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Prolonged Elevation of Interleukin-8 and Interleukin-6 Concentrations in Plasma and of Leukocyte Interleukin-8 mRNA Levels during Septicemic and Localized *Pseudomonas pseudomallei* Infection

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Patients suffering from serious bacterial infection present to the hospital after early inflammatory events, such as release of tumor necrosis factor (TNF), have been initiated. The role of other cytokines, such as interleukin-8 (IL-8), a neutrophil chemoattractant and activator, in the pathophysiology of human sepsis is not well characterized, and there are only limited data on IL-6. We studied serial concentrations of TNF, IL-6 (involved in the acute-phase response), and IL-8 in plasma and leukocyte levels of mRNA for these cytokines in patients with localized and septicemic *Pseudomonas pseudomallei* infection on admission to the hospital and during a prolonged recovery phase (up to 30 days). Of 18 patients, 8 had detectable plasma IL-8 and all had raised plasma IL-6 concentrations. In patients who died median initial concentrations of IL-8 (167 pg/ml; range, 97 to 362 pg/ml) and IL-6 (4,800 pg/ml; range, 60 to 9,245 pg/ml) in plasma were higher than those in survivors ($P < 0.008$ and $P = 0.007$, respectively). Septic patients who survived and patients with localized disease had similar cytokine levels. Plasma IL-8 and IL-6 concentrations were elevated throughout the inpatient period of recovery. Circulating leukocytes contained mRNA for IL-8 but not for IL-6 and TNF, and they may secrete IL-8. An elevated plasma IL-6 concentration (>1,000 pg/ml) had 75% mortality) was the best predictor of mortality in *P. pseudomallei* sepsis. Fifty percent of patients with detectable plasma IL-8 concentrations died. In contrast, plasma TNF bioactivity did not relate to outcome; 75% of patients who died never had detectable plasma TNF activity.

Cytokines are important mediators in the pathophysiology of septicemia, a condition associated with high mortality. Tumor necrosis factor (TNF) administered intravenously to animals mimics many of the pathophysiological changes seen in sepsis (34), and pretreatment with monoclonal antibody to TNF reduces the lethality of *Escherichia coli* injected intravenously into baboons (35). Massively elevated concentrations of TNF in plasma have previously been associated with a poor prognosis in human sepsis (7). However, TNF is not the only determinant of survival, as concentrations of this cytokine in plasma measured following endotoxin infusion into healthy human volunteers have been shown to be several times higher than those in septicemic patients, and volunteers suffered from only relatively trivial complaints (6). In addition, a raised plasma TNF level has been found to be a less useful prognostic marker in severe sepsis than conventional clinical indicators of disease severity, such as the age of the patient, urine output, and arterial pH (4). Elevated plasma interleukin-6 (IL-6) concentrations have also been associated with a poor prognosis in septic shock (38). Pathophysiological functions of IL-6 include activation of B, natural killer, and T cells (20, 23, 24) and mediation of some of the physiological changes of the acute-phase response (14).

IL-8 is likely to prove to be important in the host defense to bacterial pathogens, as it is a powerful neutrophil chemoattractant (40) which is involved in neutrophil trafficking across endothelial cells (15). Plasma IL-8 concentrations are elevated following injection of intravenous *E. coli* into primates (37) and of lipopolysaccharide (LPS) into human volunteers (25). Such increased IL-8 levels in experimental models occurred at a time similar to that of an elevation of plasma IL-6 levels and followed a transient (about 3-h) increase in the plasma TNF concentration. However, there are no previous studies of plasma IL-8 in naturally occurring severe human bacterial infections presenting to the hospital. In addition, it is not known whether plasma cytokine concentrations fluctuate over time, and thus the value of a single measurement at the time of admission to the hospital as an estimate of prognosis in septic patients may be reduced. Furthermore, there are few data concerning plasma cytokine concentrations during recovery from infection.

