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LEVELS OF SOLUBLE INTERCELLULAR ADHESION MOLECULE 1, EICOSANOIDS AND CYTOKINES IN ASCITES OF PATIENTS WITH LIVER CIRRHOSIS, PERITONEAL CANCER AND SPONTANEOUS BACTERIAL PERITONITIS

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Abstract — The levels of the eicosanoids leukotriene B₄, prostaglandin E₂, prostacycline and thromboxane B₂, the cytokines interleukin-1β, interleukin-6 and tumour necrosis factor-α and soluble intercellular adhesion molecule 1 were measured in ascites and plasma samples of patients with liver cirrhosis (53), peritoneal cancer (26) and spontaneous bacterial peritonitis (10) to assess their value as a possible diagnostic and prognostic parameter in the course of the disease. Soluble intercellular adhesion molecule 1, of the eicosanoids prostaglandin E₂ and leukotriene B₄, and the protein concentration in ascites were all significantly elevated in ascites of patients with peritoneal cancer in comparison to ascites of patients with liver cirrhosis. In ascites of patients with spontaneous bacterial infection interleukin-6 concentration was significantly elevated and the protein concentration was significantly lower in comparison to the other two groups. None of these parameters, however, seems to be of practical use as a diagnostic parameter, as there is an overlap between all the levels of these mediators in ascites of liver cirrhosis, peritoneal cancer and spontaneous bacterial peritonitis group. Soluble intercellular adhesion molecule 1 levels were much higher in plasma than in ascites, in contrast to interleukin-6 levels which were much higher in ascites than in plasma. Soluble intercellular adhesion molecule 1 in ascites correlated with soluble intercellular adhesion molecule 1 in plasma ($r = 0.6926$, $P = 0.0001$). Soluble intercellular adhesion molecule 1, interleukin-6 and the number of polymorphonuclear cells in peritoneal fluid correlated during episodes of infection in patients with a peritonitis. For this reason soluble intercellular adhesion molecule 1 and interleukin-6 could be of prognostic value for patients with peritonitis.

Keywords: soluble ICAM-1, interleukin-6, ascites, liver cirrhosis, peritoneal cancer, spontaneous bacterial peritonitis.

Ascites is the accumulation of fluid in the peritoneal cavity. The main causes of ascites are liver cirrhosis, malignancies involving the peritoneum and inflammatory processes. Early diagnosis of the probable cause of the ascites is useful for guiding further investigation and treatment (Bac *et al.*, 1993a). Ascites may be complicated by bacterial infection, a condition known as spontaneous bacterial peritonitis (SBP). SBP has a high mortality unless diagnosed and treated at an early stage (Bac *et al.*, 1993b). Ascites of all causes contain inflammatory cells, mainly macrophages (Mφ), which produce inflammatory mediators. As the pattern of mediators produced by inflammatory cells depends in part on the stimulus and on the stage of inflammation, we decided to measure in ascites a wide variety of

inflammatory mediators or products of inflammation to determine whether the different causes of ascites were associated with specific patterns of inflammatory mediators in ascites. Based on prior work in which we characterized production of inflammatory mediators by human peritoneal macrophages (hp-Mφ; Pruijboom *et al.*, 1994) we measured arachidonic acid products, several pro-inflammatory cytokines, and a soluble form of an adhesion molecule. The lipoxygenase product leukotriene B₄ (LTB₄; Lewis & Austen, 1984) and the cyclooxygenase products prostaglandin E₂ (PGE₂), thromboxane A₂ (TXA₂) and prostacycline [PGI₂ detected as 6-keto prostaglandin F_{1α} (6kPGF_{1α}); Goldyne, 1987] were measured as these are products of hp-Mφ (Prujboom *et al.*, 1994).

We also measured the pro-inflammatory cytokines interleukin-1 β (IL-1 β), IL-6 and tumour necrosis factor- α (TNF- α , Arai *et al.*, 1990; Oppenheim *et al.*, 1991), which are also produced by hp-M ϕ (Pruimboom *et al.*, 1994).

In addition, we measured the soluble intercellular adhesion molecule 1 (sICAM-1). ICAM-1 plays a crucial role in the adhesion and migration of inflammatory cells, and is a single-chain membrane-bound glycoprotein (Springer, 1990) which is expressed on endothelial and other cells (Springer, 1990; Dustin *et al.*, 1986; Temponi *et al.*, 1988). Expression of ICAM-1 is up-regulated by the cytokines IL-1, TNF- α and interferon-gamma (IFN- γ) (Dustin *et al.*, 1986; 1986; Temponi *et al.*, 1988; Pober *et al.*, 1986; Beekhuizen *et al.*, 1991) and also by the chemoattractants LTB $_4$ and C5a (Pattaroyo, 1989). Expression of ICAM-1 on tissue leads to the release of a soluble form into the circulation. The biological function of this soluble form of ICAM-1 is not yet clear and little is known about the relation between expression of ICAM-1 on tissue and its soluble form. Soluble isoforms of adhesion molecules can be found in the circulation of normal humans (Rothlein *et al.*, 1991).

