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Circulating Tumor Necrosis Factor Alpha (TNF), Soluble TNF Receptors, and Interleukin-6 in Human Subacute Bacterial Endocarditis

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Cell surface components of viridans streptococci and enterococci have been shown to stimulate the release of tumor necrosis factor alpha (TNF) and interleukin-6 from monocytes/macrophages. In the sera from 10 patients with subacute enterococcal or streptococcal endocarditis, however, the levels of both cytokines were low or undetectable, with elevated TNF levels on admission in 3 patients with complicated disease. Soluble TNF receptor levels were significantly elevated compared with those of healthy controls. When patients with malaria were used as a control group of acute intravascular infection with high circulating TNF values, the ratio between soluble TNF receptors and TNF on admission was significantly greater in the patients with subacute bacterial endocarditis. Besides different amounts of circulating TNF, enhanced TNF receptor shedding may have an important role in the pathogenesis of subacute versus acute clinical disease following human intravascular infection.

Subacute bacterial endocarditis is associated with significant morbidity and, unless treated, eventually fatal. Causative organisms are frequently certain species of enterococci or oral streptococci (12, 23, 27). Despite persistent bacteremia over weeks in the untreated patient, sepsis is an unusual feature of subacute bacterial endocarditis. Rather, the disease is characterized by a usually long-lasting illness with weight loss, arthralgia, low-grade fevers, anemia, and progressive debilitation. Recent studies indicate that lipoteichoic acids, the major cell wall components of certain viridans group streptococci and enterococci, are potent stimulators of cytokine release from human monocytes and rat macrophages in vitro (4, 15). In vivo cytokine response patterns during infection with these organisms, however, are unknown.

We serially measured circulating interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF) in the sera of 10 patients (eight males, two females; median age, 60 years) considered to have definite, culture-proven, subacute bacterial endocarditis caused by streptococci or enterococci. The patients had at least three (range, three to seven) blood cultures taken more than 8 h apart that were positive for the same organism. Three of the patients had been pretreated with antibiotics before referral to this hospital, and admission blood cultures of two of these were negative. Except for one patient with prosthetic valve endocarditis, two-dimensional echocardiography showed typical features of valvular endocarditis, and all patients presented with at least four of the following clinical features: erythrocyte sedimentation rate, >50 mm/h; C-reactive protein, >20 mg/liter (upper limit of normal, 5 mg/liter); normocytic anemia; involuntary weight loss of more than 10% in the preceding 4 weeks; prolonged fever (>2 weeks) and/or night sweats; and casts in the urine and/or microhematuria. Three patients had or developed early complications during their disease: one had purulent spondylitis on admission and developed cerebral hemorrhage, late pneumonia, and catheter-related bacteremia; one developed ischemic stroke due to cerebral emboli; and one was admitted with acute severe heart failure and died on the third hospital day. A fourth patient had mild heart failure on admission and later developed a large splenic abscess from which enterococci were cultured (Table 1). The remaining six patients had an uncomplicated course during therapy. Therapy included in all cases cell wall-active antibacterial agents combined with an aminoglycoside antibiotic.

Blood samples were obtained from all patients within 36 h of admission and 2 to 3 days later. Samples were obtained at least twice per week subsequently until patient discharge. Blood samples were centrifuged within 2 h, and appropriate serum aliquots were frozen and stored at −20°C until analysis. Samples obtained on admission from 17 nonimmune patients with *Plasmodium falciparum* malaria and from 12 healthy volunteers were included as controls. All samples were processed in an identical manner. TNF and IL-6 were measured in an immunoradiometric assay (Medgenix Ltd., Fleurus, Belgium). The lower limit of detection in these assays is 5 to 10 pg/ml, and levels of both cytokines in sera of healthy volunteers were <10 pg/ml.

On admission, elevated immunoreactive TNF levels in serum (ranging from 54 to 137 pg/ml) were found in three patients. TNF concentrations were low (median, <10 pg/ml) and remained so in the other patients except in one patient who had a late increase in circulating TNF values when he developed a splenic abscess (Table 1). Compared with those of healthy controls, IL-6 levels were slightly elevated in all patients on admission (median, 33 pg/ml) without a clear tendency for normalization during the observation period (Table 1). TNF values on admission or within the first week after admission did not correlate with the estimated duration of symptoms before diagnosis, peripheral leukocyte counts, erythrocyte sedimentation rate or IL-6 values, or vegetation size (data not shown). The highest TNF values were observed in the patient with cerebral infarction (168 pg/ml) and in the patient with acute severe heart failure (137 pg/ml) that resulted in early death (Table 1).

