Specific increase in interleukin-8 concentrations in dialysis fluid of patients with peritonitis receiving continuous ambulatory peritoneal dialysis

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Abstract

***Aims*—To evaluate the influence of interleukin-8 (IL-8) and other inflammatory cytokines (IL-6, IL-1β and tumour necrosis factor α (TNFα)) on the occurrence of peritonitis in patients receiving continuous ambulatory peritoneal dialysis (CAPD).**

***Methods*—The study population comprised 12 patients with peritonitis, 33 without peritonitis, all undergoing CAPD, and five patients undergoing peritoneal catheter implantation. Cytokine concentrations in dialysis fluid were determined by immunoassay and their values compared.**

***Results*—Concentrations of both IL-8 (median 147 pg/ml, range 20–2273 pg/ml; n = 12) and IL-6 (median 1120 pg/ml, range 96–10 600 pg/ml) were substantially elevated, while the IL-1β concentration was lower and TNFα was not detectable in patients at diagnosis. The IL-6 concentration was also elevated in patients undergoing catheter implantation as well as in those with peritonitis. The IL-8 concentration, however, was elevated only upon infection. Intraperitoneal production of IL-8 was evident on determination of paired serum and dialysis fluid cytokine concentrations, and immunostaining of peritoneal cells with monoclonal anti-IL-8 antibody.**

***Conclusions*—These results suggest that determination of the IL-8 concentration in dialysis fluid maybe useful as a specific marker for following patients with peritonitis receiving CAPD.**

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Keywords: Interleukin-8, peritonitis, peritoneal dialysis.

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Peritonitis, characterised by neutrophil infiltration in the peritoneal cavity, is one of the major complications in patients with end stage renal failure undergoing continuous ambulatory peritoneal dialysis (CAPD). We have previously demonstrated that interleukin-8 (IL-8), a potent chemotactic cytokine for neutrophils,[1 2] is associated with pyuria in patients with urinary tract infections.[3] Therefore, it is reasonable to assume that IL-8 may contribute to peritonitis in patients undergoing CAPD.

Elevated IL-6 and IL-8 concentrations have recently been reported in the peritoneal dialysis fluid of patients with peritonitis.[4 5] The present study was designed to investigate whether dialysis fluid IL-8 or IL-6 concentrations are elevated in patients with and without peritonitis and in patients undergoing peritoneal catheter implantation. The contribution of IL-1β and tumour necrosis factor α (TNFα) and evidence of the intraperitoneal production of IL-8 in patients with peritonitis receiving CAPD were also evaluated.

Methods

Twelve patients with peritonitis (10 men and two women; mean age 61·6 years, range 33–94 years) undergoing CAPD were studied. The micro-organisms isolated included *Streptococcus viridans* (three cases), *Staphylococcus epidermidis* (two cases), *S aureus* (two cases), methicillin resistant *S aureus* (two cases), *Candida albicans* (one case), and culture negative (four cases). Thirty three patients without peritonitis (25 men and eight women; mean age 47·7 years, range 23–70 years) undergoing CAPD for at least one month served as controls. Five patients undergoing peritoneal catheter implantation, three of whom subsequently developed peritonitis, were also studied. Samples from the latter patients were taken within 24 hours of catheter implantation. Evidence of peritonitis included abdominal symptoms or cloudy dialysis fluid, or both, and the presence 100 white blood cells (WBC)/mm^3 in the dialysis fluid or positive culture, or both.

Cytokine concentrations in the dialysis fluid or serum were determined by immunoassay as described in detail elsewhere.[3 6] The detection limits were 16, 10, 5, and 4 pg/ml for IL-8, IL-1β, TNFα, and IL-6, respectively.

Centrifuged sediment from dialysis fluid of patients with peritonitis was fixed on the glass slides and endogenous peroxidase activity blocked. Slides were stained using the avidin biotin complex immunoperoxidase technique and colour developed using diaminobenzidine.