Although there are publications on IL-6 and TNF- α in ascites, especially during SBP (Andus *et al.*, 1992; Pelletier *et al.*, 1992; Propst *et al.*, 1993; Deviere *et al.*, 1991), we are not aware of any reports on ascitic eicosanoid and ascitic sICAM-1 levels. Soluble ICAM-1 in plasma or serum (Rothlein *et al.*, 1991; Adams *et al.*, 1992; Banks *et al.*, 1993; Sfrikakis *et al.*, 1993; Sharief *et al.*, 1992; Harning *et al.*, 1991; Nouri-Aria *et al.*, 1993) and the cytokines IL-1, IL-6 and TNF- α in plasma, serum or ascites (Andus *et al.*, 1992; Pelletier *et al.*, 1992; Sharief *et al.*, 1992; Khoruts *et al.*, 1991; Byl *et al.*, 1993; Tilg *et al.*, 1992; McClain *et al.*, 1986; Felver *et al.*, 1990) have been found to be elevated in patients with different diseases including malignancies and liver cirrhosis. Therefore we measured the levels of sICAM-1, the representative eicosanoids LTB $_4$, PGE $_2$, PGI $_2$ and TXB $_2$ and the pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α in ascites of patients with liver cirrhosis, peritoneal cancer and SBP to increase our understanding of inflammatory mediators involved in peritoneal inflammation, to determine whether these mediators showed a specific disease-related pattern and whether this pattern could be of diagnostic and prognostic value.

Simultaneously, we measured the same parameters in blood samples of these patients to determine whether there was a close correlation between ascites and plasma values.

EXPERIMENTAL PROCEDURES

Patients

Eighty-nine ascites samples were obtained. Some patients were drained several times during the period in which samples were collected. There was usually a period of several weeks between samples (a total of 63 patients were drained). These samples were divided into three groups: the liver cirrhosis group [number of samples = 53, sex (M/F) = 33/20, age (range) = 58 (27–82)], the peritoneal cancer group [number of samples = 26, sex (M/F) = 18/8, age (range) = 61 (37–81)] and the SBP group [number of samples = 10, sex (M/F) = 7/3, age (range) = 48 (22–59)].

A patient with ascites was classified as having SBP if the number of polymorphonuclear cells (PMN) was higher than 0.25×10^9 cells/l in the ascites and the bacterial culture of the ascitic fluid was positive, or in case the culture was negative, the PMN count in the ascites should be higher than 0.5×10^9 cells/l (Bac *et al.*, 1993a). A patient with renal failure on continuous ambulatory peritoneal dialysis (CAPD) was classified as having a peritonitis if the number of PMN was higher than 0.10×10^9 cells/l in the dialysates and the bacterial culture was positive (Vas, 1989).

In the liver cirrhosis group there were 29 samples obtained from patients with alcoholic liver cirrhosis, 13 samples from patients with hepatitis B or C virus induced cirrhosis, three samples from patients with primary biliary cirrhosis or sclerosing cholangitis, five samples from patients with Budd Chiari and three samples from patients with other diseases.

Of the malignancy-related ascites group all patients had metastized disease. Eight samples were obtained from patients with colon carcinoma, seven samples from patients with pancreatic carcinoma, three samples from patients with liver carcinoma, two samples from patients with peritoneal mesothelioma, two samples from patients with gastric carcinoma, one sample from a patient with lung carcinoma, one sample from a patient with a sarcoma and two samples from patients with an unknown primary source.

In the SBP group four samples were obtained from a patient with hepatitis B virus-induced cirrhosis, and this patient had a *Staphylococcus epidermidis* infection in the ascites (PMN number varied between 0.5×10^9 and 4.2×10^9 cells/l), three samples were obtained from a patient with a pancreas carcinoma and a portal vein thrombosis, and this patient had a *Klebsiella* infection in the ascites (PMN number varied between 0.6×10^9 and 15.6×10^9 cells/l), two samples were obtained from patients with liver cirrhosis, one of these patients had an *Escherichia coli* infection in the ascites (4.3×10^9

