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Circulating antiinflammatory cytokine IL-10 in patients with inflammatory bowel disease (IBD)

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SUMMARY

IBD is characterized by increased serum concentrations of different cytokines. IL-10 inhibits the production of proinflammatory cytokines such as IL-1, tumour necrosis factor-alpha (TNF-a), interferon-gamma (IFN-γ) and IL-6 through inhibitory action on Th1 cells and macrophages, and it is thought to be a suppressor type cytokine. In the present study we determined serum concentrations of IL-10 in patients with ulcerative colitis (UC) and Crohn's disease (CD). We measured human IL-10 by our own newly established ELISA system using PharMingen antibodies. Serum antibodies were assessed in 44 patients with UC, 40 patients with CD, and in 30 healthy controls. Human IL-10 serum levels were significantly increased in patients with active UC (144 ± 34 pg/ml (mean $\pm$ s.e.m.), $P < 0{\cdot}001$) and in active CD (132 ± 32 pg/ml, $P < 0{\cdot}001$) compared with healthy controls ($44 \pm 9{\cdot}5$ pg/ml). Only patients with active CD and active UC presented with significantly increased IL-10 serum levels, while patients with inactive disease did not show any significant increase. There was no statistically significant difference between IL-10 serum levels in patients with CD or UC. Compared with clinical disease activity indices there was a significant correlation between IL-10 serum concentration and CDAI in patients with CD ($r = 0{\cdot}45$, $P < 0{\cdot}01$) and CAI in UC patients ($r = 0{\cdot}39$, $P < 0{\cdot}05$). Comparing IL-10 serum levels with serum concentrations of other proinflammatory cytokines there was a significant correlation to serum levels of sIL-2R ($r = 0{\cdot}417$, $P < 0{\cdot}05$) and IL-6 ($r = 0{\cdot}387$, $P < 0{\cdot}05$) in patients with CD. Serum cytokine levels in patients with UC did not show any significant correlation to IL-10 serum concentration. IL-10 is elevated in serum of patients with active CD and UC, suggesting that IL-10 acts as a naturally occurring damper in the acute inflammatory process of IBD.

Keywords IL-10 inflammatory bowel disease cytokines Crohn's disease ulcerative colitis

INTRODUCTION

Cytokines seem to play a key role in IBD. Proinflammatory cytokines such as interferon-gamma (IFN-γ), IL-1β, IL-6 and IL-8 are reproducibly increased in inflamed tissue in ulcerative colitis (UC) and Crohn's disease (CD) [1–4]. Although studies of serum concentrations of most cytokines have yielded conflicting results, most investigators agree that serum levels of IL-6 are increased in CD but not in UC [3,5]. Some authors raise the possibility that tissue concentrations of lymphokines can assist in the differential diagnosis of these intestinal disorders. They postulate that CD could be diagnosed by a Th1 pattern of cytokines (increased IL-2 and IFN-γ) [6,7], while UC shows a Th2 lymphokine pattern with increased IL-4, IL-5 and IL-10 [4,8,9].

In vitro results have shown that IL-10 plays a major role in the regulation of immune responses as an inhibitor of macrophage and T cell effector function [10–12].

Murine IL-10 was initially identified as cytokine synthesis inhibitory factor (CSIF), detected in the supernatant of activated Th2 clones [10]. The human counterpart is mainly produced by Th2 cells, but production by Th1 cells and monocytes has also been reported [13]. The pleiotropic activities of IL-10 include inhibition of antigen-specific T cell proliferation and down-regulation of MHC class II expression in monocytes and macrophages [14]. In B cells, up-regulation of MHC class II expression and increased immunoglobulin synthesis have been observed. Inhibitory effects of IL-10 on synthesis of cytokines such as tumour necrosis factor-alpha (TNF-α), IFN-γ [15,16], IL-1α, IL-1β, IL-6 and IL-8 on mRNA expression and protein level have been reported [17].

