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Elevated plasma interleukin-6 and increased severity and mortality in alcoholic hepatitis

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SUMMARY

Recent studies in alcoholic hepatitis have proposed a role for the cytokine tumour necrosis factor-alpha (TNF-α) a mediator of endotoxic shock in sepsis. In this study plasma levels of the closely related cytokine interleukin-6 (IL-6) were assayed in 96 samples from 58 patients with severe alcoholic hepatitis, and 69 patients in control groups (21 normal, 10 alcoholic without liver disease, 10 inactive alcoholic cirrhosis, 18 chronic liver disease, 10 chronic renal failure). Plasma IL-6 levels were markedly elevated in patients with alcoholic hepatitis when compared with all control groups ($P < 0.001$). IL-6 levels were higher in patients who died ($P = 0.04$) and correlated with the features of severe disease including: increased grade of encephalopathy, increased neutrophil count, increased prothrombin ratio, hypotension, increased serum creatinine and increased serum bilirubin. Surprisingly, no correlation was found between levels of plasma IL-6 and plasma TNF-α or endotoxin, or the presence of infection; an inverse correlation was found between plasma IL-6 and serum globulins. These findings provide further evidence that the IL-6/TNF cytokine system is activated in severe alcoholic hepatitis and may mediate hepatic or extra-hepatic tissue damage.

Keywords alcoholic hepatitis tumour necrosis factor interleukin-6

INTRODUCTION

Acute alcoholic hepatitis is a distinct clinical entity with a severity ranging from mild subclinical illness to severe disease in which one month mortality is up to 60% (Theodossi, Eddleston & Williams, 1982). Patients with encephalopathy and severe coagulopathy frequently have associated extra-hepatic features including fever, neutrophilia, activation of the acute-phase response, sepsis and acute renal failure. A clinical picture which can resemble the multisystem involvement seen in fulminant hepatic failure and severe sepsis. Paradoxically clinical and laboratory evidence of liver damage often increase over a period of 2–4 weeks after cessation of alcohol intake (Reynolds *et al.*, 1989). The exact mechanisms underlying initiation and progression of liver injury are not clear, but in endotoxic shock similar clinical features are thought to result from release of the cytokines tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6).

IL-6 is thought to be the major mediator of the acute-phase response to infection and is also produced *in vivo* in response to TNF-α (Sheron *et al.*, 1990b) which is probably the initial mediator of tissue damage in septic shock (Waage, Halstensen & Espevik, 1987). Circulating IL-6 is found in association with TNF-α in sepsis (Waage *et al.*, 1989) and in patients with paracetamol-induced fulminant hepatic failure (unpublished data). Evidence is now emerging that the cytokines TNF-α and IL-6 are involved in alcoholic liver disease. High levels of plasma TNF-α have been found to correlate with mortality in alcoholic hepatitis (Bird *et al.*, 1990; Felver *et al.*, 1990) and moderately elevated levels of IL-6 have been reported in alcoholic cirrhosis (Deviere *et al.*, 1989). To investigate the possibility that IL-6 may be implicated in the pathogenesis of alcoholic hepatitis we have assayed plasma IL-6 in patients with severe alcoholic hepatitis, and have sought correlations with levels of TNF-α, IL-1 and endotoxin.

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SUBJECTS AND METHODS

Ninety-six blood samples were studied from 58 patients admitted with acute alcoholic hepatitis. Diagnosis was based on clinical and laboratory findings including: a history of alcohol intake exceeding 80 g/day for 1 year, serum aspartate transaminase (AST) > 80 IU (range 80–240), and hepatomegaly. Percutaneous, trans-jugular or post-mortem liver biopsy specimens confirming the diagnosis were available in 38 (65%) of the 58 patients. In the remaining patients isotope colloid scans showed the typical 'white out' appearance of alcoholic hepatitis. In 38

patients a single sample was assayed, in 20 patients up to three serial samples taken on alternate days were assayed to study the time course of cytokine production. Samples were obtained a mean period of 6·3 days (95% confidence intervals (CI) 5·5–7·1 days, range 1–20) after admission.