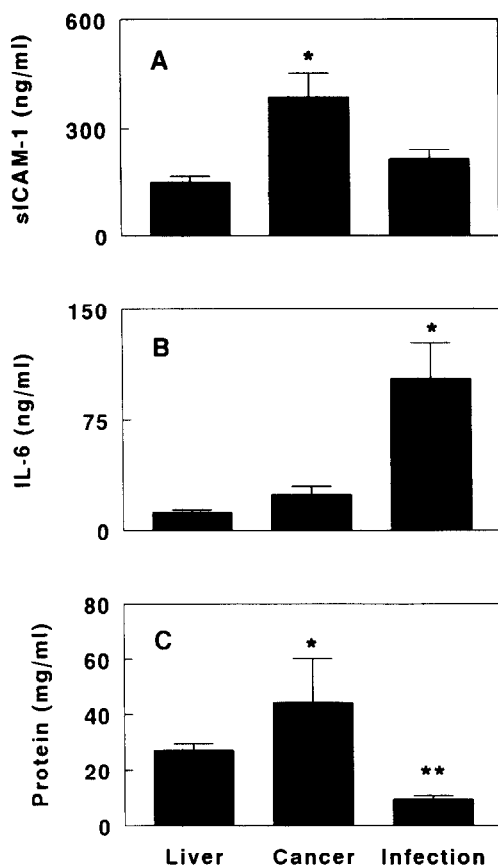


Fig. 1. (A) Soluble ICAM-1 concentration (ng/ml) in ascites of patients with ascites due to liver cirrhosis (liver, $n = 53$), peritoneal cancer (cancer, $n = 26$) and SBP (infection, $n = 10$). (B) Interleukin-6 concentration (ng/ml) in ascites of patients with ascites due to liver cirrhosis (liver, $n = 53$), peritoneal cancer (cancer, $n = 26$) and SBP (infection, $n = 10$). (C) Protein concentration (mg/ml) in ascites of patients with ascites due to liver cirrhosis (liver, $n = 46$), peritoneal cancer (cancer, $n = 25$) and SBP (infection, $n = 7$). *Significantly higher than the other two groups ($P < 0.05$); **Significantly lower than the other two groups.

PMN/l), the other patient had a *Streptococcus mitis* infection in the blood, the ascitic culture of this patient was negative (7.8×10^9 PMN/l in ascites). One sample was obtained from a patient with an acute liver failure and an *Escherichia coli* infection in the ascites (10.5×10^9 PMN/l).

In 34 of these patients with ascites, blood (24 samples from the liver cirrhosis group, six samples from the cancer group and four samples from the SBP group) was collected in EDTA-tubes on the same day or the day after ascites drainage. Blood was also obtained from 17 healthy volunteers [sex (M/F) = 12/5, age (range) = 29 (23–40)].

From two patients [sex (M/F) = 1/1, age (range) = 49 (41–57)] with renal failure on continuous ambulatory peritoneal dialysis (CAPD), the drained dialysates were collected during and after an episode of peritoneal bacterial infection. The first CAPD patient had a *Klebsiella* infection. The number of PMN varied between 0.1×10^9 and 3.8×10^9 PMN/l. The second CAPD patient had *Enterococcus faecalis* infection. The number of PMN varied between 0.2×10^9 and 1.4×10^9 PMN/l.

Assays

The ascites, dialysates and blood were centrifuged at 1500 *g* directly after withdrawal. The centrifuged ascites, dialysates and plasma were stored at -80°C until they were analysed. Soluble ICAM-1, TNF- α , IL-1 β and IL-6 were determined in the centrifuged ascites and plasma using commercially available ELISA kits (sICAM-1, British Biotechnology Products, U.K., sensitivity 2.5 ng/ml; TNF- α , Eurogenetics, U.S.A., sensitivity 10 pg/ml; IL-1 β , Eurogenetics, U.S.A., sensitivity 2 pg/ml; IL-6, Hycult, The Netherlands, sensitivity 2 pg/ml).

LTB $_4$, PGE $_2$, 6kPGF $_{1\alpha}$ and TXB $_2$ were determined by radioimmunoassays (Antibodies, Advanced Magnetics, U.S.A., ^3H -Antigen, Amersham, U.K.; standards, Sigma, U.S.A.) in centrifuged ascites and plasma which were passed through Sep Pak C $_{18}$ cartridges (Water Assoc., U.S.A.).

In the dialysates only sICAM-1 and IL-6 were determined. Protein concentrations in the ascites and plasma were determined using the pyrogallol-red-molybdate(IV) method (Instruchemie, The Netherlands).

Statistical analysis

Data are expressed as the mean \pm S.E.M. Data were statistically analysed with ANOVA followed by Student's *t*-test. The correlations were determined by the Pearson Correlation test. Data were considered significant when $P < 0.05$.

RESULTS

Soluble ICAM-1, cytokine and eicosanoid concentrations in ascites

The soluble ICAM-1 concentration was significantly elevated in ascites of patients with peritoneal cancer (385 ± 66 ng/ml, $n = 26$) compared to the concentration

Table 1. Eicosanoid concentration in ascites of patients with liver cirrhosis, peritoneal cancer and SBP

Eicosanoids	Liver cirrhosis (n)	Peritoneal cancer (n)	SBP (n)
LTB ₄ (pg/ml)	21 ± 5.4 (47)	193 ± 67** (26)	nd* (10)
PGE ₂ (pg/ml)	27 ± 3.2* (47)	122 ± 27*** (26)	79 ± 20.7 (10)
6kPGF _{1α} (pg/ml)	145 ± 62 (47)	359 ± 110**** (26)	113 ± 38 (10)
TXB ₂ (pg/ml)	57 ± 8.1 (46)	70 ± 9.4 (26)	53 ± 3.2 (8)

Data are means ± S.E.M.

nd, Not detectable; n, number of samples.