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TABLE 1. Clinical features and concentrations of circulating TNF and IL-6 in patients with subacute bacterial endocarditis

Patient no.	Age (yrs)/sex	Organism[a] and complication(s)	Peak TNF level (pg/ml) on day(s):				Peak IL-6 level (pg/ml) on day(s):			
			1	2–7	8–20	21–40	1	2–7	8–20	21–40
1	59/M	VS, spondylitis, cerebral hemorrhage, late pneumonia, and catheter-related bacteremia	54	17	<10	34	28	32	98	139
2	43/F	VS, cerebral emboli	58	168	30	18	59	124	118	107
3	75/M	EF, acute severe heart failure, death	137	88	ND[b]	ND	37	11	ND	ND
4	56/M	EF, mild heart failure, late splenic abscess	<10	<10	<10	49	166	272	71	36
5	19/M	VS	<10	<10	<10	ND	50	70	31	ND
6	60/F	VS	<10	<10	13	ND	21	23	73	ND
7	70/M	EF	<10	32	13	12	24	37	70	17
8	62/M	EF	<10	<10	<10	<10	41	80	18	<10
9	72/M	EF	<10	<10	<10	<10	21	40	23	10
10	71/M	EF	<10	<10	<10	<10	21	20	82	<10

[a] VS, viridans streptococci; EF, *Enterococcus faecalis*.
[b] ND, not determined.

In view of the known potentially deleterious effects of TNF (3, 21, 26) and the rather slowly progressive nature of the disease despite a persistent intravascular focus of infection, we also examined the possible role of TNF binding proteins (5, 13, 19) which, as two types of soluble TNF receptors (sTNF-R), have recently been found to be elevated in a variety of acute and chronic infections and inflammatory diseases (6, 10, 11, 14, 16, 20, 24, 25). sTNF-R concentrations were determined by an enzyme-linked immunobinding assay (Hoffmann-LaRoche, Basel, Switzerland), kindly donated by H. Gallati, Basel, Switzerland, designed to detect only functionally active receptor fragments with the use of mouse monoclonal antibodies against sTNF-R55 (fragment p55, clone *utr*-4) and sTNF-R75 (fragment p75, clone *htr*-20) as described previously (16). The detection limit of each of these two sTNF-R assays is 0.1 ng/ml. In healthy controls, concentrations of sTNF-R55 in serum were 1.3 ± 0.3 ng/ml and sTNF-R75 concentrations were 2.8 ± 0.5 ng/ml and thus were comparable to those reported previously with the use of this or similar assays (1, 6, 10, 11, 14, 16, 24, 25).

Concentrations of both receptor fragments in serum were increased in patients compared with those in controls, but values differed widely, ranging from 1.0 to 29.6 ng/ml for sTNF-R55 and from 1.7 to 102 ng/ml for sTNF-R75. The mean values on admission were 4.8 ± 3.3 ng/ml (median, 3.1 ng/ml) for sTNF-R55 and 20.9 ± 22.5 ng/ml (median, 9.5 ng/ml) for sTNF-R75. Values in the sera obtained 2 to 7 days after admission appeared to increase (Fig. 1). Values at 8 to 20 days after admission and later were still significantly higher than those in controls ($P < 0.001$, Wilcoxon's exact test) but indicated slowly decreasing concentrations, particularly of sTNF-R55, compared with admission values (Fig. 1). A close correlation was found between sTNF-R55 and sTNF-R75 concentrations ($r = 0.82$) and between immunoreactive TNF and both sTNF-R55 ($r = 0.77$) and sTNF-R75 values ($r = 0.79$).

