Interrelations between interleukin-6, interleukin-1 β , plasma C-reactive protein values, and in vitro C-reactive protein generation in patients with inflammatory bowel disease

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Abstract

Acute phase proteins are released from the liver in response to cytokines, and measurement of serum concentrations offers a valuable means of assessing inflammatory bowel disease. C-reactive protein (CRP) is a participating prominent component of the acute phase response in active Crohn's disease. This study aimed at determining the comparative role of the cytokines interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), in driving CRP production in inflammatory bowel disease, and to test the hypothesis that there is a difference in the profile of cytokines generated in these two conditions. Serum CRP, the release of the cytokines IL-1 β and IL-6 from monocytes, and the ability of monocyte conditioned medium to stimulate CRP synthesis by hepatocytes in an in vitro system was measured in patients with ulcerative colitis and Crohn's disease. Monocytes from patients with Crohn's disease produced more IL-1 β than monocytes from patients with ulcerative colitis or normal controls. There was no increased tendency for monocytes from Crohn's disease patients to produce more IL-6, so the greater circulating values of IL-6 reported by a number of authors in Crohn's disease may reflect the participation of a larger number of cells of the monocyte-macrophage series, or production of IL-6 by other cell types. Correlation of cytokine production by monocytes with in vitro CRP release from cultured hepatocytes in response to monocyte conditioned medium showed that, in that system, IL-1 β was the stronger stimulus to CRP production. Some of the differences in the inflammatory processes of ulcerative colitis and Crohn's disease may reflect differences in the amount of IL-1 β and IL-6 generated from macrophages and monocytes.

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Multiple nucleated cells including macrophages and circulating blood monocytes are capable of producing cytokine mediators such as interleukin-1 β (IL-1 β), tumour necrosis factor (TNF- α), and interleukin-6 (IL-6).¹ Attention has now turned to these as potential mediators of inflammation in inflammatory bowel disease. Cytokines may play this part in the mucosa, and in addition be responsible for some systemic reactions in inflammatory bowel disease. Many clinical and biochemical features of ulcerative colitis and Crohn's disease including fever,

increased production of acute phase reactants, hypoalbuminaemia, and fibroblast proliferation with fibrosis may be attributable to the actions of cytokines.²

Activated macrophages and monocytes produce a panel of cytokines with different but sometimes overlapping actions. IL-1 β and TNF- α are notably proinflammatory, being important early signals in the inflammatory process that can induce regulatory changes in a wide variety of cell types, as well as initiating the release of a cascade of other mediators.^{3,4} IL-1 β and TNF- α can also stimulate each others' production in vitro and in vivo.⁵ There are differences in the spectrum of actions of IL-6,⁶ which can be induced by IL-1 and TNF- α , does not stimulate IL-1 β or TNF production, and indeed has been shown to inhibit their synthesis by macrophages in vitro, implying that at least some of its actions down regulate the amplificatory actions of cytokines.^{6,7} A growing body of evidence, referred to later, points to an enhanced production of many cytokines in inflammatory bowel disease.

Our interest in the role of cytokines developed from two related aspects of inflammatory bowel disease. The uncertain relation between ulcerative colitis and Crohn's disease – whether different conditions or part of a continuous disease spectrum – prompts careful examination of those differences that can be defined. The interest in our laboratory on the assessment of the inflammatory activity of these conditions, particularly the use of acute phase reactants, highlighted one such difference, the tendency to find greater increases in circulating C-reactive protein (CRP) in patients with active Crohn's disease compared with ulcerative colitis.^{8,9} CRP is generated by the liver in response to cytokines, and both IL-1 β and IL-6 enhance CRP production, the first at a translational and the second at a transcriptional level.^{10,11} We reported that – for each monocyte – cells from Crohn's disease patients produce more IL-1 β than cells from ulcerative colitis patients or controls, compatible with the suggestion that there is an innate difference between the tendency for patients with Crohn's disease and ulcerative colitis to produce this cytokine when stimulated.¹²