Clinical parameters recorded were the most abnormal values in the 24-h period. The minimum systolic blood pressure was recorded after volume depletion was corrected. Hypotension was defined as a systolic blood pressure <80 mm Hg in the presence of an adequate pulmonary capillary wedge pressure. Documented infection indicated a clinically significant positive bacterial or fungal culture from body fluids or infection sites or the presence of >250 neutrophils/ml. Microbiological cultures were performed routinely and when clinically indicated. Survival was measured from the time of cytokine sampling.

Five control groups were also studied. These included: 21 healthy laboratory staff, 10 patients with inactive alcoholic cirrhosis, 10 alcoholic subjects with normal liver function, 10 patients with chronic renal failure and 18 patients with chronic liver disease of varying aetiology (seven primary biliary cirrhosis and 11 $HBsAg^+$ chronic active hepatitis).

Blood for cytokine assay was taken into endotoxin-free heparinized tubes (vacutainer) and plasma separated within 20 min by centrifugation at 200 *g* for 5 min. Samples were stored at $-20°C$ until assayed. Plasma IL-6 was assayed in all samples ($n=96$) using ELISA (R & D systems) with a sensitivity of 10 ng/*l*, TNF-α ($n=58$ from 21 patients) by radioimmunoassay (Medgenix) with a sensitivity of 5 ng/*l* ($n=58$), IL-1 by ELISA (Cistron) with a sensitivity of 20 ng/*l* ($n=58$) and endotoxin using the chromogenic LAL assay (Kabi-Vitrum) with a sensitivity of 5 ng/*l* ($n=60$, from 22 patients). All cytokine assays had an intra-assay and inter-assay coefficient of variation of less than 10%. Results of the TNF-α, IL-1 and endotoxin assays on many of the same patients have been published separately (Bird *et al.*, 1990) and are given for the purposes of comparison.

Statistical analysis

Non-parametric data were log transformed and are quoted as the geometric mean (g.m.) with 95% CI. Data were analysed using SPSS PC+ software by means of univariate Pearson correlation (daily cytokine levels *versus* clinical data) or Student's *t*-test (peak cytokine levels; patients *versus* controls, survivors *versus* non-survivors). Significant univariate associations were further analysed by stepwise multiple regression analysis. Correlations with clinical data were performed on cumulated results from all samples on a day-by-day basis irrespective of other parameters such as day of admission except where stated.

RESULTS

Of the 58 patients (Table 1), 41 (71%) had ascites at the time of study, 22 (38%) were encephalopathic, and 19 (33%) died within a period of 4 weeks; the deaths occurred at a mean of 8·7 days after the plasma samples were obtained. Of 19 deaths, five were the result of gastro-intestinal haemorrhage and 14 of hepatic failure; of the latter, 10 patients were in acute renal failure and three deaths were due predominantly to sepsis. The incidence of infection was high; 26 (45%) patients had microbiologically confirmed evidence of infection during the study period. These infections comprised nine patients with culture-negative spontaneous bacterial peritonitis, seven patients with *Candida albicans* isolated, four with *Streptococcus faecalis*, five with *Staphylococcus aureus*, two with *S. epidermidis* and one with *Escherichia coli*. Seven of these patients had evidence of infection with two or more organisms.

Table 1. Clinical details of 58 patients with alcoholic hepatitis at the time of blood sampling

		95% confidence interval
Mean age (years)	46	43–49
Sex (M/F)	35/23	—
Mortality at 4 weeks (%)	33	—
Confirmed infection during study (%)	45	—
Ascites (%)	71	—
Hepatic encephalopathy (%)	38	—
Mean sample time after admission (days)	5·3	4·5–6·1
Mean survival after sampling (days)	8·7	6·8–10·9
Mean international prothrombin ratio	1·7	1·54–1·86
Mean serum aspartate transaminase (IU)	127	117–137
Mean serum alkaline phosphatase (IU)	261	230–290
Serum bilirubin (μmol/*l*)*	169	131–223
Serum creatinine (μmol/*l*)*	131	104–165
Mean serum albumin (g/*l*)	36	24·3–47·7
Mean neutrophil count ($\times 10^6$)	13·5	11·1–15·7

* Geometric mean.