*Significantly lower than the other two groups ($P < 0.05$).

**Significantly higher than the other two groups ($P < 0.05$).

***Significantly higher than the liver cirrhosis group ($P < 0.05$).

****Significantly higher than the SBP group ($P < 0.05$).

Table 2. Soluble ICAM-1, cytokine and eicosanoid concentration in plasma of patients with liver cirrhosis, peritoneal cancer and SBP and in plasma of controls

Mediators	Liver cirrhosis (n)	Peritoneal cancer (n)	SBP (n)	Plasma control (n)
sICAM-1 (ng/ml)	781 ± 141 (24)	781 ± 367 (5)	1204 ± 271 (3)	228 ± 24* (17)
IL-6 (pg/ml)	1191 ± 358 (24)	420 ± 215 (6)	1736 ± 394 (4)	nd* (17)
IL-1β (pg/ml)	nd (17)	nd (6)	nd (4)	nd (17)
TNF-α (pg/ml)	nd (17)	nd (6)	nd (4)	nd (17)
LTB ₄ (pg/ml)	22 ± 16.3 (23)	14 ± 14.1 (6)	54 ± 54.6 (4)	nd (17)
PGE ₂ (pg/ml)	67 ± 21.1 (23)	68 ± 32.1 (6)	52 ± 48.0 (4)	70 ± 13.0 (16)
6kPGF _{1α} (pg/ml)	104 ± 67.4 (23)	14 ± 14.3 (6)	6.7 ± 4.7 (4)	8.8 ± 1.4 (16)
TXB ₂ (pg/ml)	256 ± 126 (17)	141 ± 51 (6)	127 ± 32 (2)	153 ± 67 (14)

*Significantly lower than the three patient groups ($P < 0.05$).

Data are means ± S.E.M.

n, number of samples.

nd, Not detectable.

of sICAM-1 in ascitic fluid of patients with liver cirrhosis (151 ± 16 ng/ml, $n = 53$, $P = 0.0000$) and SBP (215 ± 27 ng/ml, $n = 10$, $P = 0.0284$). There was no significant difference between the concentrations in ascites from patients with liver cirrhosis and SBP (Fig. 1A).

The IL-6 concentration was significantly elevated in ascites of patients with SBP (103 ± 24 ng/ml, $n = 10$) compared to the concentration of IL-6 in ascites of patients with liver cirrhosis (12 ± 1.8 ng/ml, $n = 53$,

$P = 0.0000$) and peritoneal cancer (24 ± 5.7 ng/ml, $n = 26$, $P = 0.0000$; Fig. 1B).

The ascites protein concentration was significantly lower in SBP (10 ± 1.6 mg/ml, $n = 7$, $P = 0.0104$) than in the liver cirrhosis group (26 ± 2.4 mg/ml, $n = 46$) and peritoneal cancer group (44 ± 2.6 mg/ml, $n = 25$, $P = 0.0000$). Ascites protein levels are significantly higher ($P = 0.0000$) in peritoneal cancer than in the other two groups (Fig. 1C).

Of the eicosanoids, LTB₄ and PGE₂ concentrations

Table 3. sICAM-1, cytokine and eicosanoid concentration in ascites and plasma of patients (grouped all together) with liver cirrhosis, peritoneal cancer and SBP

Mediators	Ascites (n)	Plasma (n)
sICAM-1	227 ± 24	820 ± 122*
ng/ml	(89)	(32)
IL-6	26 ± 4.4**	1 ± 0.3
ng/ml	(89)	(34)
IL-1β	10 ± 2.7	nd
pg/ml	(59)	(25)
TNF-α	65 ± 24.4**	nd
pg/ml	(78)	(24)
LTB ₄	72 ± 22.7	25 ± 13.0
pg/ml	(84)	(33)
PGE ₂	63 ± 10.0	65 ± 16.4
pg/ml	(84)	(33)
6kPGF _{1α}	206 ± 50	76 ± 47.3
pg/ml	(84)	(33)
TXB ₂	61 ± 5.5	218 ± 86.2
pg/ml	(81)	(25)

Data are means ± S.E.M.

nd, not detectable; n, number of samples.

*Significantly higher than the ascites values ($P < 0.05$).

**Significantly higher than the plasma values ($P < 0.05$).

were significantly higher in ascites of patients with peritoneal cancer ($n = 26$) than in the liver cirrhosis group ($n = 47$), and ascites LTB₄ and 6kPGF_{1α} concentrations of peritoneal cancer were significantly increased in comparison to SBP ($n = 10$, Table 1). The LTB₄ concentration in ascites of patients with SBP, and the PGE₂ concentration in ascites of patients with liver cirrhosis were significantly lower in comparison with the other two groups.