Patients with Crohn's disease were also recently reported to have increased plasma IL-6 concentrations compared with ulcerative colitis.¹³⁻¹⁶ The first aim of this study was therefore to confirm whether production of IL-6 by peripheral blood monocytes, as representatives of the monocyte macrophage lineage, was increased in Crohn's disease compared with

ulcerative colitis, again exploring whether there were qualitative rather than merely quantitative differences behind the differences in acute phase response in these two conditions. We therefore measured IL-6 production in patients with Crohn's disease and ulcerative colitis. We assessed *in vitro* release of cytokine from monocytes after stimulation with lipopolysaccharide, and used a fixed number of cells, to avoid the possibility that a greater cytokine response in Crohn's disease might merely reflect a greater number of cells participating in the inflammatory response. We also measured IL-1 β in this second series of patients.

The second aim of this study was to see if differences in the profile of cytokines present in Crohn's disease and ulcerative colitis could account for the greater CRP response associated with active Crohn's disease. We therefore assessed the effect of the cytokine rich monocyte conditioned media, of known IL-1 β and IL-6 concentration, in initiating release of CRP from a hepatocyte derived cell line, to find out if the CRP response could be attributed to one or other cytokine predominantly. We assessed the value of this *in vitro* model, by checking if the CRP released *in vitro* correlated with the circulating CRP concentrations in the patients from whom the monocytes had been harvested.

Patients and methods

PATIENTS AND CONTROLS

Patients with inflammatory bowel disease included in this study consisted of 22 patients with Crohn's disease (11 males and 11 females; age: mean (range) 37.9 (23–62) y) and 22 patients with ulcerative colitis (12 males and 10 females; age 45.0 (19–79) y). Eleven healthy volunteers comprising of six men and five women (age 39.9 (30–62) y) served as normal controls.

DISEASE ACTIVITY

In the Crohn's disease group 11 patients were quiescent (Harvey-Bradshaw index of 1) and 11 had active disease (Harvey-Bradshaw index: mean (SEM) 6.2 (0.8)).¹⁷ In terms of extent of the disease, nine patients had ileitis, five ileocolitis, and eight colitis. Eleven patients with ulcerative colitis were active (Truelove and Witts),¹⁸ seven mildly and four moderately. In terms of disease distribution, one patient had pancolitis, 17 had left colon involvement (four proctosigmoiditis, nine had involvement of the rectum, sigmoid, and descending colon, and four had involvement of the rectum, descending, and transverse colon), and four had disease limited to the rectum.

TREATMENT GROUPS

In the Crohn's disease group eight patients were not receiving treatment. Four patients were receiving sulphasalazine; one of these was also receiving prednisolone. Three patients were being treated with 5-aminosalicylic acid (5-ASA); two of these were also receiving prednisolone. Four patients were receiving prednisolone alone, one patient was treated with prednisolone and

azathioprine, one patient received treatment with prednisolone, azathioprine, and 5-ASA, and one patient was treated with prednisolone and cyclosporin A.

In the ulcerative group two patients were not receiving treatment. Five patients were receiving sulphasalazine; one of these was also receiving prednisolone. Twelve patients were receiving treatment with 5-ASA and six of these were also receiving steroid enemas. One patient received treatment with steroid enemas alone, one patient received treatment with prednisolone alone, and one patient was treated with prednisolone, azathioprine, and 5-ASA.