Melioidosis is a major cause of death from community-acquired septicemia in Thailand and is endemic throughout southeast Asia and northern Australia (22). This infection is caused by *Pseudomonas pseudomallei*, a motile, aerobic, non-spore-forming, gram-negative organism which is widely distributed in the soil and water of rice paddy fields. Clinically melioidosis may present as a profound septicemic illness from which about 60% of patients survive with the best current therapy (39). Mortality may be higher where the antibiotic of choice, ceftazidime, is not available. The other

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TABLE 1. Clinical characteristics of patients at point of admission to study

Patient no.	Age (yr)	Sex[a]	Diagnosis	Duration of illness (days)	Temp (°C)	Mean blood pressure (mm Hg)	Leukocyte count (10^9/liter)[b]	Blood urea nitrogen (mmol/liter)[b]	Outcome
1	53	M	*P. pseudomallei sepsis*	7	38.5	103	7.6	19	Lived
2	49	F	*P. pseudomallei sepsis*	3	36.2	60	17.6	8	Died
3	50	M	*P. pseudomallei sepsis*	2	39.0	107	8.4	3	Lived
4	61	M	*P. pseudomallei sepsis*	7	37.5	107	24.4	16	Died
5	62	M	*P. pseudomallei sepsis*	28	40.5	117	19.3	4	Lived
6	49	F	*P. pseudomallei sepsis*	7	37.6	67	15.0	18	Died
7	36	F	*P. pseudomallei sepsis*	3	38.3	80	20.4	3	Lived
8	32	M	*P. pseudomallei sepsis*	14	38.0	93	12.8	4	Lived
9	65	F	*E. coli* sepsis	1	38.5	73	20.3	6	Died
10	53	F	*E. coli* sepsis	3	36.5	126	14.8	15	Lived
11	45	M	*P. pseudomallei* pneumonia	7	37.2	100	17.0	13	Lived
12	75	F	*P. pseudomallei* pneumonia	28	37.7	133	6.8	3	Lived
13	44	M	*P. pseudomallei* pneumonia	21	37.4	97	11.3	3	Lived
14	40	M	*P. pseudomallei* pneumonia	7	39.5	117	15.6	3	Lived
15	65	M	*P. pseudomallei* pneumonia	7	36.0	90	20.2	16	Lived
16	30	M	*P. pseudomallei* pneumonia	1 y	38.0	107	8.8	3	Lived
17	36	M	*P. pseudomallei* abscess	28	39.2	107	23.1	NA	Lived
18	52	M	*P. pseudomallei* abscess	16	40.5	107	8.7	5	Lived

[a] M, male; F, female.
[b] NA, not applicable.

principal clinical form of melioidosis is a localized infection commonly manifested by visceral or soft-tissue abscess formation or pneumonia. Study of melioidosis provides an unusual opportunity for comparison between the pathogenesis of severe septicemia and that of a localized infection both caused by the same gram-negative bacterium.

We have therefore carried out a detailed longitudinal study of plasma TNF, IL-6, and IL-8 concentrations in patients with melioidosis at clinical presentation and for up to 30 days after initiation of treatment. In addition, leukocyte mRNA levels for these cytokines were assessed to determine whether circulating cytokines are secreted by fixed tissue macrophages alone or whether leukocytes in the blood may contribute to levels in plasma.

MATERIALS AND METHODS

Patients. Patients with a clinical history and findings on examination suggestive of melioidosis and who had been admitted to the medical wards of Sappasitprasong Hospital, Ubon Ratchatani, northeastern Thailand, entered this study. It was conducted over a 3-month period selected to coincide with the peak local seasonal incidence of melioidosis. All patients were over the age of 14 years, and they (or their relatives if the patient was profoundly unwell) gave informed consent to blood sampling. This study was part of a research project approved by the Ethical Review Subcommittee of the Thai Ministry of Health. Eight patients were diagnosed as suffering from septic melioidosis, and seven of those were blood culture positive for *P. pseudomallei*. The eighth patient from whom *P. pseudomallei* was cultured from sputum, obtained by tracheal aspiration, was in septic shock (blood pressure, 80/60; pulse, 126/min, with peripheral vasodilation and oliguria) and required ventilatory support prior to death 14 h after admission. Eight patients had localized melioidosis (Table 1). In addition, two patients were blood culture positive for *E. coli*, a gram-negative pathogen, and have been included in the analysis of septicemic patients.