Cloning and sequence analysis of IL-10 cDNA have shown that hIL-10 is very similar to the BCRF1 gene of Epstein–Barr virus (EBV) [15]. BCRF1 (vIL-10) and hIL-10 are closely related in amino acid sequence with 84% homology. BCRF1

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Table 1. Study population

Parameter	CD	UC
No. of patients	40	44
Age (years)	29 (18–54)	32 (16–69)
Sex (female/male)	16/24	25/19
Active/inactive disease	18/22	20/24
CDAI	110 (0–394)	(–)
Rachmilewitz index	(–)	3 (0–18)
Extraintestinal manifestations	7	3
Receiving steroids	18	17
Receiving 5-ASA only	8	11
No treatment	10	7
Previous surgery	7	2

CD, Crohn's disease; UC, ulcerative colitis; CDAI, Crohn's disease activity index.

as a viral cytokine shares many of the activities of hIL-10, although its specific activity is consistently lower than that of hIL-10 [18,19].

The suppressive effects of human and viral IL-10 suggest that this cytokine may be important in dampening an ongoing immune response, and may be a physiological counterpart to proinflammatory cytokines. Altered concentrations of IL-10 have been described in several diseases. Especially in serum of patients with malignant lymphomas [20], in ascites of patients with ovarian and other abdominal cancers [21], or in patients with septicaemia and malaria [22,23], increased concentrations of IL-10 have been found.

In the present study we have investigated circulating concentrations of this cytokine in patients with IBD compared with healthy controls. We tried to determine the role of human IL-10 in the acute immune response in IBD, and studied its relation with parameters of the acute phase response.

PATIENTS AND METHODS

Study population

Forty-four patients with UC and 40 patients with CD were included in the study. All patients had given informed consent to participate. Characterization of the patients is given in Table 1. The activity of UC was determined by the Rachmilewitz index [24]. Activity of CD was determined by CDAI [25].

Samples

Venous blood (10 ml) was collected. It was kept on ice and centrifuged at 3000 *g* for 30 min. Supernatant was then collected and aliquots were frozen at −20° C until used for assay.

Assays

To determine IL-10 concentrations, we developed a specific ELISA system using the quantitative 'sandwich'-enzyme technique. A similar assay was recently described by Peyron *et al.* [23]. We used two assays, one specific for human IL-10 (hIL-10) and BCRF-1 (viral IL-10), and another one specific for BCRF-1. The same capture antibody anti-IL-10 MoAb 9D7 was used in both assays and covered on a 96-well microtitre plate (Greiner, Solingen, Germany) overnight at 4°C. After blocking for 2 h with PBS/10% fetal calf serum (FCS), standards and samples were added. Recombinant human IL-10 (PharMingen, San Diego, CA) and BCRF-1 were used as standards. Biotinylated rat anti-IL-10 MoAb 12G8 (PharMingen) was used as detecting antibody for the hIL-10 and BCRF-1 ELISA. To detect BCRF-1 we used the tracer antibody 6B11 (PharMingen) for an incubation period of 45 min. To reveal the binding of the antibodies we used avidin-peroxidase (Sigma, Deisenhofen, Germany) and ABTS (Sigma) substrate buffer. The cross-reactivity of our assay was determined by addition of different cytokines to IL-10 standards and measuring the IL-10 concentration. In the presence of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-12, IL-13, IFN-α, IFN-γ, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF) and transforming growth factor-beta (TGF-β), no significant cross-reaction could be observed. The sensitivity of the assays was 10 pg/ml for the hIL-10/BCRF-1 ELISA and 20 pg/ml for the BCRF-1 ELISA. Intra-assay and interassay variations were <15% in both ELISA systems. Samples with a positive reaction for BCRF-1 were excluded from the study.

IFN-γ, sIL-2R, IL-1β, TNF-α and IL-6 were determined by ELISA assays developed by Immunotech (Marseille, France).

The IL-1β, IFN-γ and TNF-α assay had a sensitivity of 5 pg/ml. The IL-6 ELISA assay had a sensitivity of 3 pg/ml, while the sensitivity of the sIL-2R ELISA was 5 pM.

Serum of each patient (100 μl) was tested in duplicate. In all tests a standard cytokine preparation (recombinant cytokines with defined concentration) was used as internal control.

Statistical analysis

To calculate correlation coefficients we used the Person's correlation test. For comparison between groups the Mann–Whitney *U*-test was used.

RESULTS

Four patients with UC, two patients with CD and three healthy people were excluded from the study because of positive reactions with BCRF-1.