MONOCYTE SEPARATION

From each patient with ulcerative colitis and Crohn's disease, and normal controls 20 ml of EDTA treated blood was obtained and diluted 1:3 with Hanks's balanced salt solution (HBSS), and mononuclear cells were separated on a Isopaque-Ficoll density gradient (Lymphoprep; Nycomed, UK).¹⁹ Peripheral blood mononuclear cells were collected from the interphase and washed twice, once in HBSS and once in serum free RPMI 1640 media (Gibco, UK). Cells were then resuspended in RPMI 1640 medium supplemented with 10% (vol/vol) heat inactivated (35 minutes at 56°C) fetal calf serum, 4 mM glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, and 25 mM hydroxyethylpiperazine-ethanesulphonic acid (HEPES) buffer (complete medium). The suspension of cells containing monocytes was adjusted to contain 2×10^6 monocytes/ml, by peroxidase staining, before adherence and then plated on 60 mm tissue culture plates (Nunc) and left to adhere firmly to the plates for one hour at 37°C in a 5% CO₂: 95% air tissue culture incubator. The non-adherent lymphocytes were removed by washings with warm serum free RPMI 1640 to ensure pure monocytes were obtained.

CYTOKINE PRODUCTION

Monocytes obtained from patients and controls were cultured with or without 10 mg/ml lipopolysaccharide to induce cytokine production. After a 24 hour incubation period monocyte conditioned medium was collected, centrifuged, and stored at -70°C.

The cytokine rich monocyte conditioned medium was utilised to stimulate CRP release from a human hepatoma cell line, Alexander cells (PLC/PRF/5).^{20,21} The amount of IL-6 and IL-1 β released into these monocyte supernatants were also determined by enzyme linked immunosorbent assay (ELISA).

IN VITRO GENERATION OF CRP IN ALEXANDER CELLS BY MONOCYTE CONDITIONED MEDIUM

For stimulation of *in vitro* CRP production, confluent monolayers of Alexander cells were trypsinised and a suspension of Alexander cells in 25 ml complete medium at 100 000 viable cells/ml was established. One ml aliquots were placed into 24 well tissue culture plates and Alexander cells were allowed to reach

confluence. Equal volumes of monocyte conditioned medium and fresh complete medium were added and incubated in a 5% CO₂:95% air tissue culture incubator. After 72 hours supernatant medium was collected and stored at -20°C.

MEASUREMENT OF CRP

Serum was separated from clotted venous blood and stored at -20°C. Serum CRP estimation and the amount of *in vitro* CRP release were analysed by a double ligand sandwich ELISA performed in duplicate as previously described.¹² The lower detection limit of the CRP assay was 0.05 ng/ml, with a 7% coefficient of variation in assay and 15% between assay.

ELISA FOR IL-1 β

Quantitative estimation of IL-1 β was performed in duplicate. High binding ELISA plates (Greiner, UK: 655061) were coated with 100 μ l/well, rabbit-recombinant human IL-1 β (Universal Biologicals, UK) at a concentration of 5 μ l in coating buffer (0.05 M carbonate; pH 9.6) for at least three days (88 hours) at 4°C. After washing three times with phosphate buffered saline containing 0.05% TWEEN-20 (PBS/0.05% Tween-20; wash buffer) plates were blocked with blocking buffer (PBS with 1% BSA; 100 ml/well) for four hours at room temperature. After three washes 100 μ l of supernatant medium samples containing IL-1 β or recombinant human IL-1 β standards (British Bio-technology, UK) in doubling dilutions (20 pg/ml–100 ng/ml; standards diluted in complete medium), were added and the plates incubated overnight (16 hours) at 37°C in a moist box. After four washes 100 μ l/goat anti-human IL-1 β (British Bio-technology, UK) at a concentration of 1 mg/ml (diluted in conjugate buffer; PBS/0.5% BSA) were added and the plates incubated for two hours at 37°C. After four washes, 100 ml/well peroxidase conjugated affinipure donkey anti-goat IgG (Jackson ImmunoResearch Laboratories, USA) diluted 1:2000 in conjugate buffer (PBS/0.5% BSA) was added and the plates incubated at room temperature for a further two hours. After five washes substrate solution (H₂O₂-OPD; 100 ml/well) was added and the plates were incubated in the dark at room temperature. The reaction was terminated by adding 100 μ l 3M sulphuric acid and absorbance read at 492 nm in an ELISA plate reader. The lower detection limit was 20 pg/ml, with a 7.1% within and 5.4% between assay coefficient of variation.

