

# Neutrophil mucosal involvement is accompanied by enhanced local production of interleukin-8 in ulcerative colitis

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## Abstract

The concentration of myeloperoxidase, a neutrophil granule constituent, was measured in the perfusion fluid from sigmoid and rectal segments in patients with ulcerative colitis. The concentrations of myeloperoxidase were increased severalfold in the patients with ulcerative colitis compared with healthy controls pointing to an enhanced neutrophil activity. The release of myeloperoxidase correlated to an enhanced local release of the neutrophil activating peptide interleukin-8 (IL-8). Increased values of tumour necrosis factor (TNF- $\alpha$ ) were also found during intestinal perfusion of the patients and correlated with those of IL-8. The results obtained are compatible with the hypothesis that local mucosal recruitment/activation of neutrophils in ulcerative colitis is mediated by an enhanced IL-8 synthesis. TNF- $\alpha$  may be one relevant factor as a stimulus to IL-8 synthesis.

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Neutrophil accumulation in the inflamed intestinal mucosa is a prominent feature in ulcerative colitis. The granules of neutrophil granulocytes contain a number of enzymes, for example, myeloperoxidase, which are important in the combat against bacteria.<sup>1</sup> These granule enzymes, some of which are proteolytic, can be released upon stimulation, together with cytotoxic oxygen metabolites.<sup>2</sup> Therefore, activated neutrophils may contribute to tissue damage at sites of inflammation. The factor(s) responsible for the mucosal recruitment of neutrophils in ulcerative colitis are not certain. Human neutrophil activating peptide/interleukin-8 (NAP-1/IL-8) is a single, non-glycosylated peptide chain; its main activities include activation and chemotactic attraction of neutrophils.<sup>3-7</sup> It was originally isolated from endotoxin treated monocytes but can also be produced by a variety of other cells upon stimulation with a number of cytokines including IL-1 and tumour necrosis factor (TNF- $\alpha$ ).<sup>8-10</sup> This study was performed in an attempt to estimate the mucosal activity of neutrophils in ulcerative colitis and elucidate the possible neutrophil activating role certain cytokines may exert. For this purpose we have measured myeloperoxidase, IL-8, and some cytokines known to induce IL-8 synthesis. The measurements were performed in the perfusion fluid obtained during regional perfusion of inflamed sigmoid and rectal segments.

## Patients and methods

We studied 16 patients with proved ulcerative

colitis; eight had a disease extension to or beyond the sigmoid colon and eight had an isolated proctitis (eight women and eight men, with a mean age of 35 years, range 20 to 75). The diagnosis was based on established criteria including a typical endoscopic appearance supported by histological findings.<sup>11</sup> All patients had endoscopically active disease of various degrees. The clinical symptoms varied from mild disease with little blood in stools to more active disease with frequent diarrhoea with blood and mucus in all stools. The four degree score of Binder<sup>12</sup> was used to classify clinical and endoscopic findings. Histological scoring of biopsy specimens was performed on formaldehyde fixed sections. Six patients were not receiving any treatment. Six patients were receiving treatment with oral or enema steroids, or both and two had recently finished treatment with steroids. One patient was treated with sulphasalazine and one with lidocaine enema. The patients were compared with 18 healthy volunteers (11 women and seven men, with a mean age of 33 years, range 21 to 64). The study was approved by the ethics committee of the medical faculty of Uppsala University.