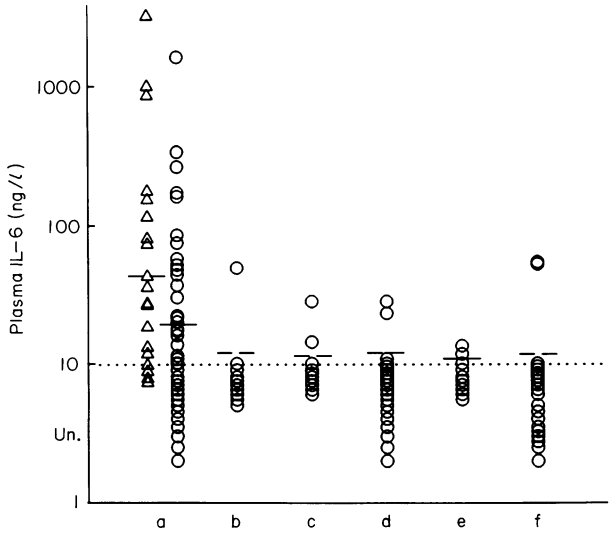

Fig. 1. Peak plasma IL-6 levels in subjects with: a, alcoholic hepatitis; b, alcoholic; c, inactive cirrhosis; d, chronic liver disease; e, chronic renal failure; and f, healthy controls. ○, survivors; △, non-survivors. Bars are geometric means. Un., undetectable.

IL-6

Plasma IL-6 was detectable in 37 (63·7%) of 58 patients with alcoholic hepatitis and in 10 (17·3%) levels of over 100 ng/*l* were detected (Fig. 1). Levels were significantly higher in alcoholic hepatitis than in all control groups ($P<0·001$). In none of the

Table 2. Plasma levels of IL-6 samples (ng/L) from patients with alcoholic hepatitis and control groups

	n	Geometric mean	95% confidence interval
Alcoholic hepatitis	58	27·5	20·8–36·3
survivors (>28 days)	39	19·9	14·9–26·6
non-survivors	19	43·2	25·5–73†
Normal controls	21	11·7	<10–14·8*
Inactive alcoholic cirrhosis	10	11·4	<10–14·2*
Chronic liver disease	18	12·1	<10–14·1*
Alcoholics (no liver disease)	10	11·7	<10–16·1*
Chronic renal failure	10	10·5	<10–11·2*

* $P<0·001$ compared with all patients with alcoholic hepatitis.
† $P=0·04$ compared with survivors (peak levels).

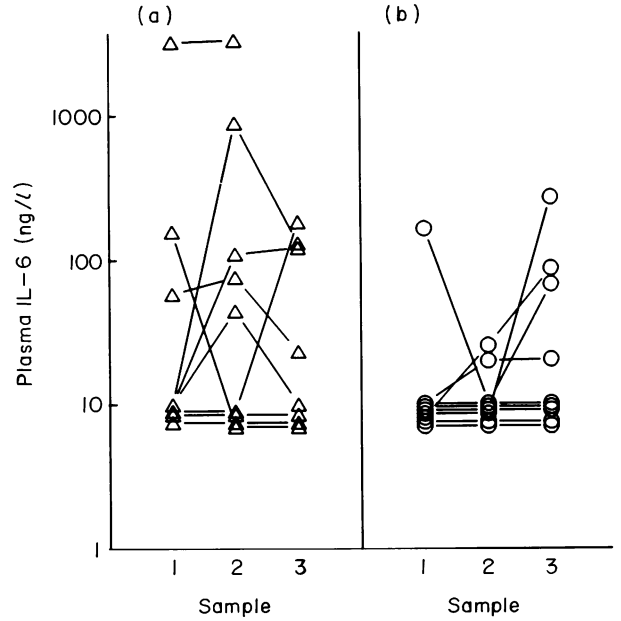

Fig. 2. Serial plasma IL-6 levels in 20 patients with alcoholic hepatitis: a, non-survivors; b, survivors.

disease control groups was plasma IL-6 significantly higher than the normal controls (Table 2). In those patients ($n=20$) from whom more than one sample was obtained, levels of IL-6 rose slightly over the 6 days from the time of the first sample with mean levels in the first, second and third samples being 23·7, 25·4 and 28·5 ng/*l*, respectively. Analysing all patients ($n=58$) there was a univariate correlation between duration of hospital admission and plasma IL-6 level ($P=0·014$), with levels tending to rise with time: In many patients the levels of IL-6 also varied markedly from day to day (Fig. 2).