Soluble ICAM-1, cytokine and eicosanoid concentrations in plasma

In Table 2 the plasma levels of sICAM-1, cytokine and eicosanoid concentrations per patient group and controls were compared. No significant differences were seen between the three patient groups. Soluble ICAM-1 and IL-6 concentrations in plasma of the patients were significantly elevated in comparison to the controls. IL-1β and TNF-α were not detectable in plasma of patients; IL-1β, IL-6, TNF-α and LTB₄ were not detectable in control plasma.

Comparison between the sICAM-1, cytokine and eicosanoid concentrations in ascites and plasma

As we had only a small number of plasma samples from the peritoneal cancer and SBP groups and there were no significant differences between the plasma levels of the three patient groups, all the plasma samples

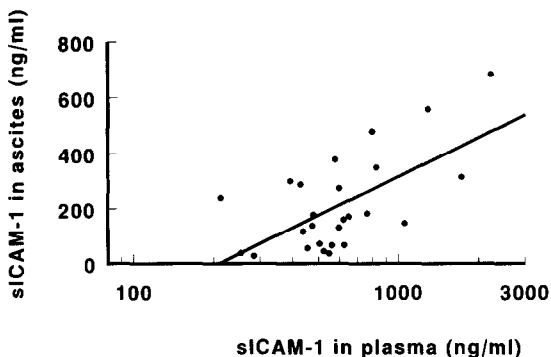


Fig. 2. Correlation between soluble ICAM-1 in ascites and plasma of patients with ascites due to liver cirrhosis, peritoneal cancer and SBP ($r = 0.6926$, $P = 0.0001$, $n = 27$).

of patients were compared with the ascites samples, without subdivision, as calculated and shown in Table 3. There were differences between plasma and ascites values: sICAM-1 concentration was much higher in plasma, while IL-6 and TNF-α concentrations were higher in ascites (Table 3).

There was a good relation between ascites sICAM-1 and plasma sICAM-1 ($r = 0.6926$, $P = 0.0001$, $n = 27$, Fig. 2); for the other metabolites there was no correlation.

Soluble ICAM-1 and IL-6 concentrations during peritonitis

In two patients with SBP, the ascites was drained several times (patient 1: five times, days 0, 25, 28, 32, 38, and patient 2: four times, days 0, 6, 18, 24) over 5–6 weeks. For both these patients sICAM-1 and IL-6 concentrations in ascites changed during this period: patient 1 had no SBP on the first day (day 0) of drainage (negative culture, 0.1×10^9 PMN/l). The sICAM-1 and IL-6 concentrations in the ascites were low (77 ng/ml sICAM-1 and 1 ng/ml IL-6). On day 25 the patient had a subtotal hepatic resection. After this operation the number of PMN (1.7×10^9 PMN/l), sICAM-1 (190 ng/ml) and IL-6 (72 ng/ml) had increased in the ascites. A *Staphylococcus epidermidis* infection was diagnosed on day 28 in the ascites (0.5×10^9 PMN/l, 242 ng/ml sICAM-1, 56 ng/ml IL-6). During the peritonitis sICAM-1 and IL-6 concentrations peaked at day 32 (316 ng/ml sICAM-1, 119 ng/ml IL-6, 4.2×10^9 PMN/l). Antibiotics were given after day 32, after which the sICAM-1 and IL-6 concentrations decreased (195 ng/ml sICAM-1, 79 ng/ml IL-6, 2.2×10^9 PMN/l, Fig. 3A).

Patient 2 had a *Klebsiella* peritonitis on the first day

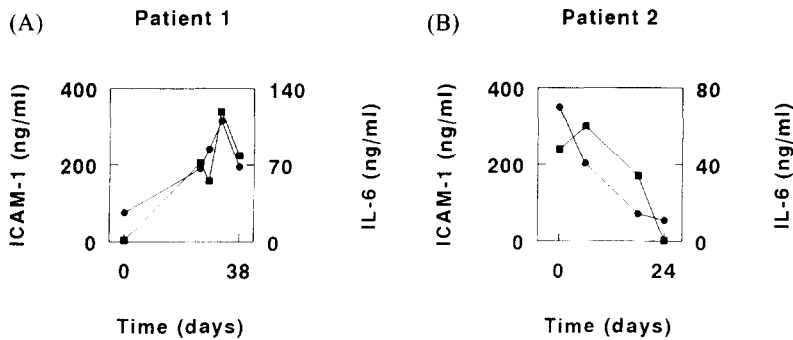


Fig. 3. Soluble ICAM-1 (●) and IL-6 (■) concentrations (ng/ml) in ascites of two patients with SBP. (A) Patient 1 had no SBP on day 0 of drainage. The patient was operated on day 25, and a *Staphylococcus epidermidis* infection started after this operation. Antibiotics were given after day 32. (B) Patient 2 had a SBP on the first day of drainage (*Klebsiella* infection). Antibiotics were given for several weeks. At day 24 the patient was free of infection.