ELISA FOR IL-6

IL-6 estimation was performed in duplicate. High binding ELISA microtitre plates (Greiner, UK; 655061) were coated with 100 μ l/well rabbit anti-recombinant human IL-6 (genzyme) at a concentration of 5 mg/ml in coating buffer (0.05 M carbonate; pH 9.6) for at least two days (64 hours) at 4°C. After washing three times with phosphate buffered saline containing 0.05% TWEEN-20 (PBS/0.05% Tween-20; wash

buffer) plates were blocked with blocking buffer (PBS with 1% BSA; 100 ml/well) for two hours at room temperature. Plates were then washed again three times before adding 100 μ l of supernatant medium samples containing IL-6 or IL-6 standards in doubling dilutions (50 pg/ml–50 ng/ml; standards diluted in complete medium). The plates were then incubated for two hours at 37°C in a moist box. After four washes 100 μ l goat anti-human IL-1 β (British Bio-technology) at a concentration of 1 mg/ml (diluted in conjugate buffer; PBS/0.5% BSA) was added and the plates incubated for a further two hours at 37°C. After four washes, 100 μ l/well peroxidase conjugated affinipure donkey anti-goat IgG (Jackson ImmunoResearch Laboratories, USA) diluted 1:2000 in conjugate buffer (PBS/0.5% BSA) was added and the plates then incubated for a further two hours at room temperature. After five washes 100 μ l/well substrate solution (H₂O₂-OPD) was added and the plates incubated in the dark at room temperature to the desired extinction. The reaction was stopped by adding 100 μ l 3M sulphuric acid and absorbance read 492 nm in an ELISA plate reader.

In this assay system recombinant human interleukin-1 β used for the standard curve was obtained from British Bio-technology. Recombinant human interleukin-6 (code 88/514) and interleukin-1 α standards (code 86/632) used in these assays were also kindly provided by National Institute for Biological Standards and Controls (NIBSC), Blanche Lane, South Mimms, Potters Bar, Hertfordshire, England. The antibodies used include rabbit anti-recombinant human IL-1 β (Universal Biologicals, UK) and goat anti-human IL-1 β (British Bio-technology), which has no cross reactivity with IL-1 α , IL-2, IL-6, and TNF (α or β). Rabbit anti-recombinant human IL-6 (genzyme; LP-716) and goat anti-human IL-6 (British Bio-technology) do not cross react with IL-1 (α or β), TNF (α or β), and GM-CSF.

The lower detection limit of the IL-6 assay 50 was pg/ml, with a 14% between and within assay coefficient of variation. There was no cross reaction between these assays; and in addition IL-1 α did not interfere with the assay.

STATISTICS

Non-parametric procedures were used for statistical significance testing. Comparisons between groups were assessed by Mann-Whitney U test, and correlation coefficients were calculated using Kendall rank correlation test.

Results

IN VITRO PRODUCTION OF IL-1 β

The potential of monocytes from Crohn's disease to generate IL-1 β was found to be significantly higher than ulcerative colitis.

Spontaneous IL-1 β release by monocytes in Crohn's disease (mean (SEM) 562 (282) pg/ml) was significantly raised compared with ulcerative colitis (75 (29) pg/ml; $p < 0.05$) and normal controls (29 (7) pg/ml; $p < 0.05$). Lipopolysaccharide stimulated IL-1 β generation in Crohn's

disease (mean (SEM) 16 573 (5879) pg/ml) was also significantly higher compared with ulcerative colitis (4505 (1403); $p<0.005$) and normal controls (3678 (1000) pg/ml; $p<0.05$).