Peak IL-6 levels were higher in those patients with alcoholic hepatitis who died within 4 weeks of the study period than in survivors ($P=0·04$, Table 2). There were also significant correlations between plasma IL-6 and concurrent clinical and laboratory features of alcoholic hepatitis (Table 3). Increased levels of plasma IL-6 correlated positively with markers of liver

Table 3. Correlation matrix given as *r* value [*P* value] for plasma levels of IL-6 and TNF-α, and clinical and laboratory features in patients with alcoholic hepatitis

	IL-6 ($n=96$)	TNF-α ($n=58$)
Plasma TNF-α	NS	—
Plasma IL-6	—	NS
Plasma endotoxin	NS	NS
Encephalopathy grade	0·37 [<0·001]*	0·36 [<0·001]
Neutrophil count	0·34 [<0·001]	0·54 [<0·001]
Serum creatinine	0·28 [0·003]	0·73 [<0·001]*
Death (<28 days)	0·27 [0·004]	0·48 [<0·001]
Shock	0·24 [0·009]	0·46 [<0·001]
Day of admission	0·23 [0·014]	NS
Serum globulin	−0·22 [0·022]	NS
INR	0·20 [0·024]	NS
Male sex	0·19 [0·031]	NS
Age	−0·19 [0·034]	0·25 [0·031]
Serum bilirubin	0·18 [0·038]	0·41 [0·001]
Serum IgA	NS	NS
Serum IgG	NS	NS
Serum IgM	NS	NS
Confirmed infection	NS	0·46 [<0·001]

Factors remaining significant after multiple regression analysis are shown (*$P<0·001$). NS, not significant.

failure, including grade of encephalopathy ($P<0·001$), and, less strikingly, international standardized prothrombin ratio ($P=0·024$) and serum bilirubin ($P=0·038$). There were no associations between plasma IL-6 levels and serum albumin or serum AST and alkaline phosphatase.

Plasma IL-6 levels also correlated positively with extrahepatic features, including hypotension ($P=0·009$), serum creatinine ($P=0·003$), and neutrophil count ($P<0·001$). There were negative correlations between plasma IL-6 and age ($P=0·034$) and levels were higher in men than in women ($P=0·031$). There was no association between plasma IL-6 and serum immunoglobulins IgA, IgM and IgG ($n=27$). There was a negative correlation between plasma IL-6 and serum globulin ($P=0·022$) as estimated from serum total protein and albumin levels. In a multiple regression analysis performed to look for independent associations, plasma IL-6 correlated only with grade of encephalopathy ($P<0·001$, $n=96$). The power of this analysis was however limited in view of the relatively low numbers of samples.

Relationship with plasma TNF-α and endotoxin

Plasma levels of TNF-α (g.m. 21·9, CI 19–25·2 ng/*l*) and endotoxin (g.m. 26·9, CI 19·4–37·2 ng/*l*) were significantly elevated in patients with alcoholic hepatitis, whereas IL-1 was undetectable in any of the samples. Levels of TNF-α and endotoxin correlated with the clinical features of severe alcoholic hepatitis (Table 3); the pattern of correlation of TNF-α was similar to that found with IL-6. There was no correlation between the plasma level of IL-6 and plasma levels of TNF-α, IL-1 or endotoxin. Whereas the level of plasma TNF-α correlated positively with microbiologically confirmed infection, there was, perhaps surprisingly, no association between such infection and plasma levels of either IL-6 or endotoxin. Within the subset of patients with confirmed infection there was no

association between plasma IL-6 and any particular type of infection.

DISCUSSION

Alcoholic hepatitis is associated with elevated levels of plasma IL-6. These generally correlate with the clinical features of severe disease, most notably the level of encephalopathy, but also the presence of renal failure, hypotension and neutrophilia, and resemble the associations found previously (Bird *et al.*, 1990) with plasma TNF-α levels (Table 3). Although it is not possible to identify a direct relationship between plasma IL-6 and any single clinical feature of alcoholic hepatitis, the general association of high plasma IL-6 levels with both disease severity and mortality is quite clear. Similar elevations in plasma IL-6 and TNF-α are found in sepsis and fulminant hepatic failure, perhaps reflecting common disease mechanisms.