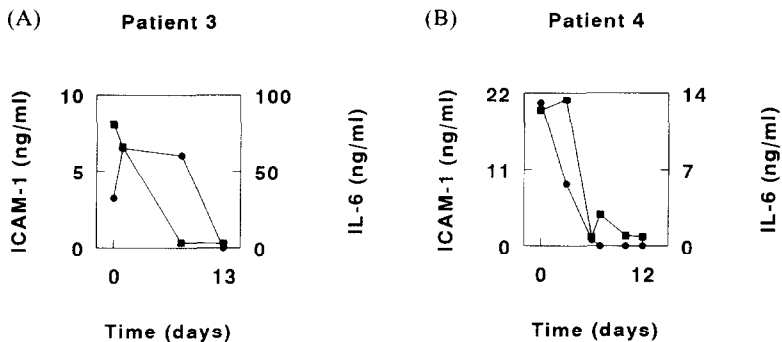


Fig. 4. Soluble ICAM-1 (●) and IL-6 (■) concentrations (ng/ml) in drained dialysates of two CAPD patients during an episode of peritoneal infection. (A) Patient 3 had a peritonitis (*Klebsiella*) on day 0 and no antibiotics had yet been given. On day 1 antibiotics were given. On day 13 the patient was free of infection. (B) Patient 4 had a peritonitis (*Enterococcus faecalis*) on day 0 and no antibiotics had yet been given. On day 12 the patient was free of infection.

of drainage. In the ascites of this patient the sICAM-1 and IL-6 concentrations and the PMN number were high (351 ng/ml sICAM-1, 48 ng/ml IL-6, 15.6×10^9 PMN/l). Antibiotics were given for several weeks and the sICAM-1 concentration decreased to a low level (56 ng/ml). IL-6 concentration slightly increased (day 6, 60 ng/ml), but then also decreased to a very low level (0.3 ng/ml). At the same time the number of PMN decreased (3.4×10^9 PMN/l on day 6, 0.6×10^9 PMN/l on day 18). At the last drainage the bacterial culture of the ascites was negative and the number of PMN had further decreased (0.5×10^9 PMN/l; Fig. 3B).

The ICAM-1 and IL-6 concentrations were also measured in drained dialysates of two CAPD patients during an episode of peritoneal infection (patient 3: four times, days 0, 1, 8, 13) and patient 4: six times, days 0, 3, 6, 7, 10, 12). Patient 3 had an untreated bacterial infection on day 0 (*Klebsiella*, 1.4×10^9 PMN/l), the

sICAM-1 concentration was 3 ng/ml and the IL-6 concentration 80 ng/ml. On day 1 antibiotics were given and the IL-6 concentration decreased (66 ng/ml), and the sICAM-1 concentration (6 ng/ml) and the number of PMN (3.8×10^9 PMN/l) increased. On day 8 the sICAM-1 concentration had decreased only slightly, but the IL-6 concentration (3 ng/ml) and the number of PMN (0.1×10^9 PMN/l) had decreased. On day 13 the patient was free of infection (negative culture, $<0.1 \times 10^9$ PMN/l), sICAM-1 was not detectable and the IL-6 concentration stayed low (3 ng/ml; Fig. 4A).

Patient 4 had a bacterial infection on day 0 (*Enterococcus faecalis*, 1.4×10^9 PMN/l) and before antibiotics were given, the sICAM-1 concentration was 21 ng/ml and the IL-6 concentration 12 ng/ml. On day 3 after antibiotics were given, the IL-6 concentration increased slightly (13 ng/ml), but sICAM-1 concentration (9 ng/ml) and also the number of PMNs in the

dialysates (0.8×10^9 PMN/l) decreased. Between days 6 and 10 the infection was still present, but the concentration of sICAM-1 and IL-6 and the number of PMN decreased (day 6: 0.9 ng/ml sICAM-1, 0.8 ng/ml IL-6, 0.5×10^9 PMN/l; day 7: 0 ng/ml sICAM-1, 2 ng/ml IL-6, 0.2×10^9 PMN/l; day 10: 0 ng/ml sICAM-1, 0.9 ng/ml IL-6, 0.1×10^9 PMN/l). On day 12 the patient was free of infection (negative culture, PMN $<0.1 \times 10^9$), sICAM-1 was not detectable and the IL-6 concentration (0.8 ng/ml) was also low (Fig. 4B).

The correlation between sICAM-1, IL-6 and the number of PMN in the ascites and the drained dialysates in those patients with a peritonitis was for sICAM-1 and PMN 0.7201 ($P = 0.0002$), for IL-6 and PMN 0.4454 ($P = 0.0378$) and for sICAM-1 and IL-6 0.4800 ($P = 0.0238$).