Results were expressed as medians and ranges. The values of each parameter at diagnosis of each episode of peritonitis or during catheter implantation were used for comparison. Specimens with values below the detection limit were excluded from comparisons involving that variable. Data analysis was performed using Hollander and Wolfe's method; p values less that 0·05 were regarded as significant.

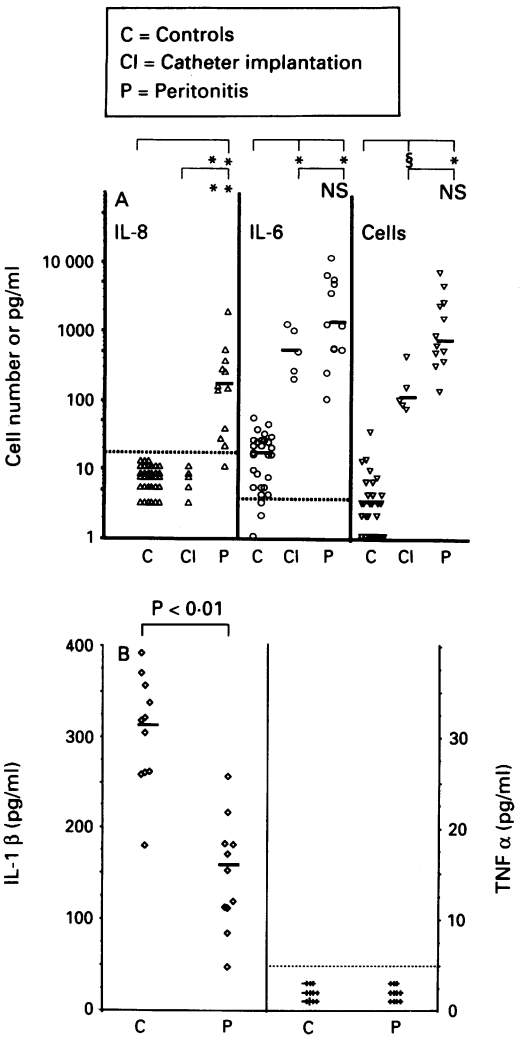

*Figure 1 Concentrations of cytokines in dialysis fluid of patients with (P; n = 12 for panel A and n = 11 for panel B) and without (C; n = 33 for panel A and n = 11 for panel B) peritonitis and five patients undergoing catheter implantation (CI). The dotted lines denote detection limits of immunoassay. The horizontal lines denote median values. (**P<0·001, *P<0·01, §P = 0·05 v control; NS = not significant)*

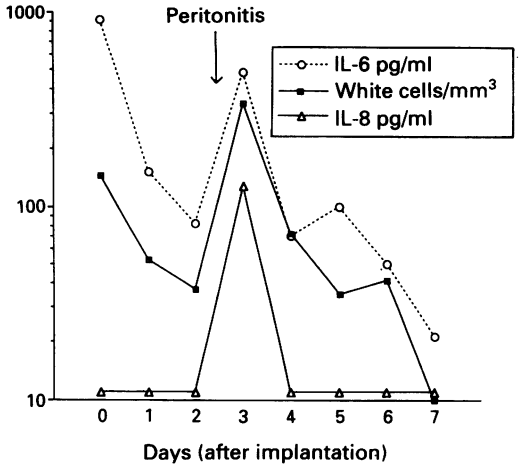

Figure 2 Change in dialysis fluid IL-8 and IL-6 concentrations and WBC counts in patients following implantation of the catheter and the subsequent development of peritonitis.

Results

As shown in fig 1A, IL-8 concentrations were below the detection limit (<16 pg/ml) in the dialysis fluid of all patients without peritonitis (n = 33). These patients also had low IL-6 concentrations (median 15·5 pg/ml, range 0–52 pg/ml) and WBC counts (6·8 cells/mm^3, range 1·2–65 cells/mm^3). In patients with peritonitis (n = 12) IL-8 and IL-6 concentrations and the WBC counts were significantly elevated at diagnosis with median values of 147 pg/ml (range 20–2273 pg/ml), 1120 pg/ml (96–10 600 pg/ml), and 680 cells/mm^3 (125–6500 cells/mm^3), respectively.