Animal studies (Castell *et al.*, 1988) have shown that 80% of radiolabelled recombinant IL-6 are cleared via the liver, suggesting that the elevation in plasma IL-6 in alcoholic hepatitis may be a consequence of decreased hepatic clearance. However, the fluctuation in plasma IL-6 levels in individual patients (Fig. 2) is more in keeping with a short plasma half-life, as found previously (Sheron *et al.*, 1990b) in human studies (100 min). Although decreased clearance or even decreased levels of an IL-6 inhibitor may be important, it is more probable that levels of IL-6 are mainly elevated as a result of increased production. The source of IL-6 and TNF-α in alcoholic hepatitis is unclear; increased production of TNF-α from peripheral blood mononuclear cells has been demonstrated previously in alcoholic hepatitis (McClain & Cohen, 1989), and increased hepatic vein concentrations of TNF-α, but not IL-6, have been demonstrated in humans given intravenous endotoxin infusions (Fong *et al.*, 1990). The marked elevation in plasma IL-6 found in alcoholic hepatitis may reflect hepatic inflammation, or non-specific factors unrelated to underlying mechanisms of disease pathogenesis; moderate levels of serum IL-6 have, for example, been detected on bioassay following elective surgery (Cruickshank *et al.*, 1990). However, plasma IL-6 was not significantly elevated in any of the disease control groups which included patients with chronic liver disease, nor was there any association between plasma IL-6 and infection, another potential confounding factor.

A study of patients with alcoholic cirrhosis (from which patients with biopsy-proven alcoholic hepatitis were excluded) found that levels of serum IL-6 were mildly elevated and correlated positively with serum IgA levels; only two out of 15 patients had levels in excess of 100 U/ml on bioassay. The absence of detectable plasma IL-6 in inactive alcoholic cirrhosis in the present study may reflect a difference in sensitivity between the ELISA and the bioassay. In patients with alcoholic hepatitis there was no association between plasma IL-6 and serum IgA, and a negative correlation was identified between plasma IL-6 and serum globulin levels.

Endotoxin and TNF-α are potent stimuli to IL-6 production *in vitro* (Sehgal *et al.*, 1988) and *in vivo* (Sheron *et al.*, 1990b), and although high levels of these substances were detected in alcoholic hepatitis, there were no correlations between levels of IL-6 and either TNF-α or endotoxin. In contrast, in meningococcal sepsis close correlations were found between levels of TNF-α and IL-6 (Waage *et al.*, 1989), suggesting IL-6 release may have occurred in direct response to circulating TNF-α. It appears that in alcoholic hepatitis factors other than TNF-α and endotoxin are of importance in mediating IL-6 production.

The majority of cytokines, including IL-6, are multifunctional. In addition, many cytokine effects are probably mediated by a synergistic action or antagonism between different cytokines (Le & Vilcek, 1989). For example, TNF-α is a vital component of the mechanism leading to both endotoxic shock and galactosamine induced fulminant hepatic failure (Lehman, Freudenberg & Galanos, 1987) and anti-TNF antibodies protect animals from otherwise lethal injections of these agents (Beutler, Milsark & Cerami, 1985). However, recombinant TNF has been used in clinical studies in humans with no evidence of serious toxicity despite plasma levels in excess of those seen in disease states (Sheron *et al.*, 1990a). Similarly in animal studies IL-6 has few toxic effects even in very high doses (Helle *et al.*, 1988). IL-6 is present in association with TNF-α in both disease states and in animal models of TNF-α-associated toxicity, and anti-TNF-α antibodies also prevent IL-6 release (Fong *et al.*, 1989). It is possible therefore that both TNF-α and IL-6 in synergy with other factors have a role in mediating tissue damage. If elevated levels of plasma cytokines prove to have a causative role in the mediation or perpetuation of tissue damage in alcoholic hepatitis, this would raise the possibility of immunomodulatory therapy. Corticosteroids—powerful suppressors of the immune system and of TNF-α production—have recently been shown to improve survival in selected cases of severe alcoholic hepatitis (Imperiale & McCullough, 1990). The development of anti-cytokine antibodies and antagonists offers another approach worthy of consideration.

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