In patients with inactive Crohn's disease both spontaneous and lipopolysaccharide-stimulated IL-1 β (mean (SEM) 967 (545) and 11 791 (4995) pg/ml, respectively) release were significantly greater than in active ulcerative colitis (41 (21) pg/ml; $p<0.05$ and 3404 (1378) pg/ml; $p<0.05$, respectively).

In active Crohn's disease both spontaneous and lipopolysaccharide-stimulated IL-1 β (mean (SEM) 158 (66) and 21 355 (10 755) pg/ml, respectively) release from monocytes were raised compared with active ulcerative colitis (108 (53) pg/ml and 5606 (2476) pg/ml, respectively; $p>0.05$) although the difference did not reach statistical significance.

PERIPHERAL BLOOD MONOCYTE COUNT

The number of peripheral blood monocytes for Crohn's disease (mean (SEM) $5.3 (0.4) \times 10^9$ /ml) was greater compared with ulcerative colitis ($4.6 (0.35) \times 10^9$ /ml; $p>0.05$) and normal controls ($3.3 (0.25) \times 10^9$ /ml; $p<0.05$), although the difference was only significant when compared with normal controls. Among the patients with inflammatory bowel disease, those who had active inflammation (mean (SEM) $5.4 (0.4) \times 10^9$ /ml; $p<0.05$) had significantly greater monocyte counts than those without ($4.6 (0.35) \times 10^9$ /ml) similar to the findings described above.

IN VITRO PRODUCTION OF IL-1 β RELATED TO TOTAL MONOCYTE COUNT

Calculated spontaneous and lipopolysaccharide stimulated production of IL-1 β , related to total count of monocytes per 10 ml blood, were both strikingly and statistically significantly raised in Crohn's disease compared with ulcerative colitis and normal controls (Table).

Both spontaneous and lipopolysaccharide stimulated IL-1 β release related to total monocyte count per 10 ml blood from patients with inactive Crohn's disease were significantly raised compared with inactive ulcerative colitis. In active Crohn's disease lipopolysaccharide stimulated IL-1 β production related to total count of monocytes per 10 ml of patients blood was raised compared with active ulcerative colitis. There was no statistical difference

between the concentrations of IL-1 β generated spontaneously in active Crohn's disease and active ulcerative colitis.

IN VITRO PRODUCTION OF IL-6

Both spontaneous and lipopolysaccharide stimulated IL-6 production by monocytes from patients with Crohn's disease (mean (SEM) 1606 (249) and 3244 (363) pg/ml, respectively) were similar to ulcerative colitis (1064 (155) and 2457 (250) pg/ml, respectively) and normal controls (1473 (295) and 2802 (352) pg/ml, respectively).

In patients with inactive Crohn's disease both spontaneous and lipopolysaccharide stimulated IL-6 (mean (SEM) 1720 (450) and 2802 (352) pg/ml, respectively) production were not significantly different to inactive ulcerative colitis (1001 (242) and 2361 (354) pg/ml, respectively).

Similarly in patients with active Crohn's disease both spontaneous and lipopolysaccharide stimulated IL-6 (mean (SEM) 1492 (237) and 3008 (346) pg/ml, respectively) release were not significantly different compared with active ulcerative colitis (1127 (205) and 2553 (368) pg/ml, respectively).

IN VITRO PRODUCTION OF IL-6 RELATED TO TOTAL MONOCYTE COUNT

No difference was found between the capacity of the same number of monocytes from patients with ulcerative colitis and Crohn's disease to generate IL-6. To assess the capacity of a given volume of blood from a patient in each group to generate IL-6, the in vitro production described above was related to the number of circulating monocytes.

Calculated spontaneous and lipopolysaccharide stimulated IL-6 production related to the total count of monocytes per 10 ml blood from patients with Crohn's disease were both significantly greater than from patients with ulcerative colitis (Table). In normal controls both spontaneous and lipopolysaccharide stimulated IL-6 release related to total monocyte count were similar to ulcerative colitis. Lipopolysaccharide stimulated IL-6 generation from controls was less than from patients with Crohn's disease ($p<0.005$), but spontaneous IL-6 generation did not differ significantly between these two groups. When results in active Crohn's disease were compared with active ulcerative colitis, and inactive Crohn's disease compared with inactive ulcerative colitis, although mean values were higher in Crohn's disease, statistical differences did not emerge. Similarly, calculated IL-6 production did not rise with disease activity in either group.