Patients with malaria had elevated levels of TNF and of both sTNF-R fragments on admission (Table 2). TNF values were much higher than in the patients with endocarditis ($P < 0.001$), and sTNF-R values were slightly higher ($P < 0.05$) (Table 2). For comparison of the excess of sTNF-R relative to TNF, we calculated the ratios between sTNF-R and TNF values (on a weight basis) of both groups on admission using 9 pg/ml for TNF values below the detection limit (<10 pg/ml). For the endocarditis patients, the ratios on admission

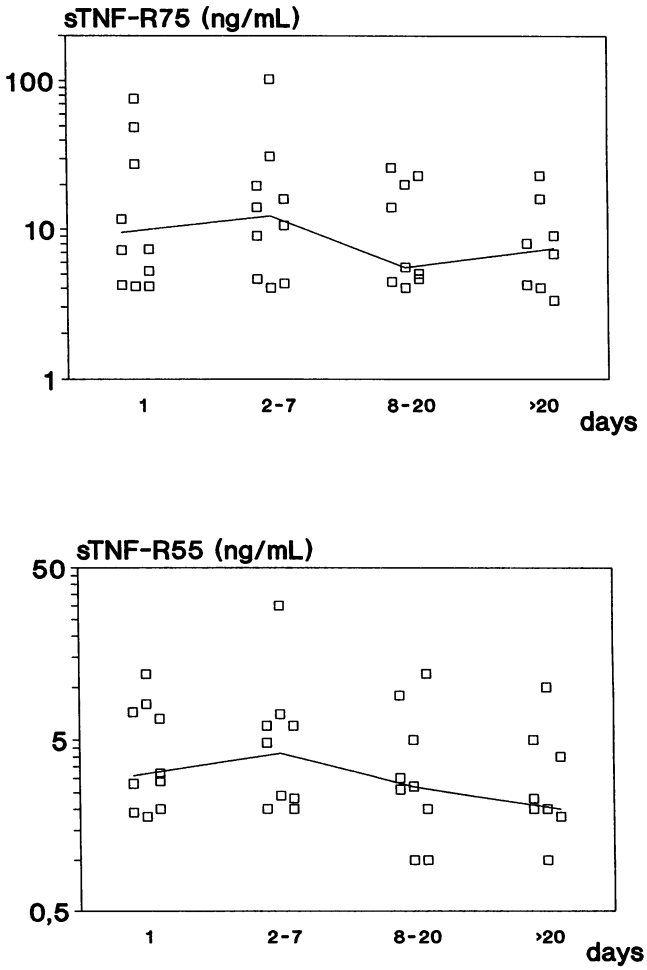

FIG. 1. Concentrations of sTNF-R55 and sTNF-R75 in serially obtained serum samples from patients with subacute streptococcal or enterococcal endocarditis. Only the highest values during the indicated periods of time are shown. The line represents the course of median values over time. The concentrations of functionally active sTNF-R fragments were measured by an enzyme-linked immunobinding assay.

TABLE 2. TNF and sTNF-R concentrations in serum and the ratio between both on admission: comparison between patients with subacute endocarditis and those with acute malaria

Disease	Amt (range) of:			Ratio[a] (range) of:	
	TNF (pg/ml)	sTNF-R55 (ng/ml)	sTNF-R75 (ng/ml)	sTNF-R55 to TNF	sTNF-R75 to TNF
Subacute endocarditis	<10 (<10–137)	3.1 (1.8–11.9)	9.5 (4.1–75.5)	319 (87–800)	849 (509–2,925)
Acute malaria	107 (44–518)	5.6 (3.4–33.1)	24.5 (11.1–127)	56 (32–92)	247 (134–475)
P value[b]	<0.0001	0.03	0.04	<0.00001	<0.00001

[a] For TNF concentrations below the detection limit (<10 pg/ml), a value of 9 pg/ml was used for calculation of the ratio.
[b] Wilcoxon's exact test.

ranged from 87 to 733 (sTNF-R55; median, 217), and from 455 to 1,989 (sTNF-R75; median, 689) (Table 2). These ratios were significantly lower in the patients with acute malaria (median, 56 [sTNF-R55] and 247 [sTNF-R75]; $P < 0.00001$, Wilcoxon's exact test) (Table 2). The ratios remained significantly different for patients of both groups with uncomplicated disease (endocarditis versus malaria: 311 versus 73 [sTNF-R55, $P < 0.001$] and 800 versus 246 [sTNF-R75, $P < 0.001$]).