The mean serum concentrations of IL-10 were significantly higher in patients with active UC (144 ± 34 pg/ml (mean $\pm$ s.e.m.), $P < 0{\cdot}001$) and in active CD (132 ± 32 pg/ml, $P < 0{\cdot}001$) than in healthy controls ($44 \pm 9{\cdot}5$ ng/ml) (Fig. 1). In contrast, patients with inactive CD (52 ± 17 pg/ml) and inactive UC (73 ± 19 pg/ml) did not show any significant increase in IL-10 serum concentrations. Between CD and UC we could not find statistically significant differences concerning IL-10 serum levels.

Serum concentrations of IL-10 were correlated with clinical disease activity indices in patients with IBD. There was significant correlation between IL-10 serum concentration and clinical disease activity in patients with UC using the Rachmilewitz index ($r = 0{\cdot}399$, $P < 0{\cdot}05$). In patients with CD we compared IL-10 serum levels with clinical disease activity using the Crohn's disease activity index (CDAI). Similar to patients with UC, we could find significant correlation between IL-10 and CDAI ($r = 0{\cdot}45$, $P < 0{\cdot}01$).

To determine the dependence of IL-10 on steroid treatment we examined daily variations of IL-10 under medical treatment in individual patients. Out of nine patients with CD and 10 patients with UC, time courses of four patients with IBD (two

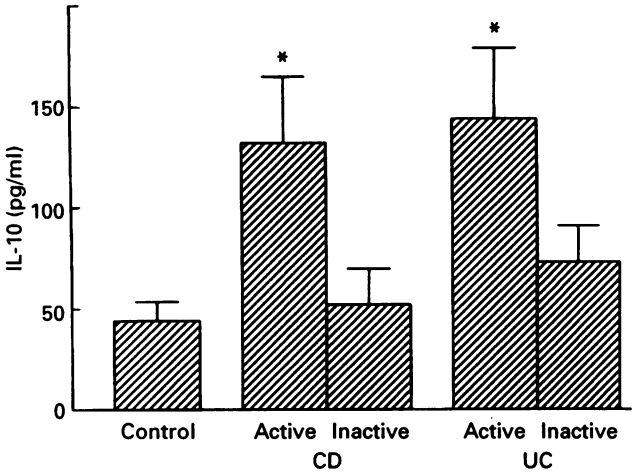

Fig. 1. Serum concentrations of IL-10 in patients with IBD and in healthy controls. IL-10 was determined in the serum of 44 patients with ulcerative colitis (UC) (20 with active and 24 with inactive disease), 40 patients with Crohn's disease (CD) (18 active, 22 inactive disease) and in 30 healthy controls. Active disease in UC was defined as Rachmilewitz index (CAI) $\geqslant 4$. Active disease in CD was defined as Crohn's disease activity index (CDAI) $\geqslant 150$. $^*P < 0{\cdot}001$ compared with control.

patients with CD and two with UC) are shown in Fig. 2. Under treatment with steroids we could initially find an increase of IL-10 values compatible with an elevated clinical disease activity index, and afterwards together with an attenuation of disease activity a decline of IL-10 serum concentration. There was no significant difference between patient groups with and without steroid therapy. This was true for patients with and without active or inactive disease (Table 2). Therefore, steroid treatment does not seem to have an influence on IL-10 level.

Because of the *in vitro* effects of IL-10, with important immunosuppressive effects including inhibition of the production of several cytokines, we determined serum levels of other lymphokines and compared them with serum IL-10 concentrations. In UC patients we could not find any significant correlation between IL-1β, TNF-α, IFN-γ, sIL-2R or IL-6 and the serum level of IL-10 (Table 3). In contrast, in acute CD enhanced concentrations of sIL-2R and IL-6 were encountered. Between IL-10 and IL-6 serum levels we could find a weak but significant correlation in individual patients ($r = 0{\cdot}387$, $P < 0{\cdot}05$). Serum levels of sIL-2R also showed significant correlation compared with IL-10 ($r = 0{\cdot}417$, $P < 0{\cdot}05$), while other cytokines such as IFN-γ, TNF-α and IL-1β did not show any significant correlation.

DISCUSSION

Increased serum concentrations of different cytokines have been described in CD and in UC. However, serum concentrations of cytokines with immunosuppressive effects such as IL-10 have not been investigated in IBD yet. The present study was performed to elucidate the role of IL-10 in IBD.