IN VITRO CRP

Release of CRP from Alexander cells by unstimulated and lipopolysaccharide stimulated monocyte conditioned medium from patients with inactive Crohn's disease (mean (SEM) 0.79 (0.20) ng/ml and 1.50 (0.32) ng/ml respectively) was not significantly different compared with inactive ulcerative colitis (0.68 (0.14) ng/ml and 2.0 (0.19) ng/ml respectively) and normal con-

In vitro interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) production related to total monocyte count per 10 ml blood obtained from patients with ulcerative colitis, Crohn's disease, and controls

	Spontaneous IL-1 β pg/ml (mean (SEM))	LPS stimulated IL-1 β pg/ml (mean (SEM))	Spontaneous IL-6 pg/ml (mean (SEM))	LPS stimulated IL-6 pg/ml (mean (SEM))
CD (n=22)	1949 (929)	49 151 (19 716)	3992 (603)	8540 (1276)
UC (n=22)	134 (43)†	8 613 (2362)‡	2372 (363)*	5479 (546)*
Controls (n=11)	50 (13)§	6 730 (2066)†	25 49 (579)	4220 (552)*
Inactive CD (n=11)	3412 (1770)	35 329 (20 253)	3828 (991)	8354 (2107)
Inactive UC (n=11)	77 (33)¶	6 620 (2349)¶	2198 (592)	5033 (773)
Active CD (n=11)	486 (257)	62 973 (34 412)	4155 (737)	8727 (1547)
Active UC (n=11)	192 (77)	10 607 (4139)¶	2546 (445)	5924 (783)

* $p<0.05$ compared with Crohn's disease, † $p<0.01$ compared with Crohn's disease, ‡ $p<0.005$ compared with Crohn's disease, § $p<0.001$ compared with Crohn's disease, ¶ $p<0.05$ compared with active Crohn's disease, ¶ $p<0.05$ compared with inactive Crohn's disease. CD=Crohn's disease, UC=ulcerative colitis.

Figure 1: Release of C-reactive protein (CRP) from Alexander cells by unstimulated and lipopolysaccharide (LPS) stimulated monocyte conditioned medium from patients with ulcerative colitis (UC) and Crohn's disease (CD). CRP release in response to active Crohn's disease conditioned medium is significantly increased (see text) in both cases, compared with active ulcerative colitis and controls.

controls (0.61 (0.09) ng/ml and 1.30 (0.12) ng/ml respectively) (Fig 1).

Both unstimulated and lipopolysaccharide stimulated monocyte conditioned medium from patients with active Crohn's disease (mean (SEM) 1.73 (0.40) ng/ml and 3.48 (0.87) ng/ml respectively) caused significantly greater CRP release from Alexander cells than monocyte conditioned medium from active ulcerative colitis (0.67 (0.17) ng/ml; $p<0.02$ and 2.01 (0.38) ng/ml; $p<0.05$ respectively) and normal controls (0.61 (0.09) ng/ml; $p<0.02$ and 1.30 (0.12) ng/ml; $p<0.002$ respectively) (Fig 1).

CORRELATION OF IL-1 β AND IL-6 IN CONDITIONED MEDIUM WITH STIMULATION OF IN VITRO CRP SYNTHESIS

Among individual patient groups, Crohn's disease and ulcerative colitis, active and inactive, significant correlations between in vitro CRP values and the value of either IL-1 β or IL-6 in conditioned medium did not emerge.