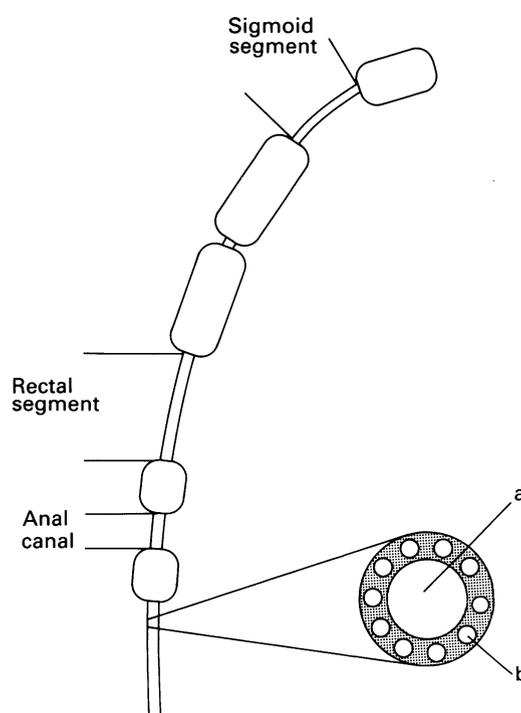


Figure 1: A schematic presentation of the colorectal perfusion system. The perfusion tube with five balloons delimiting one rectal and one sigmoid segment. Two balloons control the position of the tube by surrounding the anal canal. The central channel of the tube (a) was used for the introduction of the tube with an endoscope and decompression of gas and fluids during perfusions; the smaller channels (b) were used to inflate air into the balloons and deliver perfusion fluid to and from the segments and marker dye (for detection of leakage) above the tube.

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The concentrations of interleukin-8 (IL-8), myeloperoxidase (MPO), and tumour necrosis factor (TNF- $\alpha$ ) in perfusion fluid from sigmoid/rectal segments in patients with ulcerative colitis and proctitis and controls. The appearance rates of these substances are also given

Segment	IL-8 concentration appearance rate	MPO concentration appearance rate	TNF- $\alpha$ concentration appearance rate	
				pg/ml ng/cm/h
Proctitis (n=8)	Sigmoid	74 (48-117)** 1.6 (1.0-2.5)	28 (12-66)** 0.6 (0.2-1.5)	9.8 (7.1-14) <sup>NS</sup> 0.21 (0.16-0.28)
	Rectum	257 (110-589)*** 5.1 (2.5-10.2)	132 (30-575)*** 2.2 (0.4-13.8)	36 (13-98)*** 0.7 (0.3-1.7)
Colitis (n=8)	Sigmoid	195 (79-468)*** 4.2 (1.7-10.5)	195 (55-708)*** 3.1 (0.8-12.6)	25 (11-58)** 0.5 (0.2-1.3)
	Rectum	166 (87-316)*** 4.3 (2.2-8.3)	234 (68-832)*** 4.9 (1.2-20.4)	34 (16-72)*** 0.9 (0.4-1.9)
Controls (n=18)	Sigmoid	35 (26-47) 0.7 (0.5-1.1)	11 (10-13) 0.20 (0.17-0.23)	8.1 (6.0-10.7) 0.17 (0.13-0.25)
	Rectum	43 (30-62) 1.0 (0.6-1.5)	16 (11-23) 0.3 (0.2-0.5)	7.2 (6.8-7.8) 0.16 (0.14-0.19)

Values are given as means and 95% confidence intervals (differences between patients and controls are also expressed as p values where \*, \*\*, \*\*\* are p<0.05, 0.01, and 0.001, respectively).

We used a perfusion system where segments of the sigmoid colon and rectum were isolated. Before colorectal perfusion studies, the patients and controls had a bowel preparation as for flexible rectosigmoidoscopy, that is – diet restriction and oral purgative the day before and a non-irritative enema two hours before perfusion. The perfusion instrument was a plastic tube (16 mm in external diameter), which was 60 cm long and contained smaller channels in the tube wall and one large central channel; five latex balloons were attached to the tube (Fig 1). The investigation started with an endoscopic examination up to the junction of the sigmoid descending colon. Before insertion of the tube, it was threaded onto the endoscope. The tube was then introduced up to the sigmoid colon with the endoscope acting as a guide. When the balloons were inflated with air, two 8 cm long segments were established (Fig 1); one in the rectum (rectal segment) and another in the sigmoid colon (sigmoid segment). After withdrawal of the endoscope, the position of the tube was checked fluoroscopically. To stabilise the position of the