Our data show that most patients with UC and CD have significantly increased serum concentrations of IL-10 compared with healthy controls (Fig. 1). There was also a significant correlation between serum IL-10 levels and disease activity indices using the Rachmilewitz index (CAI) and the CDAI. Therefore, IL-10 serum levels seem to reflect disease activity in UC and CD. Similar correlation has also been described for serum levels of IL-6 and sIL-2 [26,27]. We examined daily variations of IL-10 levels in highly active UC and CD patients under treatment with intravenous steroids. All of the patients showed an increase in IL-10 serum levels, with a decline with the advent of remission. In patient groups with and without steroid therapy we could not find any significant difference (Table 2). Our results concerning the decline in IL-10 levels in

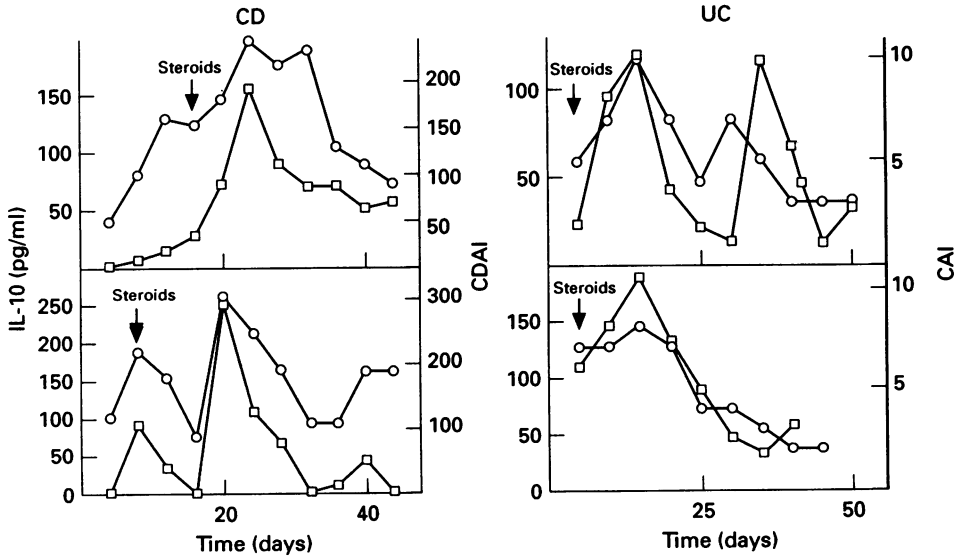

Fig. 2. Daily variations of IL-10 levels in four patients (two with Crohn's disease (CD) and two with ulcerative colitis (UC)) representative of 10 patients with highly active UC and nine patients with active CD under treatment with intravenous steroids. □, IL-10; ○, Crohn's disease activity index (CDAI)/Rachmilewitz index (CAI).

Table 2. Serum concentrations of IL-10 in Crohn's disease (CD) and ulcerative colitis (UC) in relation to clinical activity and steroid therapy

		n	IL-10 (pg/ml) ± s.e.m.
Active disease			
CD, CDAI ⩾ 150	Total	18	132 ± 32
	Steroids +	11	157 ± 41
	Steroids −	8	119 ± 27
UC, CAI ⩾ 4	Total	24	144 ± 34
	Steroids +	13	161 ± 41
	Steroids −	11	128 ± 28
Inactive disease			
CD, CDAI < 150	Total	22	52 ± 17
	Steroids	7	42 ± 19
	Steroids −	15	57 ± 17
UC, CAI < 4	Total	20	73 ± 19
	Steroids +	4	71 ± 11
	Steroids −	16	74 ± 23

CDAI, Crohn's disease activity index; CAI, Rachmilewitz index.

both IBD groups argue against an effect of steroids on IL-10 concentrations. Obviously an effect of the decreased disease activity can not definitely be ruled out in our clinical study.