Although IL-1β and TNFα stimulate the production of both IL-6 and IL-8, IL-1β concentrations in patients with peritonitis were significantly lower (152 pg/ml, range 48–256 pg/ml; n = 11) than those in the controls (317 pg/ml, range 180–392 pg/ml; n = 11) (fig 1B). TNFα concentrations were below the detection limit (<5 pg/ml) in patients with (n = 11) and without (n = 11) peritonitis.

Catheter implantation elicited a substantial increase in IL-6 concentrations (460 pg/ml, range 185–1130 pg/ml) and WBC counts (93 cells/mm^3, range 71–400 cells/mm^3), but not in IL-8 concentrations (<16 pg/ml), in patients without infection compared with controls (fig 1A). No significant differences were noted when the WBC counts and IL-6 concentrations in the dialysis fluid of patients undergoing catheter implantation were compared with those of patients with peritonitis, although these levels tended to be higher in the latter. Patients undergoing catheter implantation are capable of producing IL-8, as IL-8 concentrations increased in those who subsequently developed peritonitis. As shown in fig 2, IL-8 and IL-6 concentrations and WBC counts increased on development of peritonitis; IL-8 concentrations were the first to return to normal in response to antibiotic treatment.

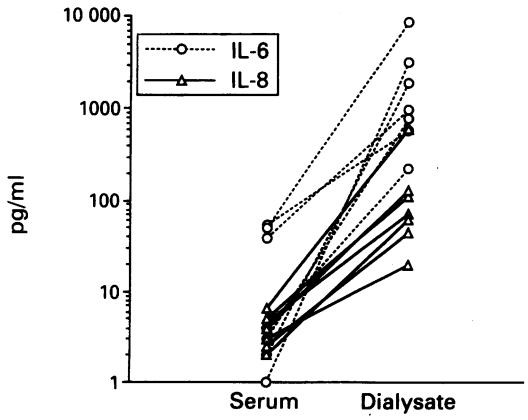

Figure 3 Concentrations of IL-8 and IL-6 in paired dialysis fluid and serum specimens from patients with peritonitis undergoing CAPD.

Paired IL-6 and IL-8 concentrations in serum and dialysis fluid were determined (fig 3). Both IL-6 and IL-8 concentrations were substantially higher in dialysis fluid than in serum, favouring the local production of IL-8 within the peritoneal cavity. To strengthen