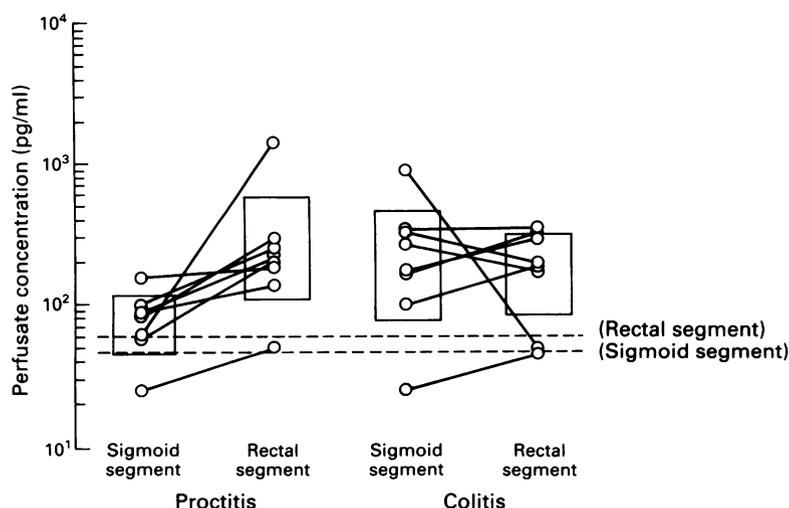


Figure 2: The individual interleukin-8 (IL-8) concentrations in the perfusion fluid from rectal and sigmoid segments in patients with ulcerative colitis. Boxes indicate the confidence intervals of the means in patients grouped with respect to disease extension. The broken lines represent the upper limits for the confidence intervals for IL-8 during perfusion of rectal and sigmoid segments in healthy controls.

tube, two balloons surrounding the anal canal were inflated (Fig 1).

Using the inflow channels, the rectal and sigmoid segments were continuously perfused at a rate of 3 ml/min with a perfusion solution containing 120 mM NaCl, 5.4 mM KCl, 2 mM Na<sub>2</sub>HPO<sub>4</sub>, 10 mM glucose, 35 mM mannitol, and 1 g/l polyethylene glycol (Mw 4000). The pH of the solution was 7.2 and the osmolarity 290 mmol/l. Aprotinin (10000 IU/ml; Bayer, Leverkusen, Germany) was added to the perfusion solution (10 ml aprotinin/l) to inhibit possible proteolytic activity in the effluent.

The perfusate fluid was collected by the outflow channels from the isolated segments. After 50 minutes of continuous perfusion to obtain steady state conditions, samples of the effluent were collected on ice. The total recovery of perfusion fluid was almost equal to that infused. After centrifugation of the samples, the supernatants were immediately frozen at -70°C until they were analysed. As the assays might even be affected by the presence of small amounts of proteases, phenylmethylsulfonyl fluoride to a final concentration of 2 mM (Sigma Chemical, St Louis) was added to all samples before they were thawed on ice and analysed. Further details of the perfusion system is presented elsewhere.<sup>13</sup>

The granulocyte granule component myeloperoxidase was measured by a radioimmunoassay from Pharmacia Diagnostics, Uppsala, Sweden. IL-8 was determined by a solid phase double ligand ELISA.<sup>14</sup> TNF- $\alpha$  was measured by an enzymatic immunoassay obtained from Medgenix, Brussels, Belgium. Interleukin-2 (IL-2) was determined by a radioimmunoassay (Medgenix). Granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-1 (IL-1 $\beta$ ) were measured by immunometric assays (Medgenix). The perfusion fluid samples were analysed in duplicate and in sequence to avoid inter day variation. The variability was less than 10% for all methods. The specificity of the assays applied to perfusion fluid was assured by dilution and by recovery experiments showing correlation to the standard curves.

#### STATISTICS

To obtain normally distributed values, all values were logarithmically transformed before calculations and statistical analyses. Means and 95% confidence intervals are presented in text and figures. Differences between groups were calculated with the two tailed Student's *t* test; p<0.05 was considered significant. Clinical findings related to perfusion data were tested by one way analysis of variance.

