·6 pg/μg total RNA (median, 0·2 pg/μg total RNA). In active CD IFN-γ mRNA expression ranged from 0·025 to 0·6 pg/μg total RNA (median, 0·05 pg/μg total RNA).

DISCUSSION

For quantification of mRNA, different PCR-based methods have been established. Initially, attempts were made using external RNA standards such as β_2-microglobulin or actin, which were amplified in separate reactions allowing comparative but not absolute measurements [19–22]. Absolute quantification requires that target and standard are amplified with equal efficiency at each step of the PCR reaction. Several approaches [23–25] have been developed to solve this problem. One possibility is that the competitive standard differs from the analysed template by the generation of a new restriction site. However, this can lead to errors in quantification of absolute values due to an incomplete digestion of the competitor. Studies utilizing an internal standard that differs from the unknown target by the addition of a small intron demonstrated that this standard could be amplified with the same efficiency as the target over a wide range of cycles and initial target concentrations [18,26,27].

For quantification of cytokine mRNAs present in the intestinal mucosa, synthetic competitor mRNAs were constructed through the insertion of small introns in cDNAs encoding for the native cytokines. The use of competitor RNA instead of competitor DNA allows for the simultaneous reverse transcription of standard and target mRNA in the same tube, and eliminates effects due to differences in the reverse transcription reaction.

The present study demonstrates that the concentration of

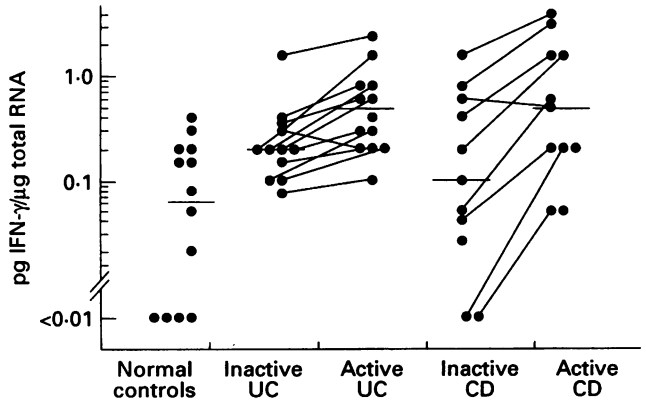

Fig. 5. IL-10 mRNA levels in mucosal tissue from normal control patients, patients with histologically active and inactive ulcerative colitis (UC) and active and inactive Crohn's disease (CD). The bar indicates median values of IL-10 mRNA levels in each group. Values obtained from samples derived from histologically active and inactive disease of the same patients are connected by thin lines.

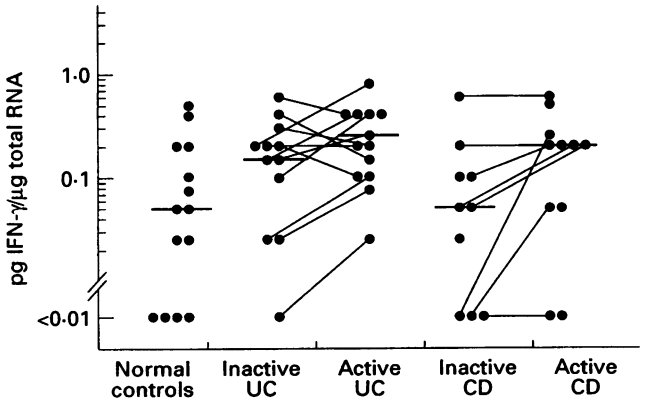

Fig. 6. IFN-γ mRNA levels in mucosal tissue from normal control patients, patients with histologically active and inactive ulcerative colitis (UC) and Crohn's disease (CD). The bar indicates median values of the IFN-γ mRNA levels in each group. Values obtained from samples derived from histologically active and inactive disease of the same patients are connected by thin lines.

most of the cytokine mRNAs analysed can be quantified in mucosal biopsy specimens even in control patients without histological evidence of intestinal inflammation. This finding is in agreement with previous results demonstrating high numbers of activated T cells already present in the mucosa under normal conditions [28,29]. Furthermore, there are quantitative differences in cytokine mRNA transcripts between tissue derived from patients with histologically active and inactive disease and from normal controls. In accordance with observations from Gilberts *et al.* [30], we did not observe significant differences in the amount of CD3δ PCR products in tissue derived from histologically active and inactive sites of individual patients with IBD. Thus, it is unlikely that the amount of activated T cells was responsible for the altered cytokine mRNA levels observed in the inflamed mucosa. However, it cannot be excluded that other immune cells beside T cells, which are present in the inflamed tissue, contribute to the Th1/Th2-like cytokine mRNA expression in the intestinal mucosa.

