

# High circulating concentrations of interleukin-6 in active Crohn's disease but not ulcerative colitis

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## Abstract

Peripheral venous plasma concentrations of interleukin-6 were studied in 21 patients with active Crohn's disease, 20 patients with ulcerative colitis, and 16 control subjects. Interleukin-6 was detected in the plasma of 18 of 21 patients with Crohn's disease (median 47 (range <20-250) pg/ml) but in only two with ulcerative colitis and two control subjects. In the patients with Crohn's disease there was a significant negative correlation between the plasma interleukin-6 and the serum albumin concentrations. In eight patients with Crohn's disease and five patients with ulcerative colitis undergoing resection plasma from peripheral circulation and mesenteric vein draining diseased intestine was studied. Interleukin-6 was detected in seven of eight peripheral and mesenteric samples from the patients with Crohn's disease but was not detected in any of the samples from the patients with ulcerative colitis. There was no significant difference between mesenteric and peripheral samples in the concentrations of interleukin-6.

Interleukin-6 has multiple biological actions which led to many initial designations by several laboratories. These include B cell stimulatory factor 2/B cell differentiation factor, interferon- $\beta_2$ , hybridoma/plasmacytoma growth factor, and 26 kDa protein.<sup>1</sup> It has a variety of functions<sup>2</sup> which include induction of differentiation of activated B cells to immunoglobulin secreting plasma cells and induction of acute phase proteins by hepatocytes<sup>3</sup> which is characterised by a rise in C reactive protein and a fall in albumin synthesis. Interleukin-6 has been shown to have an accessory role in T cell activation and proliferation. It also preferentially stimulates granulocyte and macrophage colony formation.

In inflammatory bowel disease there is an increase in the mucosal population of T cells, B cells, plasma cells, and macrophages as well as neutrophils. Circulating as well as mucosal lymphocytes and cells of the mononuclear phagocyte system are in an enhanced state of activation.<sup>4-7</sup> An acute phase response also occurs in both diseases in relapse and this is a useful way of assessing the activity of the disease and response to treatment. As interleukin-6 has been shown to induce the synthesis of acute phase proteins, we have investigated circulating concentrations of this cytokine in patients with active ulcerative colitis and Crohn's disease and studied their relation with some of the parameters of the acute phase response.

## Methods

### STUDIES ON PLASMA

#### Study population

Twenty one patients with active Crohn's disease (12 men, nine women; age range 16-74 years) were studied. Thirteen had small bowel disease only, five colonic disease only, and three ileocolonic disease. Twenty patients had active ulcerative colitis (eight men, 12 women; age range 17-79 years). Seven patients with inflammatory bowel disease (five with Crohn's disease and two with ulcerative colitis) were taking oral steroids, 18 a 5 aminosalicylic acid (five with Crohn's disease and 13 with ulcerative colitis), and five azathioprine (four with Crohn's disease). Sixteen normal healthy control subjects were also studied (six men, 10 women; age range 21-54 years).

The clinical activity of Crohn's disease was assessed using the Harvey-Bradshaw index<sup>8</sup> and of ulcerative colitis by a scoring system modified from Powell-Tuck *et al.*<sup>9</sup> Active disease was considered to be present if the score was over 5.

In eight patients with Crohn's disease and five with ulcerative colitis undergoing resection plasma from peripheral circulation and mesenteric vein draining diseased intestine was studied. All of these patients were receiving intravenous steroids at the time of the operation.

#### Samples

Ten ml of venous blood was collected in ethylenediamine tetra-acetic acid and 0.3 ml aprotinin (Sigma). It was kept in ice and within 10 minutes centrifuged at 400 $\times$ g for 10 minutes. Supernatant was then centrifuged at 2000 $\times$ g for 10 minutes to remove platelets. Aliquots were frozen at -70°C until used for assay.

Blood was also obtained for full blood count, erythrocyte sedimentation rate, C reactive protein, and  $\alpha_1$  acid glycoprotein. C reactive protein was measured by latex enhanced immunoassay.<sup>10</sup>  $\alpha_1$  Acid glycoprotein concentrations were measured using polyethylene glycol enhanced immunoturbidimetric assay.