High counts of bacterial organisms within the valvular vegetation and prolonged, persistent bacteremia contrasted with slow evolution of symptomatic generalized disease and inflammatory changes are among several unique features of endocarditis due to so-called viridans group streptococci and enterococci. Although the lesion on the heart valves itself is virtually acellular and the bacteremia is often of low grade, sustained systemic inflammatory disease clearly indicates a definite though as yet ill-defined host immune cell involvement. The finding of relatively low TNF values in our patients somehow contrasts with the results of previous in vitro experiments (4, 15) since it would suggest rather limited capacity of the organisms for in vivo activation of host immune cells to release proinflammatory cytokines. Only IL-6 levels were consistently elevated in our patients. Circulating TNF, which, we hypothesized, would have explained much of the clinical disease of endocarditis and would be consistent with the ability of cell surface components of viridans streptococci and enterococci to effectively activate monocytes/macrophages, was elevated only in patients who developed complications such as acute severe heart failure or severe tissue damage subsequent to cerebral hemorrhage or infarction. TNF levels remained low during treatment with cell wall-active antibiotics, which presumably may have increased the concentration of local and circulating lipoteichoic acid (9).

The reasons for these apparent discrepancies are unknown. Methodological problems appear unlikely to account for the relatively low levels of circulating IL-6 and TNF in our patients since samples were processed in an identical manner and clotting times, freezing and thawing, storage time, and temperature did not significantly influence the results of these cytokine assays in multiple control experiments with pooled control samples with high and low values (data not shown). Also, the ability of the assays to detect greatly elevated levels of TNF and IL-6 such as those found in sepsis and septic shock was documented in sera obtained from several such patients (data not shown).

It is possible that long-lasting stimulation of host immune cells during persistent bacteremia leads to progressive unresponsiveness of cells to bacterial antigens in terms of TNF release. The amount of bacterial antigen may be too small, or antigens are inefficiently presented to host cells owing to immune complex formation. Alternatively or concomitantly, cells may become sensitive to shed soluble cytokine receptors such as sTNF-R55 and sTNF-R75 (22). Both receptors have been found to be rapidly released and detectable in the circulation after experimental endotoxinemia (24, 25), experimental TNF infusion (20), and acute infection with meningococcemia (11) or *P. falciparum* parasitemia (16). These receptors may functionally inactivate circulating TNF (1, 16, 24, 25) and, given as therapy, may prevent endotoxin-induced death (2). To entirely block TNF activity, a large molar excess of TNF receptors is required (24, 25); in acute endotoxinemia, malaria, or meningococcemia, such an excess may not be adequately met to abolish toxic consequences of TNF (11, 16, 24, 25). Thus, the ratio between TNF receptors and TNF and the evolution of this ratio might be a useful indicator of disease severity and, as the results of this study suggest, perhaps also of disease acuteness or chronicity.

In the present study, the ratio of TNF receptors to immunoreactive TNF was much higher in the patients with subacute endocarditis than in patients with acute malaria. Subacute disease following intravascular infection might, therefore, be characterized as differing from acute disease not only in circulating TNF but also in a relative excess of sTNF-R, and the causative organisms, although potent stimulators of cytokine release in vitro, may be more potent stimulators of TNF receptor shedding in vivo, thus protecting the host from acute potentially lethal toxicity. The relatively low IL-6 levels compared with those measured in human sepsis may be only secondary to this counterregulation (25). Consistent with this view would also be recent results from this laboratory of TNF receptor studies in semi-immune patients with benign *Plasmodium vivax* malaria who showed large excesses of TNF receptor levels relative to circulating TNF (17) and with Mediterranean spotted fever rickettsiosis, in which the ratio of sTNF-R to TNF correlates well with clinical disease severity (unpublished observations).

Although the possibility cannot be excluded, it appears unlikely that the strains causing endocarditis in this series differ significantly from previously studied organisms in their in vitro ability to activate immune cells. Also, it is unknown what cells are involved in the in vivo cytokine response in human endocarditis. Different cell lines have different ratios of the two TNF receptors (14). Although the vegetation itself usually does not contain immune cells, the involvement of cardiac valve endothelial and stromal cells in cytokine response, modulation, and perhaps receptor shedding might be relevant in vivo (7) and should be studied further.

Acute infections with clinical sepsis due to viridans streptococci or enterococci are extremely rare. We were therefore unable to include such patients as a perhaps more suitable control group. It is noteworthy that an important exception appears to be viridans streptococcal bacteremia in

certain patients with profound granulocytopenia and monocytopenia, which may, for presently unexplained reasons, lead to full-blown sepsis with respiratory distress syndrome and shock (8, 18). It is tempting to speculate that in such patients the serum cytokine profile might be much different from that seen in the endocarditis patient, and we are currently evaluating the concentrations of and ratios between TNF receptors and TNF in such patients.

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