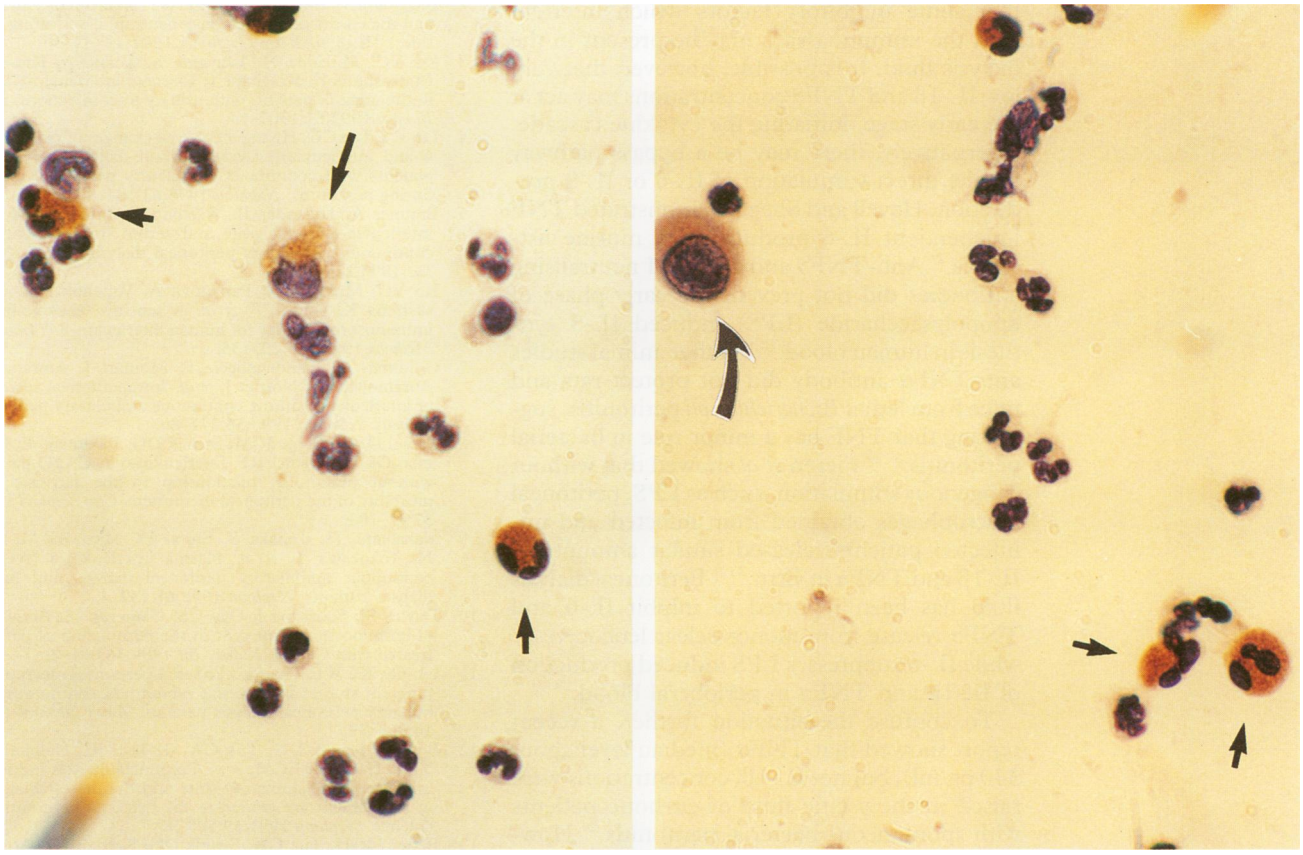

Figure 4 Positive immunostaining reaction with monoclonal antibody to IL-8 in peritoneal macrophages (arrow), mesothelial cells (curved arrow), and neutrophils with three or less nuclear segments (arrowheads) of patients with peritonitis undergoing CAPD (original magnification × 1000).

these obversations, peritoneal macrophages and mesothelial cells, identified by their characteristic morphology, together with neutrophils in the dialysis fluid of patients with peritonitis, were positively stained with monoclonal anti-IL-8 antibody WS-4 (fig 4). Neutrophils with four or more nuclear segments generally did not stain. These findings are specific, as immunostaining with monoclonal anti-CD15 antibody, which serves as a positive control for granulocytes, did not discriminate between these neutrophils with respect to the nuclear segment pattern (data not shown).

Discussion

Accumulating evidence suggests that inflammatory cytokines have a vital role in the response to infection. The present study demonstrates the specific intraperitoneal release of IL-8 in patients with peritonitis undergoing CAPD, and confirms previous reports on IL-6 and IL-8 in peritoneal dialysis fluid.[4 5 7-9]

In patients undergoing CAPD for the first time exposure to dialysis fluid and foreign material such as the catheter presumably induces a local inflammatory response within the peritoneal cavity,[10-13] as indicated by the raised IL-6 concentrations found in these patients. The failure to detect IL-8 in these patients is not unexpected as mononuclear, rather than neutrophil, cell numbers predominate. On initial infection of the peritoneum, IL-8 concentrations surge, and provided the infection does not persist, IL-8 concentrations return to normal. One patient with peritonitis whose dialysis fluid IL-8 concentrations were normal at diagnosis was consequently found to have significantly elevated IL-8 concentrations the following day. These characteristic kinetics permit the use of IL-8 as a marker when diagnosing and monitoring the course of patients with perintonitis receiving CAPD. This may also apply to other localised infectious diseases such as urinary tract infections.