Actually the source of IL-10 in serum of patients with IBD remains unknown. IL-10 is produced by various cell types. Human IL-10 is predominantly secreted by $CD4^+$ T cells. Most Th2, Th1 and Th0 cell clones are able to produce considerable amounts of this cytokine [13]. In addition, $CD8^+$ T cell clones, human monocytes and EBV-transformed lymphoblastoid cell lines also secrete IL-10 following activation [17,28,29]. Concerning the source of circulating IL-10 in IBD, there is evidence that both peripheral blood mononuclear cells (Kucharzik *et al.*, unpublished data) as well as intestinal mucosal cells [8,9] are capable of producing IL-10.

The activity of IL-10 was initially described as cytokine synthesis inhibitory factor [10]. Inhibitory effects on production of different cytokines and on monocyte and T cell effector function have been reported [11,12]. These data suggest that IL-10 acts as a natural damper of immune proliferation and inflammatory response. In IBD, IL-10 seems to be up-regulated as a response to proinflammatory cytokine activity. It could be that the immunosuppressive effect of IL-10 is probably not strong enough for dampening inflammatory activity in the mucosa.

In contrast to our results, some authors determined increased IL-10 mRNA levels in patients with UC but not CD [8,9]. They postulate that UC is mediated by a Th2 cell response, while CD is associated with a Th1 response. These data raise the possibility that acute phase responses in CD and UC are mediated by different cytokines. We determined increased hIL-10 concentrations both in active CD and in active UC. Therefore, we cannot follow the hypothesis of different lymphokine production in UC and CD. Furthermore, we assessed serum levels of other cytokines in correlation to the IL-10 level (Table 3). No significant positive correlation between serum IL-10 and TNF-α, IFN-γ, IL-1β, sIL-2R and IL-6 in UC could be measured. In contrast, we were able to find positive correlation between the cytokines IL-6 and sIL-2R and serum concentrations of IL-10 in patients with CD. It is conceivable that both proinflammatory and anti-inflammatory cytokines are up-regulated in IBD and influence each other. The missing correlation to other cytokines does not mean that there is no regulatory influence of IL-10 on the production of these cytokines. Several reports have demonstrated increased mRNA levels of different cytokines or altered cytokine production by lamina propria mononuclear cells in patients with IBD [1,2]. Serum levels do not usually reflect alterations in mucosal immunity and spontaneous lymphokine secretion in monocytes [3,4]. There are reports of increased mRNA levels of IL-1β, IL-4, IL-6 and IL-8 in UC [4]. These lymphokines are down-regulated *in vitro* by human and viral IL-10 [10,11,13]. IL-10 and other immunosuppressive cytokines such as IL-13 [30] may down-regulate these cytokines in IBD.

Table 3. Correlation between IL-10 serum levels and other cytokine serum concentrations

	IL-1β	sIL-2R	IFN-γ	TNF-α	IL-6
CD	0·09	0·417*	−0·094	−0·059	0·387*
UC	−0·086	0·097	0·102	−0·003	−0·089

* $P < 0{\cdot}05$.
CD, Crohn's disease; UC, ulcerative colitis.

Important evidence for the role of IL-10 as immunoregulator in the intestinal tract is provided by data from IL-10-deficient mice who developed chronic enterocolitis [31,32]. These mice showed aberrant expression of MHC class II molecules on intestinal epithelia, indicating an uncontrolled immune response stimulated by enteric antigens. Similar results have been reported in IL-2 gene disrupted mice and T cell receptor mutant mice who developed chronic bowel inflammation [33,34]. Although it is not possible to transfer the situation of animal models to the human system, these data might contribute to further understanding of the pathogenesis of human idiopathic inflammatory bowel disease. It remains unknown, however, whether increased serum concentrations of IL-10 reflect physiological anti-inflammatory activity or dysregulation of cytokines in the inflamed mucosa.

Our data for the first time demonstrate the occurrence of anti-inflammatory cytokines like human IL-10 in serum of patients with IBD. Correspondingly, other investigators have previously described increased serum levels of different proinflammatory cytokines in patients with active CD as well as in active UC. Others found continuously increased serum concentrations of cytokines such as IL-6 in active as well as in inactive CD [35]. Our data show that the naturally occurring counterpart of these cytokines, i.e. IL-10, is also increased in the serum of active IBD patients. It needs, however, further elucidation whether and to what extent the anti-inflammatory activity, i.e. the down-regulatory action of IL-10 on the mucosal immune system, contributes to the overall presentation of the inflammatory process in IBD.

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