Study protocol. An initial blood sample was taken for measurement of electrolytes and glucose, renal and liver function tests, and a full blood count. Three separate blood cultures were performed. Sputum, a rectal swab, and any pus were also collected for bacterial culture before antibiotics were administered. *P. pseudomallei* was identified as an oxidase-positive organism giving characteristic wrinkled colonies on Ashdown's medium (2), and identification was confirmed with the API2ONE system (API Laboratories, Basingstoke, United Kingdom). Routine laboratory microbiological methods were employed for detection of other organisms. Initial antibiotic therapy for patients with suspected melioidosis was either intravenous ceftazidime (120 mg/kg/day) or intravenous amoxicillin (120 mg/kg/day)-clavulanic acid combination (Augmentin). These antibiotics were being compared in an ongoing randomized, prospective controlled trial at the time of this study.

Blood was taken immediately prior to administration of antibiotics ($T = 0$) for measurement of plasma cytokine concentrations and leukocyte mRNA extraction. Subsequent blood samples for cytokine analyses and mRNA extraction were taken 1, 3, 6, 12, 24, 36, and 48 h later via an indwelling peripheral venous catheter and then on days 3, 4, 5, 6, 7, 10, 15, 20, 25, and 30 or until death or discharge from hospital. Blood sampling was undertaken at frequent intervals immediately after treatment was initiated to detect any effect of administration of bacteriocidal antibiotics on cytokine release. Each 5-ml blood sample was collected into an endotoxin-free tube containing potassium EDTA and 100 μl of trasylol (2,000 Kallikrein inactivator units). This was immediately centrifuged at 1,000 × *g* for 5 min, and the plasma was stored at −25°C in Ubon (1 to 3 weeks) and then at −70°C in Bangkok and London prior to cytokine analyses (within 3 months). Control experiments showed no loss of cytokine bioactivity over this period when samples are frozen in this manner (data not shown).

Cytokine analyses. All assays of plasma cytokine concentrations were carried out in a blinded fashion by a laboratory unaware of the clinical details of individual patients. Bioassay measurements were obtained from standard curves by using known concentrations of TNF and IL-6 which were run concurrently. Additional positive control samples and ones with appropriate (anti-TNF and anti-IL-6) and control

antibodies added were also assayed. Plasma TNF was measured by bioassay as previously described by using the WEHI 164 subclone 13 cell line (the kind gift of A. Waage, University of Trondheim, Trondheim, Norway) (11). Plasma IL-6 was measured by using the B-9 cell line proliferation assay (the kind gift of J. Gauldie, McMaster University, Hamilton, Canada) (1). We have previously demonstrated the specificity of this assay (36). The lower limit of sensitivity of the TNF assay was 23 pg/ml, and that of the IL-6 assay was 1 pg/ml. IL-8 was measured with an enzyme-linked immunosorbent assay (ELISA) with a lower limit of detection of 95 pg/ml (9).

mRNA analysis. The buffy coat was removed from centrifuged blood samples after plasma had been removed. Contaminating erythrocytes were lysed at room temperature with a solution of 0.8% ammonium chloride, 10 mM sodium bicarbonate, and 1 mM tetrasodium EDTA. Leukocytes were pelleted by centrifugation (1,000 × *g* for 5 min at 4°C), homogenized in RNA extraction buffer (4 M guanidine thiocyanate, 25 mM Tris [pH 7.0], 0.5% *N*-lauroylsarcosine, and 0.1 M 2-mercaptoethanol), and frozen first at −25°C and then at −70°C. Subsequently RNA underwent double phenol-chloroform and chloroform-isoamyl alcohol extraction and precipitation in isopropanol and then in ethanol before being resuspended in DEPC water. After quantification on an optical densitometer (Pye/Unicam SP6-450) equal amounts of RNA (10 to 15 μg) were run on denaturing formaldehyde–1% agarose gels, transferred by capillary blotting to Hybond-N (Amersham), and fixed by exposure to UV light. The blots were prehybridized with 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 sodium citrate)–1× Denhardt's solution–0.5% sodium dodecyl sulfate–0.05% sodium PP_i–50 μg of polyadenylic acid per ml–100 μg of transfer RNA per ml and then hybridized with γ-^{32}P-end-labelled oligonucleotide probes: for TNF, a 25-mer (21); for IL-6, a 30-mer (8); for IL-8, a 30-mer; and for β-actin, a 42-mer (32). Blots were washed and then autoradiographed with intensifying screens at −70°C for 24 to 48 h. Between probings, blots were stripped by heating for 1 h at 65°C in a solution of 0.005 M Tris-HCl (pH 8.0), 0.002 M Na_2EDTA, and 0.1× Denhardt's solution.