Analysis performed on all inflammatory bowel disease patients combined showed statistically significant correlations between in vitro CRP synthesis in response to monocyte conditioned medium, and the IL-1 β and IL-6 values in the medium, when the conditioned medium was obtained in the absence of lipopolysaccharide ($r=0.74$; $p<0.001$ and $r=0.18$; $p<0.05$ respectively) but not in the presence of lipopolysaccharide ($r=0.14$; $p>0.05$ and $r=0.14$; $p>0.005$ respectively).

SERUM CRP CONCENTRATIONS

Serum CRP concentration in inactive Crohn's disease (mean (SEM) 5.7 (2.6) mg/l) was not significantly different from inactive ulcerative colitis (3.0 (1.5) mg/l) although it was significantly more than normal controls (1.6 (0.6) mg/l; $p<0.05$).

Serum CRP concentration in patients with active Crohn's disease (53.6 (16.2) mg/l) was however considerably raised compared with active ulcerative colitis (10.7 (2.3) mg/l; $p<0.02$) and normal controls ($p<0.002$), as well as inactive Crohn's disease ($p<0.005$) (Fig 2).

CORRELATION OF SERUM CRP WITH IN VITRO CRP RELEASE

In inflammatory bowel disease patients we found a significant correlation between serum CRP, and in vitro CRP release in response to monocyte conditioned medium in the absence of lipopolysaccharide ($r=0.28$; $p<0.005$) but not in the presence of lipopolysaccharide ($r=0.17$; $p>0.05$).

Discussion

In this study we investigated differences in the following parameters between ulcerative colitis and Crohn's disease: (a) serum CRP concentrations, (b) release of the cytokines IL-6 and IL-1 β from monocytes, and (c) the effects of these monocyte conditioned media on an in vitro model of CRP release.

Figure 2: Serum C-reactive protein (CRP) concentration is considerably raised in patients with active Crohn's disease (CD) ($n=11$) compared with active ulcerative colitis ($p<0.02$) and normal controls ($p<0.002$). In inactive CD ($p>0.05$) serum CRP is not significantly different compared with inactive ulcerative colitis (UC). Results expressed as mean (SEM).

This study confirms previous reports that serum CRP concentration is significantly higher in patients with active Crohn's disease compared with active ulcerative colitis.^{8-9,22} Further findings were then made using a human hepatoma cell line, Alexander cells, as a means of assessing the comparative contribution of different cytokines to the CRP response,²³ albeit by the use of a cell line whose responses to cytokines may not fully represent physiological events. In this system we showed that cytokine rich medium conditioned by monocytes from patients with active Crohn's disease resulted in significantly greater CRP release than monocyte conditioned medium from active ulcerative colitis and normal controls. The results support the hypothesis that differences in serum CRP concentrations in ulcerative colitis and Crohn's disease may reflect differences in cytokine production in the two diseases.

There is emerging evidence from a number of studies of differences in the tendency of patients with Crohn's disease and ulcerative colitis to produce cytokines IL-1 β and IL-6. Raised serum concentrations of IL-1 β have been reported in patients with acute exacerbations of Crohn's disease, but not those with quiescent disease.^{14,24,25} In patients with ulcerative colitis IL-1 β activity was rarely found in the plasma even during active disease.²⁶ Several groups report that circulating IL-6 concentrations are higher in Crohn's disease compared with ulcerative colitis.¹³⁻¹⁶ We have investigated one source of these cytokines, the monocytes from peripheral blood as a representative of the monocyte-macrophage series, and have attempted to discover if there are differences in IL-1 β and IL-6 production between the two diseases.

In this second study, in a new series of patients, we confirmed our previous report that interleukin-1 β production by peripheral blood monocytes is greater in Crohn's disease than ulcerative colitis.¹² We showed this with a fixed number of monocytes from each patient, so the difference in IL-1 β production between the two conditions was not due to the trivial explanation that a greater number of monocytes were studied in the cell population sampled in Crohn's disease. The results suggest a genuine difference in the capacity of Crohn's disease monocytes to produce IL-1 β . In addition, however, the greater number of circulating monocytes implies that in Crohn's disease IL-1 β expression may be even greater, and at the tissue level macrophages are particularly prominent in Crohn's disease. Thus, a higher IL-1 β circulating concentration in Crohn's disease compared with ulcerative colitis probably reflects both a greater potential for cells of the monocyte-macrophage series to generate this cytokine and also a greater number of cells affected.