#### Results

The patients were grouped into those who had a disease extension to or beyond the sigmoid colon (colitis) and those who suffered from an isolated proctitis. In patients with colitis the concentrations of myeloperoxidase were increased by an average of 14 and 18 times in the perfusion fluids from the sigmoid and rectal segments compared with controls (p<0.001). In patients with isolated proctitis, there was an eightfold increase

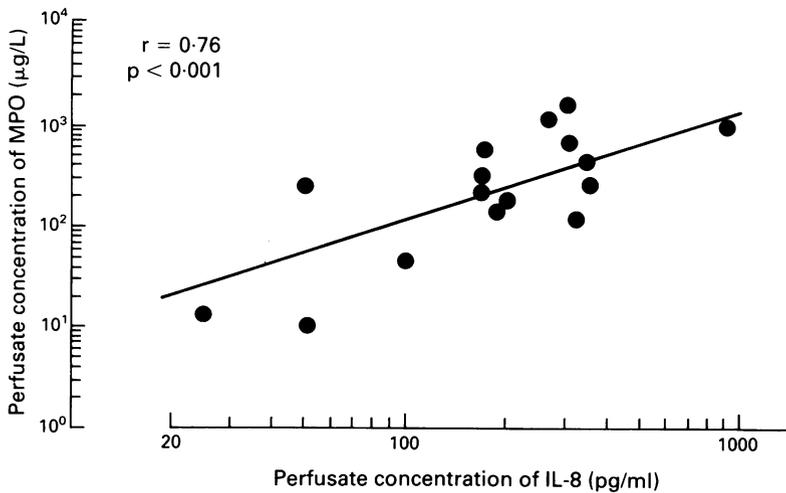


Figure 3: The interleukin-8 (IL-8) concentrations v the myeloperoxidase (MPO) concentrations in the perfusion fluids from the rectal and sigmoid segments in patients with colitis.

of the mean myeloperoxidase concentration in the rectal segment ( $<0.001$ ) and a twofold increase in the sigmoid segment (Table). The perfusion fluid concentrations of IL-8 were also significantly increased in both segments in colitis and proctitis (Fig 2 and Table). The myeloperoxidase and IL-8 concentrations in sigmoid/rectal segments were correlated in patients with colitis ( $r=0.76$ ,  $p<0.001$ ; Fig 3) and in proctitis ( $r=0.46$ ,  $p<0.05$ ). Significantly increased values of TNF- $\alpha$  were found in perfusates from both segments in colitis but only from the rectal segment in proctitis (Table). The recovered sigmoid-rectal amounts of TNF- $\alpha$  correlated with the recovered amounts of IL-8 in patients with colitis ( $r=0.75$ ,  $p<0.001$ ; Fig 3) and proctitis ( $r=0.62$ ,  $p<0.001$ ). To compensate for differences in flow rates during perfusion, the appearance rates of myeloperoxidase and cytokines were calculated. The differences between the perfusion fluid concentrations of the components in the patients and those in the controls were similar to the differences in the appearance rates (Table). The values of IL-2, GM-CSF, and IL-1 $\beta$  were below the detection limits.