In addition to patient samples, mRNA extraction was performed in control experiments with LPS-stimulated whole blood and THP-1 cells (a monomyelocytic cell line) which were then probed as described above and previously (8).

Statistical analysis. Clinical data and plasma cytokine levels are given as median values with ranges and were compared by using the Mann-Whitney U test. Proportions were tested by Fisher's exact test. A *P* value of <0.05 was regarded as significant.

RESULTS

Patient clinical characteristics. Thirty-five patients entered into the study, and 18 of them had either septicemia caused by a gram-negative organism or localized *P. pseudomallei* infection and therefore completed the protocol. The patients had a mean age of 50 years; 12 were male, and 6 were female. Clinical details are shown in Table 1. Ten patients had blood culture-proven sepsis (eight *P. pseudomallei* and two *E. coli* infections), and localized melioidosis was diagnosed in eight patients by culture of sputum or pus aspirated from abscesses. Four patients, all of whom were septicemic, died within 24 h of admission to the hospital. On admission to the study, the mean blood pressure of patients who subsequently died was 70 mm Hg (range, 60 to 107 mm Hg) compared with 107 (80 to 133) mm Hg in survivors (*P* = 0.03) (Table 2). Although patients who survived had a slightly greater febrile response, leukocytosis, and blood urea nitrogen on admission to the study (Table 1), none of these differences reached statistical significance in this small sample. Blood pressure, fever, leukocyte count, and blood urea nitrogen in patients with localized disease and survivors of septicemia were similar.

TABLE. 2. Associations among clinical parameters, admission plasma cytokine concentrations, and patient mortality

Paramater or cytokine concn	No. of patients		*P* value
	Survived	Died	
Plasma TNF			
>25 pg/ml	2	0	NS[a]
>25 pg/ml	12	4	
Plasma IL-6			
>1,000 pg/ml	0	3	0.011
<1,000 pg/ml	14	1	
Plasma IL-8			
>95 pg/ml	4	4	0.049
<95 pg/ml	10	0	
Mean blood pressure			
<75 mm Hg	0	3	0.011
>75 mm Hg	14	1	
Temp			
<37°C	2	1	NS
>37°C	12	3	
Leukocytosis			
>15 × 10^9/liter	6	3	NS
<15 × 10^9/liter	8	1	
Blood urea			
>15 mmol/liter	2	2	NS
<15 mmol/liter	11	2	

[a] NS, not significant.

Plasma cytokine concentrations. Elevated plasma IL-8 concentrations were detectable in 8 of the 18 patients studied and included all 4 patients who subsequently died (Fig. 1). In healthy human subjects there is no measurable IL-8 (or TNF or IL-6) in the circulation. The highest plasma IL-8 concentrations (161 to 362 pg/ml) were found in the three patients who also had very elevated plasma IL-6 concentrations and who died. Plasma IL-8 concentrations were significantly higher in patients who died compared with those in patients who survived (*P* = 0.008). Plasma IL-8 concentrations were a significant predictor of mortality (Table 2). Plasma IL-8 concentrations remained raised throughout the duration of hospital treatment, in which time there was clinically apparent recovery (Fig. 2). There was no clear trend of reduction in plasma IL-8 concentrations of individual patients during recovery.

The most elevated plasma IL-6 concentrations were detected at presentation in three of four patients who subsequently died (Fig. 1). The highest IL-6 concentration recorded at admission to the study was 9,245 pg/ml, which rose to 19,930 pg/ml 3 h after the administration of antibiotics. Plasma IL-6 concentrations in patients who died were significantly higher than in those who survived (*P* = 0.0065). A raised plasma IL-6 concentration (>1,000 pg/ml) was a good predictor of mortality (Table 2). Elevated plasma IL-6 levels were detectable in all patients, including those who survived. Plasma IL-6 remained elevated at approximately constant concentrations during the entire admission