In contrast with the above findings we found that IL-6 production by a given number of monocytes, both spontaneously and after stimulation with lipopolysaccharide, did not differ between Crohn's disease, ulcerative colitis, and normal controls. The greater number of circulating monocytes, however, in the group of patients studied implies the potential for more IL-6 release from this source, and may contribute to

the raised circulating IL-6 concentration in Crohn's disease. Interleukin-6 expression can also be induced in many other cell types. In normal subjects the IL-6 gene is transcribed at high values in the spleen, liver, and kidney, and endothelial cells, fibroblasts, T cells, B cells, and other cell types can produce IL-6 in response to a wide range of stimuli.²⁷⁻²⁹ With this diversity of cells and signals stimulating IL-6 production, it is clearly difficult to establish the contribution of individual cell types to circulating IL-6 concentrations.

The concentration of IL-1 β and to a lesser extent IL-6 in monocyte conditioned medium correlated significantly with the ability of those media to induce in vitro CRP release from the human hepatocyte derived cell line. The correlation was with unstimulated cytokine production, as would be expected if in vitro cytokine production from monocytes reflects contemporaneous in vivo cytokine production; the stimulated production with maximal lipopolysaccharide doses is likely to reflect the maximum potential capacity to respond. While both cytokines may therefore induce CRP expression in inflammatory bowel disease, the correlation between IL-1 β and CRP release was stronger than that between IL-6 and CRP release, suggesting that the in vitro system that we studied for IL-1 β is probably the more powerful stimulant of CRP release. On these grounds, the higher circulating CRP concentrations in Crohn's disease may largely be attributed to the greater level of IL-1 β drive.

One may speculate that there are other clinical differences between ulcerative colitis and Crohn's disease that reflect differences in the profiles of cytokines between Crohn's disease and ulcerative colitis. The tendency to transmural granulomatous inflammation and fibrosis and resultant gut stenosis are among the most striking differences between Crohn's disease and ulcerative colitis. IL-1 is possibly important in the initiation of granuloma. This has been reported in animal models.³⁰ Shikama *et al*³¹ induced granulomas in vitro by culturing murine spleen cells with artificial microparticles and showed that culture supernatants contained high values of IL-1 activity, correlating with granuloma size. Additionally, granulomas were produced by culturing spleen cells in the presence of agarose beads coupled to recombinant IL-1.³¹ Increased fibrosis reflects enhanced collagen synthesis by fibroblasts that can be regulated by a number of inflammatory mediators. Several macrophage derived cytokines are potentially fibrogenic, including IL-1 β and TNF- α . IL-1 β and TNF, individually, stimulate the proliferation and collagen production although findings differ among various studies.³²⁻³⁶ They can directly increase the transcription of type I, III, and type IV collagen,³⁷⁻³⁹ and increase fibroblast proliferation.⁴⁰ Another intriguing aspect with regard to cytokine release is the potential involvement of smoking habits. Smokers, as a population, have higher CRP concentrations than non-smokers,⁴¹ and alveolar macrophages from smokers produce more IL-1 on stimulation⁴²; could the striking association with Crohn's disease reflect the effect of this environ-

mental agent on IL-1 β release? It may be that patients who have Crohn's disease manifest this form of inflammatory disease in the bowel reflecting a combination of a genetically determined tendency and environmental influences towards the generation of a cytokine network that leads to transmural inflammation and considerable fibrosis, while patients with ulcerative colitis have a tendency to generate a cytokine network that causes diffuse mucosal inflammation.

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