#### Assay of interleukin-6

Interleukin-6 present in samples was assayed by enzyme linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA). The assay uses the quantitative 'sandwich' enzyme technique. A monoclonal antibody specific for interleukin-6 was coated onto the microtitre plate. Immobilised interleukin-6 in the samples

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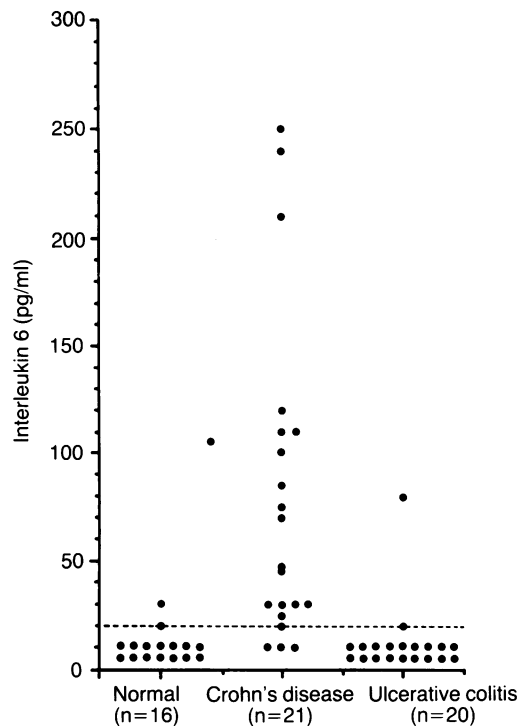


Figure 1: Plasma concentrations of interleukin-6 in normal control subjects and patients with active ulcerative colitis and Crohn's disease. Dotted line indicates detection limit of the assay. Crohn's disease v normal controls:  $p < 0.001$ ; Crohn's disease v ulcerative colitis:  $p < 0.001$ ; ulcerative colitis v normal subjects: not significant.

was detected using an enzyme linked polyclonal anti-interleukin-6 antibody. This assay recognises both natural and recombinant interleukin-6 with no measurable cross reactivity to other cytokines (information from R&D Systems).

In our laboratory the detection limit of this assay is 20 pg/ml with interassay and intra-assay variations of less than 13%. Mean (SEM) plasma recovery of interleukin-6 was 101.7 (7.5)%.

#### STATISTICS

Except where indicated, comparisons between groups were made by the Mann-Whitney U test. Spearman's rank test was used for correlation.

## Results

### PLASMA SAMPLES

#### Disease activity

Mean (SEM) scores of disease activity of ulcerative colitis and Crohn's disease were 8.2 (0.7) and 7.6 (0.5) respectively. There were no significant differences between Crohn's disease and ulcerative colitis in C reactive protein concentrations (mean (SEM) 72.1 (11.4) and 98 (38.2) mg/l respectively),  $\alpha_1$  acid glycoprotein (1.7 (0.2) and 2.7 (0.8) g/l), albumin (37.1 (1.6) and 39.4 (1.9) g/l), or platelet count (463 (44.4) and 450.8 (33.8)  $\times 10^9/l$ ) (Student's *t* test for analyses).

#### Interleukin-2 receptor concentrations

There was no significant difference in the concentrations of interleukin-2 receptor (measured by ELISA) between ulcerative colitis patients

and Crohn's disease patients (median 1160 (range 675–7150) v 1135 (480–9000) U/ml respectively). These data form part of another publication.<sup>11</sup>

#### Interleukin-6 concentrations (Fig 1)

Interleukin-6 was detected in plasma of 18 of the 21 patients with Crohn's disease (median 47 (range <20–250) pg/ml) but in only two of the 20 patients with ulcerative colitis and two of the 16 normal control subjects. Of the two patients with ulcerative colitis who had detectable interleukin-6 concentrations (20 and 80 pg/ml), one (80 pg/ml) had toxic megacolon with severe total colitis which required colectomy three days after the sample was taken.