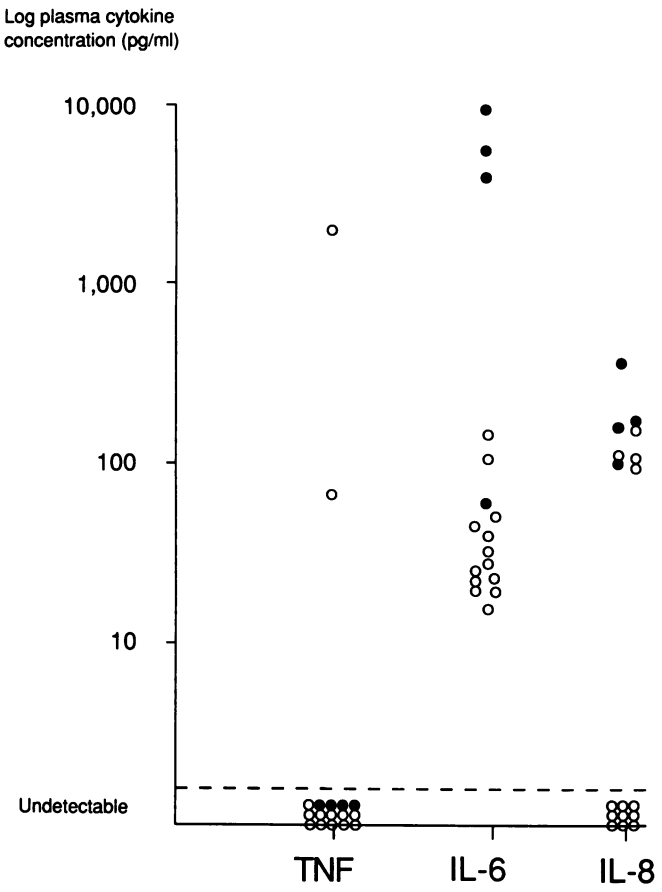

FIG. 1. Cytokine concentrations in patients with melioidosis at admission to study. ●, patients who died; ○, survivors.

to the hospital in a fashion similar to that of plasma IL-8 (Fig. 2).

In contrast, plasma TNF bioactivity was raised in only two patients at admission, and neither of these died (Fig. 1). Plasma TNF concentrations did not correlate with either plasma IL-8 or plasma IL-6 concentrations. Transient rises in plasma TNF up to concentrations of 150 pg/ml were detected in eight patients during their illness. However, in three of the patients who died, no TNF was detected, and in the fourth a level of 35 pg/ml was recorded 3 and 6 h after admission. One patient who did not die had an initial plasma TNF concentration above 2,000 pg/ml and presented with lung abscesses and an abscess in the subcutaneous tissue overlying his left tibia, both due to *P. pseudomallei* infection. TNF bioactivity was detectable in the plasma after day 10 in just one patient.

Peripheral leukocyte cytokine mRNA content. Raised levels of mRNA for IL-8 in peripheral blood leukocytes were detected in all patients (Fig. 3). In contrast, no mRNA could be detected for either TNF or IL-6 in circulating leukocytes. The amount of leukocyte IL-8 mRNA compared with that of 28S and 18S ribosomal RNA bands and the expression of β-actin mRNA (a housekeeping reference mRNA) was greater in those patients who had higher circulating concentrations of the cytokine. IL-8 mRNA in peripheral blood leukocytes from control subjects not suffering from infection was only occasionally detectable with prolonged autoradiography.

DISCUSSION

This study documents for the first time plasma IL-8 concentrations in human patients with sepsis. Elevated plasma IL-8 concentrations are associated with poor patient prognosis (Fig. 1 and Table 2). The presence of circulating IL-8 in patients with bacterial infection is consistent with in vitro data (30) that IL-8 gene expression occurs in response to bacterial LPS as well as TNF and IL-1, another early proinflammatory cytokine (10). IL-8 is secreted into plasma after injection of bacterial LPS into human volunteers (25) and of *E. coli* into baboons (28). The known properties of IL-8 of neutrophil chemotaxis and activation make it probable that high levels of this cytokine reflect an important role for IL-8 in the pathophysiology of an infection, such as melioidosis, caused by a gram-negative organism. In this study, both IL-8 and IL-6 concentrations in plasma were better predictors of mortality than was the plasma TNF concentration. Plasma IL-8 concentrations did not differ between septic patients who survived and patients with localized disease, possibly because patients in the latter group may have had an episode of bacteremia prior to admission.