The most substantial finding in this study was the significantly increased expression of IL-10 mRNA transcripts in biopsies derived from sites with active UC and CD. IL-10 is a cytokine with potent anti-inflammatory capacities, mainly due to the inhibition of the synthesis of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 [31], and the fact that IL-10 is able to suppress macrophage-induced cytokine production by Th1 cells [32]. In addition, IL-10 has recently been shown to reduce TNF-α levels and mortality of experimental endotoxaemia in mice [33,34]. Increased IL-10 mRNA and protein levels have been described in a variety of inflammatory diseases such as rheumatoid arthritis, septicaemia and malaria [35–37]. Furthermore, mice deficient for IL-10 develop colitis-like chronic enterocolitis raised under conventional, non-pathogen-free conditions [38]. Thus, in order to maintain an appropriate immune response and to counteract the effects of the proinflammatory cytokines present in inflamed mucosa of patients with IBD, increased IL-10 levels might be important in the down-regulation of inflammatory events.

Studies examined the expression of IFN-γ in the intestinal mucosa revealed contradictory results. In some studies decreased levels of IFN-γ mRNA have been reported in the lamina propria of patients with IBD [39,40]. Our results, obtained by the sensitive method of competitive RT-PCR, confirm data showing an increase of IFN-γ mRNA concentrations in mucosal lesions of patients with IBD compared with normal mucosa [9,41,42]. Since HLA expression is strongly induced by IFN-γ [43], the increased IFN-γ mRNA expression might provide a rational explanation for the finding that epithelial cells derived from inflamed mucosa of patients with IBD expressed higher levels of HLA class II antigens compared with those from normal mucosa. Furthermore, the increased IFN-γ mRNA expression might be responsible for the tissue damage observed in IBD, since IFN-γ is able to sustain cytotoxic reactions [44]. Quantification of IL-2 mRNA concentration showed a significant increase in the mucosa of patients with active CD compared with control patients and patients with UC. This finding is consistent with a previous report from Mullin *et al.* [16].

The biological importance of IL-2 for the mucosal immune response and chronic intestinal inflammation was demonstrated in IL-2 'knock-out' mice [45]. The surviving animals developed (like IL-10-deficient mice) symptoms of an intestinal inflammation which corresponds to UC in humans. Recently it has been shown that the production of IL-4 and IL-10 in these animals is significantly increased without preventing the chronic inflammation [46]. Thus, it appears of significance that alterations in the Th1 and Th2 profiles are able to influence the inflammation, especially when the immune system of the mucosa is constantly stimulated by environmental antigens.

IL-4 mRNA expression in the intestinal mucosa was too low for quantification, and did not allow for a conclusive assessment of alterations between patients with active and inactive disease and normal control subjects. To avoid false-negative results of IL-4 mRNA transcripts in the intestinal mucosa due to the choice of the primer pairs used for quantification, we initially performed a competitive RT-PCR using stimulated intestinal T lymphocytes. These T cells expressed IL-4 mRNA within a range of 1–20 pg/μg total RNA (data not shown). Thus, the inability to detect IL-4 mRNA transcripts seems to be a consequence of a very low expression of this cytokine in the intestinal mucosa. This finding is consistent with data from McCabe *et al.* [6], who suggested that IL-4 is produced in the intestinal mucosa in extremely minute amounts. However, the data obtained here indicate that mucosal IL-4 mRNA expression is decreased in patients with IBD. This is in agreement with work by Karttunnen *et al.* [47], who demonstrated a reduced number of IL-4-secreting cells in children with IBD using the ELISPOT technique.

Interestingly, in patients with UC without histological evidence of acute inflammation, for all cytokines examined mRNA expression was already increased compared with normal control subjects. This is consistent with data reported by Reinecker *et al.* [48], who found increased production of IL-6 in lamina propria mononuclear cells derived from sites with inactive UC. Thus, the increased mRNA expression might be explained by early 'priming' and/or an increased activation of cytokine/secreting cells present in uninvolved mucosa. This might underline a potential susceptibility of the complete large bowel to develop UC.

On the assumption that most of the IL-10 mRNA is not only expressed by Th2 cells but also by monocytes and Th1 cells [49], the results of the present study suggest that in contrast to patients with UC and normal controls, the intestinal immune response in patients with CD is characterized by a Th1 cytokine profile. This finding is consistent with preliminary results by Romagnani and co-workers [14], who established intestinal T cell clones from patients with CD which mainly produced a Th1 cytokine profile. Since the nature of the triggering antigen is critical for the type of specific immune response, it is tempting to speculate that bacterial antigens might be involved in the pathogenesis of CD.

In conclusion, competitive RT-PCR permits determination of cytokine mRNA levels in a single biopsy specimen, and allows for their comparison at sites of active and inactive disease. The assessment of the concentration of different cytokines *in vivo* might provide insights into the pathophysiologically relevant mechanisms leading to chronic inflammation. More complete understanding of these factors should provide means to develop strategies to inhibit proinflammatory cytokines by, for example, enhancing endogenous suppressive pathways.

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