DISCUSSION

The present study shows that there are significant differences in the concentrations and patterns of inflammatory products in ascites due to different disorders. Soluble ICAM-1, the eicosanoids PGE₂ and LTB₄ all clearly differentiated between peritoneal cancer and liver cirrhosis: these metabolites were all higher in ascites of patients with peritoneal cancer. Ascitic sICAM-1 and ascitic IL-6 also increased during episodes of peritoneal infection in two patients with SBP as well as in infected dialysates of two CAPD patients.

Soluble ICAM-1 levels were much higher in plasma than in ascites, in contrast to IL-6 levels which were much higher in ascites than in plasma. Soluble ICAM-1 in ascites of all the patients correlated with sICAM-1 in plasma of these patients. The sICAM-1 levels in plasma of patients were significantly higher than the plasma levels of the controls. The cytokines IL-1 β and TNF- α were low or not detectable in ascites or plasma of patients with liver cirrhosis, peritoneal cancer or SBP.

Soluble ICAM-1 concentration in ascites and plasma

Although there were significant differences in concentrations of sICAM-1 in ascites, in the different disorders plasma levels of sICAM-1 were not significantly different, probably due to the small number of plasma samples in the peritoneal cancer group and the SBP group. Other studies showed that sICAM-1 levels were elevated in sera of patients with different type of diseases in comparison to controls (Rothlein *et al.*, 1991; Sfrikakis *et al.*, 1993; Sharief *et al.*, 1992)

including acute liver disease (chronic hepatic C virus infection, Nouri-Aria *et al.*, 1993), chronic liver diseases (primary biliary cirrhosis and primary sclerosing cholangitis, Adams *et al.*, 1992) and patients with different forms of cancer (Banks *et al.*, 1993; Harning *et al.*, 1991). Soluble ICAM-1 is probably released more to the bloodstream circulation than locally within the peritoneal cavity as the sICAM-1 levels in plasma of all the patients was significantly higher than levels in ascites of all the patients. Adhesion to and penetration through the vascular endothelium is a mandatory step for leucocyte migration and accumulation at sites of inflammation. *De novo* or up-regulated expression of adhesion molecules on sinusoidal lining cells in inflamed liver biopsy specimens indicates that these endothelial cells actively modulate their phenotype in response to environmental factors, thus playing a key role in the recruitment of leucocytes in acute and chronic liver inflammation (Volpes *et al.*, 1992) and probably leading to the release of ICAM-1 locally. Malignant melanomas are usually surrounded by a heavy inflammatory infiltrate; the inflammatory cells often express activation-associated markers (Ralfkiaer *et al.*, 1987) and the malignant melanomas express ICAM-1 (Hansen *et al.*, 1991). An explanation for the significant differences of sICAM-1 concentration in ascites of the different groups in our study could be that cancer cells are also a source of sICAM-1. A study showed that an unstimulated human melanoma cell line expressed ICAM-1 on the cell surface but did not release significant levels of sICAM-1. Stimulation with TNF- α , IL-1 and IFN- γ , but not IL-6, then caused the release of sICAM-1. The same group showed in the same publication that in nude mouse models bearing s.c. human melanoma tumours, the serum contained human sICAM-1 (Giavazzi *et al.*, 1992). These two studies show clearly that only the cancer cells in these models could have been the source of the soluble form of ICAM-1. In humans, cancer cells are probably an important source for the release of sICAM-1 locally into the peritoneal cavity. Assuming that cancer cells are a source of sICAM-1, this may provide cancer cells with additional escape mechanisms, e.g. ineffective recognition of cell surface ICAM-1 by cytotoxic lymphocytes or blocking immune recognition by blocking the ligand for ICAM-1, lymphocyte-function-associated antigen 1 (LFA-1), on the leucocytes (Springer, 1990; Rothlein *et al.*, 1986).

All our patients with peritoneal cancer had high levels of sICAM-1 in the ascites. As all these patients have late stage cancer, it was not possible to correlate the disease progression of these patients with the sICAM-1 level in the ascites or plasma of these patients. Other studies did show that the levels of sICAM-1 in

humans correlated with liver metastasis in a variety of tumours, and with disease progression in melanoma cells (Banks *et al.*, 1993; Harning *et al.*, 1991).