IL-8 mRNA was detected in circulating leukocytes of all patients, and levels were greater in patients with higher plasma IL-8 concentrations. The presence of IL-8 mRNA in circulating leukocytes does not necessarily mean that these cells are actively secreting this cytokine. However, stimulation of whole blood inoculated in vitro with bacterial LPS resulted in generation of IL-8 mRNA and was followed by secretion of IL-8 detectable by ELISA (8). IL-8 has been shown to be secreted in vitro by phagocytosing neutrophils (3), activated macrophages (40), endothelial cells (32) and fixed tissue cells (33). All of these cell types might contribute to IL-8 detected in the plasma of patients with melioidosis. The plasma IL-8 concentration remained elevated throughout the inpatient period of recovery. This unexpected result could not be followed up, as the study design did not include an investigation at 3 months (complete recovery) and this would have been logistically impracticable in rural Thailand. We have also found prolonged IL-8 secretion in an in vitro model of infection by *Mycobacterium tuberculosis* (13). Intravascular IL-8 may be important in limiting neutrophil trafficking across the endothelium at sites of inflammation (18), and this may be an important mechanism in resolution of an inflammatory response. The prolonged elevation of IL-8 during the resolution of infection and presumed clearance of bacteria and bacterial LPS suggests that additional stimuli, possibly including other cytokines, such as IL-1 (37), are involved in IL-8 secretion in vivo. However, immunological detection of IL-8 has not always correlated with bioactivity of this cytokine (42). It is also possible that some IL-8 circulates in an inactive form or bound to an inhibitor or soluble receptor, as does TNF.

In this study there is a clear association between very high levels of IL-6 in plasma and fatal sepsis caused by a gram-negative organism. This confirms similar data reported for meningococcal meningitis (38) and sepsis in an intensive-care unit (16). Three of the four patients who died from septicemia had plasma IL-6 concentrations about 100-fold higher than those in the patients in the study who survived. The other patient who died had an elevated plasma IL-6 concentration of 60 pg/ml on admission that increased to 342 pg/ml. All four patients died within 24 h of admission to the study. A high IL-6 level was the best indicator of poor prognosis in this group of patients (Table 2). Although