#### Crohn's disease

There was no significant difference in plasma interleukin-6 concentrations between patients with only colonic disease (median 30 (range <20–110) pg/ml;  $n=5$ ) and those with only small bowel disease (median 45 (range 15–250) pg/ml;  $n=13$ ). Of the three patients with both small bowel and colonic disease, two had high concentrations (120 and 210 pg/ml), while in the third it was 30 pg/ml.

Five patients with Crohn's disease were taking oral prednisolone and their plasma concentrations of interleukin-6 (median 45 (range 25–100) pg/ml) were not significantly different from patients not taking steroids (40.5 (<20–250) pg/ml).

Five patients with Crohn's disease were taking a 5 aminosalicylic acid (four taking this only and one also taking oral prednisolone). In one of these patients no interleukin-6 was detected in plasma and in all the rest the concentration was 30 pg/ml. The difference between patients with Crohn's disease taking a 5 aminosalicylic acid and those not taking one (median 56, range <20–250) fell just short of significance ( $p=0.056$ ).

For the whole group there was a significant negative correlation between plasma interleukin-6 concentrations and albumin concentrations ( $RS=-0.57$ ;  $p=0.028$ , Fig 2). There were no significant correlations between interleukin-6 concentrations and C reactive protein and  $\alpha_1$  acid glycoprotein concentrations and platelet count, or clinical activity score.

In one patient the interleukin-6 concentration fell with clinical response to treatment with steroids (plasma interleukin-6 (clinical score): day 1: 70 pg/ml (10); day 2: oral prednisolone started; day 3: <20 pg/ml (5); day 5: 30 pg/ml (6); day 7: 30 pg/ml (4)).

### PERIPHERAL AND MESENTERIC SAMPLES

Interleukin-6 was detected in seven of the eight peripheral and mesenteric venous plasma samples from the patients with Crohn's disease (median (range) peripheral: 35 (<20–120) pg/ml; mesenteric: 40 (<20–160) pg/ml). Paired analysis showed no significant differences between the peripheral and mesenteric samples. Interleukin 6 was not detected in any of the plasma samples (peripheral or mesenteric) from patients with ulcerative colitis. This included the patient with toxic megacolon in whom the

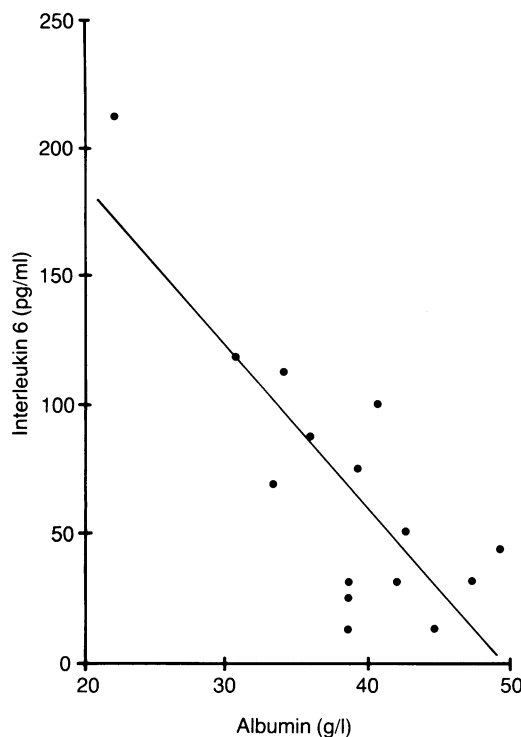


Figure 2: A negative correlation between plasma concentrations of interleukin-6 and serum albumin ( $RS = -0.57$ ;  $p = 0.028$ ) is shown.

plasma interleukin-6 concentration three days before operation was 80 pg/ml.

None of the patients with Crohn's disease were taking a 5 aminosalicylic acid but all those with ulcerative colitis, except the patient with toxic megacolon, were taking one.