Most of the patients had clinically mild

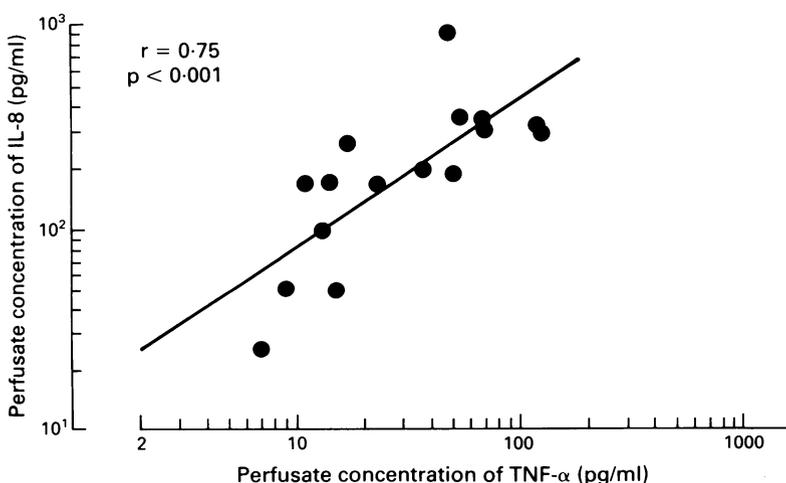


Figure 4: The correlation between the measured amounts of tumour necrosis factor (TNF- $\alpha$ ) and interleukin-8 (IL-8) in the perfusion fluids from rectal and sigmoid segments in patients with colitis.

disease. On a four degree scale, the median clinical score was 2 (range 1–3) and the median endoscopic and histologic scores of the affected sigmoid and rectal segments were 2 (range 2–3) and 3 (range 2–3), respectively. We found no significant relation between these scores and the measured substances in perfusion fluid.

## Discussion

During regional perfusion of defined sigmoid and rectal segments from healthy controls and patients with ulcerative colitis we were able to detect measurable amounts of myeloperoxidase. The severalfold increase of the local release of myeloperoxidase in patients should reflect the increased neutrophil accumulation/activity in the inflamed colonic-rectal mucosa. Previous investigators have also shown, by tissue extraction, an increased release of neutrophil granule constituents in the inflamed mucosa in ulcerative colitis.<sup>15</sup> The potential hazard of persistent local neutrophil activation relates to the ability of the neutrophil to generate toxic oxygen radicals and release myeloperoxidase and other preformed azurophilic enzymes including collagenase, elastase, and cathepsin G with the capacity to destroy the connective tissue matrix.<sup>2</sup> The very toxic hypohalide anion catalysed by the enzyme myeloperoxidase may also cause tissue damage.<sup>1</sup>

The mechanism recruiting and activating the neutrophils in the intestinal mucosa in ulcerative colitis is probably multifactorial. The finding that increased intestinal release of IL-8 was accompanied by increased release of myeloperoxidase, however, makes IL-8 a possible factor for local activation of polymorphonuclear granulocytes in this disease. IL-8 presumably plays an important part in neutrophil diapedesis through vascular endothelium and focal recruitment at inflamed sites.<sup>3-7</sup> Thus, intradermal injection in animals results in plasma exudation and massive neutrophil infiltration.<sup>16</sup> Besides being chemotactic for neutrophils, IL-8 also activates these cells to degranulate and show respiratory burst. The cellular source of enhanced IL-8 synthesis in the inflamed mucosa has not been identified in this study but bacterial lipopolysaccharides, immune complexes, and several cytokines including IL-2, IL-3, TNF- $\alpha$ , GM-CSF, and IL-1 are strong inducers to IL-8 production in mononuclear phagocytes.<sup>7,17</sup> Endothelial cells and fibroblasts also produce IL-8 in response to IL-1 and TNF- $\alpha$ .<sup>8,9,18</sup> The finding that TNF- $\alpha$ , by contrast with IL-1 $\beta$ , IL-2, and GM-CSF, was seen in increased concentrations in colonic-rectal perfusion fluid from patients with ulcerative colitis and also correlated with the increased IL-8 release supports the idea that TNF- $\alpha$  is one possible mediator of enhanced IL-8 synthesis in the inflamed mucosa.

The lack of correlation between differences in myeloperoxidase and cytokine values and disease activity should be interpreted with caution because of the narrow ranges of endoscopic and histological scores in our patients and the treatment they were receiving; steroids definitely affect the inflammatory response and probably also IL-8 generation. The finding that some of the patients with isolated proctitis also had

increased release of myeloperoxidase and IL-8 in the seemingly unaffected sigmoid segment might reflect a low degree sigmoid inflammation not detectable by endoscopy.

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