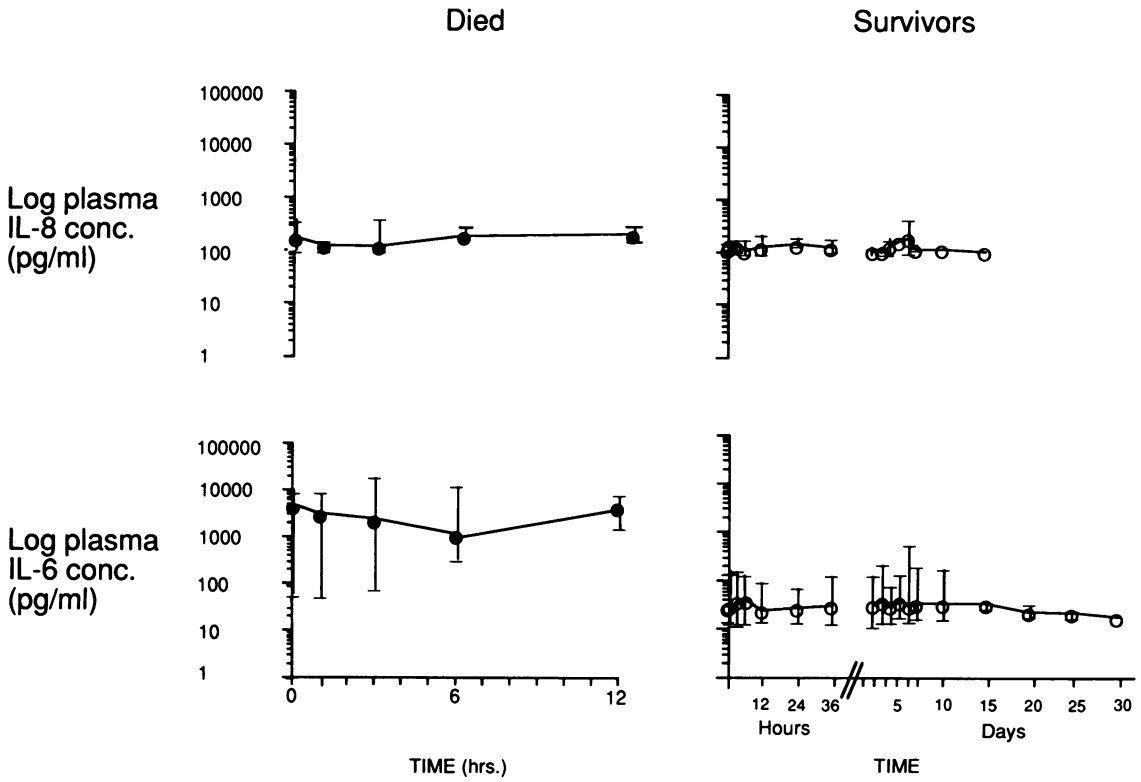

FIG. 2. Longitudinal data showing plasma IL-8 and IL-6 concentrations in survivors (○) and nonsurvivors (●) with melioidosis. Values are medians, with ranges shown as error bars. The lower limit of detection of IL-6 was 1 pg/ml, and that of IL-8 was 95 pg/ml. Data for the surviving patients who had undetectable IL-8 levels (i.e., below the lower end of range as illustrated) are not shown. The IL-6 level was raised in all patients.

relatively few pathophysiological features of sepsis can be attributed directly to the known effects of IL-6 (19), it has been shown that anti-IL-6 monoclonal antibodies protect against lethal infective doses of *E. coli* in an animal model (31). Thus, it is likely that IL-6 contributes to pathophysiology in human sepsis, possibly acting synergistically with other cytokines. The fact that IL-6 rather than TNF was more commonly detected in the plasma of patients in this

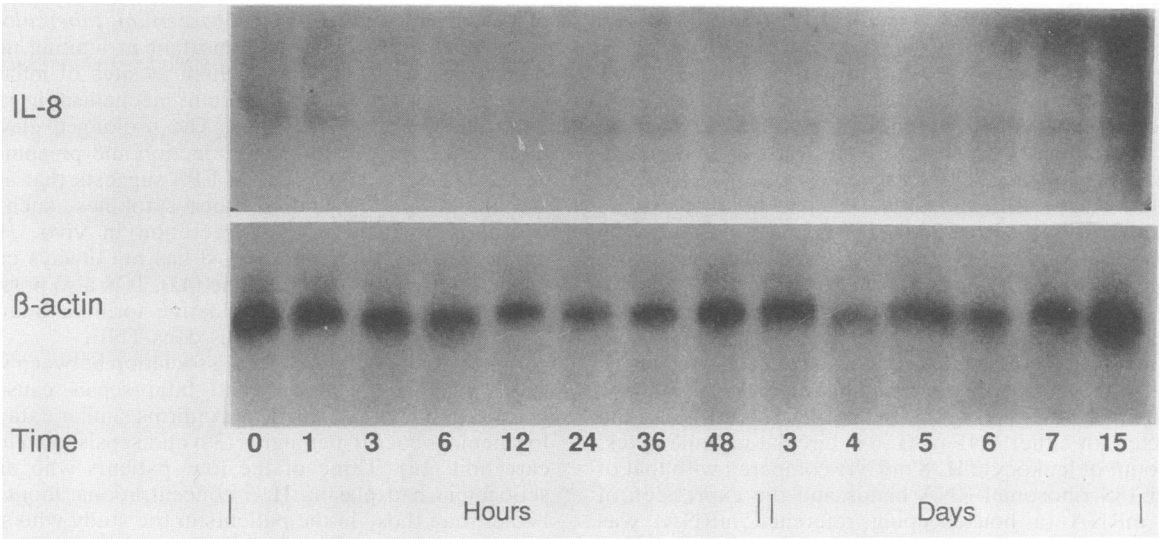

FIG. 3. Autoradiographs of RNA extracts (Northern blots) from circulating leukocytes from a patient with nonfatal septicemic melioidosis probed to demonstrate the presence of mRNA for IL-8 and β-actin, a housekeeping (reference) gene. Stripping of these blots and reprobing for TNF and IL-6 revealed no mRNA for these cytokines. Control experiments (not shown) with the same probes demonstrated TNF, IL-6, and IL-8 mRNA in extracts of whole blood stimulated by LPS (8). Similar results were obtained on Northern blot analysis of RNA extracted from circulating leukocytes of all other patients in the study.