IL-1, IL-6 and TNF- α concentrations in ascites and plasma

In previous studies we isolated M ϕ from ascitic fluid of patients with liver cirrhosis. These M ϕ had a high IL-6 production capacity (Pruimboom *et al.*, 1994). The IL-6 levels in the ascites of the patients used for this study were always much higher in the peritoneal cavity than in the plasma, indicating that IL-6 was produced locally in the peritoneal cavity by M ϕ . Endothelial cells are also able to produce IL-6 (Aarden *et al.*, 1991; Jirik *et al.*, 1989). IL-1 β and TNF- α were hardly detectable in ascites or plasma of patients with liver cirrhosis, peritoneal cancer and PBS. Other reports, however, show that besides elevated IL-6 levels, elevated IL-1 and TNF- α plasma levels were found in patients with ascites caused by liver diseases, in comparison to controls (Khoruts *et al.*, 1991; Tilg *et al.*, 1992). In SBP patients, in comparison to non-infected patients, elevated TNF- α levels in ascites (Deviere *et al.*, 1991) and plasma (Byl *et al.*, 1993) were also found. Similarly to our results, one other report also described significant elevated IL-6, but not IL-1 or TNF- α levels in ascites and serum of SBP patients (Propst *et al.*, 1993).

Besides M ϕ and endothelium, tumour cells should also be considered as potential sources of IL-6. Some tumour cells have been shown to produce IL-6 (Heinrich *et al.*, 1990; Kawano *et al.*, 1988). IL-6 is a potent growth factor for myeloma/plasmacytoma cells and its deregulated expression may be involved in the oncogenesis of human multiple myelomas (Kishimoto, 1989). In this way IL-6 can be involved in the growth pattern of malignancy.

The liver is an important site of synthesis and is the main clearance organ of circulating cytokines. The rapid hepatic clearance of circulating cytokines may normally constitute an important mechanism limiting the systemic action of cytokines. Lack of hepatic clearance may be one reason for disturbances of the immune system in patients with hepatic cirrhosis (Andus *et al.*, 1991) and probably also in patients with liver metastasis. IL-6 is important in liver diseases, as it induces acute-phase protein synthesis, particularly fibrinogen production, in hepatocytes (Kishimoto, 1989). The high IL-6 production during the bacterial infection is probably a direct effect of the bacteria on the macrophages in the peritoneum: lipopolysaccharide (LPS), a bacterial membrane component, is known to be one of the most potent stimuli of M ϕ (Lynn & Golenbock, 1992). Increasing LPS concentrations

increase the IL-6 production capacity of human peritoneal M ϕ *in vitro* (Pruimboom *et al.*, 1994).

Correlation between IL-6 and sICAM-1

The high sICAM-1 and IL-6 levels and low IL-1 β and TNF- α levels in the patients with ascites could indicate that there is a connection between IL-6 and sICAM-1: IL-6 could induce ICAM-1 expression and also the release of the soluble form. In an auto-immune mouse model ICAM-1 was newly induced in the hepatic sinusoids of controls by intravenous injection of recombinant mouse IL-6, and elevated production of IL-6 by hepatic mononuclear cells also correlated with this ICAM-1 expression (Ohteki *et al.*, 1993). However, incubation of cultured monolayers of human endothelial cells with IL-6 did not affect their adhesiveness for human monocytes (Beekhuizen *et al.*, 1991). IL-1 α/β , TNF- α and IFN- γ , but not IL-6, increase the permeability of human endothelial cells *in vitro* (Burke-Gaffney & Keenan, 1993).

It has not been shown previously that both sICAM-1 and IL-6 levels in ascites of patients with SBP and in the dialysis fluid of CAPD patients with SBP correlated with episodes of infection. sICAM-1 and IL-6 levels were high when the patients had an infection, decreased when antibiotics were given and were hardly detectable when the patients were free of infection.

Eicosanoids in ascites and plasma

It is not clear why LTB₄ production is much higher in ascites of the peritoneal cancer group than in the liver cirrhosis group. It is likely that cancer cells in the peritoneal cancer group activate the inflammatory cells to produce LTB₄. This elevation may in turn stimulate ICAM-1 expression (Holt *et al.*, 1993).

The release of the vasodilating agents PGE₂ and PGI₂ (Goldyne, 1987) could act as mediators involved in the induction of ascites (Hamilton *et al.*, 1982). In patients with a liver disease, endothelial cells, macrophages and hepatocytes or the disability of the liver to clear these vasodilators (Keppler *et al.*, 1985) could be responsible for the PGE₂ and PGI₂ production. In patients with cancer, the cancer cells themselves could also be responsible for the high concentration of the PG in ascites. A number of experimentally induced tumours in animals, as well as those occurring naturally in humans, have been found to produce high amounts of PG, in particular PGE₂ (Strelkov *et al.*, 1989; Husby *et al.*, 1977).

In conclusion, the levels of sICAM-1, the eicosanoids LTB₄ and PGE₂ and the protein concentration in ascites are parameters which showed a specific

disease-related pattern; however, for diagnostic use they may not be suitable as all the parameters of the three different patient groups overlapped. However, as sICAM-1 levels in ascites correlated with sICAM-1 levels in plasma and both sICAM-1 and IL-6 correlated with episodes of infection (in ascites of patients with SBP and drained dialysates of CAPD patients with SBP),

sICAM-1 and IL-6 seem to be parameters which could be used in the future to monitor patients with SBP.

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