Both IL-8 and IL-6 are produced by a variety of cell types, some of which produce both cytokines.[1 14] Immunostaining for IL-8 identified peritoneal macrophages, mesothelial cells and neutrophils as the local producing cells within the peritoneal cavity during peritonitis. This is consistent with previous in vitro findings.[1 15 18] Similar findings have been reported for IL-6.[19 20] Other probable sources of IL-8 production include endothelial cells and fibroblasts within the peritoneum.

Another important finding was that neutrophils with four or more nuclear segments did not stain for IL-8. The lack of IL-8 production by these cells may indicate negative regulation of further neutrophil recruitment. Additional studies are required to confirm this hypothesis.

As in cases of urinary tract infection there were no significant increases in IL-1β and TNFα concentrations in the dialysis fluid of

infected patients.[3] It is postulated that these cytokines are produced at a very low level or that some inhibitory factors which interfere with the immunoassays may be present in the dialysis fluid. It is possible, however, that very low IL-1β and TNFα concentrations may act at the early stage, initiating the cytokine cascade. Alternatively, there may be a bypass pathway for the direct stimulation of IL-6 or IL-8 production. Havell and Shegal demonstrated TNF independent IL-6 production in murine listeriosis.[21] Anti-TNFα and anti-IL-1 neutralising antibodies did not prevent the early phase of lipopolysaccharide (LPS) induced IL-8 synthesis in human blood.[22] In other animal studies anti-TNFα antibody did not protect rats and mice from lethal *Escherichia coli* peritonitis, suggesting that TNF has a minor role in bacterial peritonitis.[23,24] Fieren *et al* showed that without exogenous stimulation, such as LPS, peritoneal macrophages obtained from infected and uninfected patients released similar amounts of IL-1β and TNFα in vitro.[25,26] Peritoneal dialysis fluid has been reported to inhibit IL-6 and TNFα release from mononuclear leucocytes,[27] while IL-6 suppresses LPS induced production of IL-1β and TNFα in peripheral blood.[28]

To confuse the situation further, a recent report showed that TNFα (median level about 340 pg/ml), but not IL-1β, concentrations were raised in the ascitic fluid of cirrhotic patients with spontaneous bacterial peritonitis.[29] However, these results must be interpreted cautiously as IL-6 concentrations in the patients with cirrhosis were very high (about $1{\cdot}7 \times 10^5$ ng/ml). Taken together, the involvement of IL-1β and TNFα in peritonitis cannot be completely ruled out, and studies using in situ hybridisation would have been useful. Therefore, measurement of IL-1β and TNFα concentrations in dialysis fluid for the diagnosis of peritonitis seems to be of no advantage.

The influence of IL-8 on neutrophil chemotaxis and activation (intraperitoneal administration of human recombinant IL-8 causes neutrophil infiltration in mice[30]) suggests that this cytokine plays an important role in the response to bacterial infection of the peritoneal cavity. Intraperitoneal administration of IL-8 may be beneficial in patients undergoing CAPD as is the case with interferon-α, which is effective in preventing infection when administered prophylactically.[31] Nevertheless, a long term study to compare the occurrence of probable complications, such as peritoneal fibrosis, in patients with or without cytokine treatment is mandatory.

In conclusion, IL-8 concentrations are significantly raised in the dialysis fluid of patients with peritonitis undergoing CAPD. Administration of recombinent IL-8 may be useful for prophylaxis in patients undergoing CAPD.

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