### Discussion

Our study shows that circulating concentrations of interleukin-6 are increased in most patients with active Crohn's disease. In contrast, no interleukin-6 was detected in the plasma of patients with active ulcerative colitis apart from two, where the range of values was not significantly different from those in normal control subjects. Interleukin-6 was detected in peripheral circulation and in mesenteric venous blood from patients with Crohn's disease undergoing resection but not in those undergoing colectomy for active ulcerative colitis. These findings seem to show a genuine difference between the two diseases since patients in both groups had active disease and a closely similar acute phase response characterised by C reactive protein,  $\alpha_1$  acid glycoprotein, and albumin concentrations.

The reason for the difference in circulating concentrations of interleukin-6 between ulcerative colitis and Crohn's disease is not clear. Interleukin-6 can be produced by a variety of cell types, but when inflammation occurs monocytes,<sup>12</sup> endothelial cells,<sup>13</sup> and fibroblasts<sup>14</sup> are predominant sources. The high concentration in Crohn's disease could therefore be due to enhanced production by any of these cell types in the inflamed mucosa. Recent studies, however, have shown interleukin-6 mRNA transcripts in mucosal biopsy specimens from both

ulcerative colitis and Crohn's disease.<sup>15,16</sup> In addition, concentrations of interleukin-6 in mesenteric plasma were not significantly different from those in peripheral circulation, unlike our previous findings for interleukin-2 receptor.<sup>11</sup>

It is possible that the differences between ulcerative colitis and Crohn's disease in circulating concentrations of interleukin-6 could be due to enhanced synthesis of this cytokine by circulating monocytes in the latter. Recently, monocytes have also been shown to express receptors for interleukin-6 which are downregulated upon stimulation.<sup>17</sup> Thus, with stimulation monocytes will produce interleukin-6, and with fewer receptors for the peptide on its surface, more will be available to hepatocytes for synthesis of the acute phase proteins.

Interleukin-1 is a potent inducer of interleukin-6 synthesis,<sup>18</sup> and an alternative explanation is that increased concentrations of interleukin-6 in Crohn's disease reflect changes in interleukin-1. While no significant difference in *in vitro* synthesis of interleukin-1 by mononuclear cells isolated from mucosa with active Crohn's disease or ulcerative colitis has been shown,<sup>19</sup> it is possible that circulating monocytes may have a greater capacity to produce interleukin-1 in Crohn's disease.

We have previously reported inhibition of interleukin-1 $\beta$  production by 5 aminosalicylic acid and corticosteroids in organ cultures of colonic biopsy specimens.<sup>20</sup> In the present study the small number of patients with Crohn's disease taking a 5 aminosalicylic acid seemed to have lower interleukin-6 concentrations than those not taking this drug. As a large proportion of patients with ulcerative colitis were taking one of these drugs, this may explain the differences between the two diseases. However, seven patients with ulcerative colitis were not taking the drug and only one of these had detectable concentrations of interleukin-6 despite active disease and an acute phase response.

Another explanation for the differences in interleukin-6 concentration might be the high circulating concentrations of receptors to interleukin-6 in ulcerative colitis but not Crohn's disease.

We found a significant negative correlation between the interleukin-6 and albumin concentrations in patients with Crohn's disease. The lack of a positive correlation between the interleukin-6 and the C reactive protein and  $\alpha_1$  acid glycoprotein concentrations may be due to the fact that the acute phase response is the result of more than one circulating cytokine – that is, also interleukin-1 and tumour necrosis factor  $\alpha$ . In addition, the acute phase proteins may be regulated differentially by mediators like interleukin-6, interleukin-1, and tumour necrosis factor  $\alpha$ .<sup>3</sup> This also raises the possibility that the acute phase responses in ulcerative colitis and Crohn's disease are mediated by different cytokines. Another consideration is that there is a time lag in acute phase protein synthesis occurring in response to interleukin-6.<sup>21</sup>

Regardless of the source of interleukin-6, further studies to investigate the highly significant differences between the two diseases may

provide more information on the pathogenesis of Crohn's disease. In addition, interleukin-6 concentrations may be of practical value in distinguishing between ulcerative colitis and Crohn's disease and in monitoring disease activity in the latter.

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