study is consistent with experimental observations in vitro and in vivo. The peak expression of IL-6 mRNA in leukocytes after incubation of human blood with bacterial LPS in vitro occurs several hours after the peak of TNF mRNA (8). Similarly, intravenous injection of monoclonal antibody against TNF into baboons attenuates the rise in plasma IL-6 concentration that follows intra-aortic infusion of *E. coli*, which suggests that TNF production is important in IL-6 generation (12). It is of interest that all patients had increased plasma IL-6 concentrations which remained elevated throughout inpatient treatment. This reflects the sensitivity of our bioassay, as in another study only 2% of septicemic patients had elevated serum IL-6 concentrations 10 days after admission to the hospital (5). The persistently raised plasma IL-6 level may have resulted from continuing low levels of inflammation in tissues. There were no differences in plasma IL-6 concentrations in severely ill patients with septicemia who subsequently recovered and in those patients with localized abscesses or pneumonia.

The fact that we were unable to detect any mRNA for IL-6 in circulating leukocytes indicates that this cytokine is unlikely to be secreted by these cells. We cannot exclude having missed a peak of gene expression earlier in the course of illness. It is more probable that plasma IL-6 reflects transcription and secretion of this cytokine in other tissues. Studies using in situ hybridization and immunohistochemical staining would have been helpful. However, no biopsies were performed in these patients as part of their clinical management, and it was not considered ethical to biopsy for research purposes in seriously ill patients.

TNF bioactivity detected in plasma did not correlate with mortality in septic patients in the present study, although data obtained by immunological assays suggests an association (4, 6). Measurement of cytokine concentrations by bioassay as opposed to immunological measurement by ELISA or radioimmunoassay may be critical in assessing the pathophysiological role of cytokines in human disease. Specifically, TNF forms biologically inactive polymers which are detectable in immunological assay systems (26), and it binds to soluble receptors. The role of soluble receptors and cytokine antagonists in sepsis remains to be investigated in a prospective study. Plasma TNF concentrations did not correlate with the raised plasma IL-8 or IL-6 levels associated with death. In this study, TNF bioactivity, at a low concentration, was detected in only one of the four septic patients who subsequently died. It is therefore not surprising that no mRNA for TNF was found in circulating leukocytes from any of the patients during their hospital admission.

TNF appears to have been cleared from the circulation of patients by the time they were admitted to this study. TNF has a short half-life in plasma. Plasma TNF concentrations in mice peak within 1 h of intravenous injection of bacterial LPS and are undetectable 8 h after injection (29). The fact that TNF was detected in some patients highlights the difference between animal models using a discrete infective challenge and the more complicated situation of an ill person presenting relatively late in the course of infection. Even when detectable, plasma TNF concentrations did not remain elevated throughout the period of clinical recovery, unlike IL-6 and IL-8 concentrations. However, it is possible that TNF production in tissues contributes to the prolonged elevation of IL-6 concentrations detected in plasma. In a murine listeriosis, TNF and IL-6 were present in the spleen, a site of bacterial replication in infected animals, although only IL-6 was measurable in plasma (17). In the present study, there was no evidence of an increase in plasma TNF concentration following administration of antibiotics as has been documented in the Jarisch-Herxheimer reaction that follows treatment of relapsing fever (27).

The results from this study have possible therapeutic implications for patients with sepsis caused by a gram-negative organism. Currently there is much interest in treating septic patients by intravenous administration of monoclonal antibodies against TNF or drugs, such as oxypentifylline, which decreases TNF but not IL-6 production in response to bacterial LPS (41). Our findings suggest that efforts might need be directed towards one or more other cytokines, such as IL-6, antibodies for which have been demonstrated to be protective against *E. coli* infection in mice (31), and IL-8. However, any therapy involving immunosupression which interferes with the incompletely understood cytokine cascade that is part of the host defense system is potentially hazardous for patients with severe sepsis.

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