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Release of Soluble Tumor Necrosis Factor Receptors in Mediterranean Spotted Fever Rickettsiosis

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Tumor necrosis factor alpha (TNF) is a key cytokine in the defense against many intracellular pathogens, including *Rickettsia conorii*, the causative agent of Mediterranean spotted fever (MSF). The levels of two soluble TNF receptors (sTNFR-p55 and sTNFR-p75), the extracellular domains of the two cell surface receptors for TNF, were elevated in the acute-stage plasma samples from 20 patients with serologically confirmed MSF. The median values were 3.1 and 7.8 ng/ml for sTNFR-p55 and sTNFR-p75, respectively. sTNFR values correlated significantly with plasma TNF concentrations. Patients with severe MSF had higher values for both receptor fragments than patients with nonsevere disease. The differences were statistically significant for sTNFR-p55 (median, 5.8 versus 2.0 ng/ml; $P = 0.008$). Given the proportionately higher values for both TNF and sTNFR-p55 in patients with severe MSF, the sTNFR-p55/TNF ratios for the two patient subgroups did not differ ($P = 0.5$), while the sTNFR-p75/TNF ratios were significantly different ($P = 0.01$), with disproportionately lower values in patients with severe disease.

Tumor necrosis factor alpha (TNF) is a key cytokine in the defense against many intracellular pathogens, including *Rickettsia conorii*. TNF is released from *R. conorii*-infected mononuclear cells (11) and exerts a direct antirickettsial activity in vitro (12). In an animal model of spotted fever rickettsiosis, elevated levels of immunoreactive TNF can be measured in the plasma during the acute stage of infection (4). Depletion of TNF in mice with *R. conorii* infection by intravenous administration of antibodies to TNF has been shown to result in fatal infection, with high titers of rickettsiae in endothelial cells, macrophages, and hepatocytes (3). A role for TNF in the pathogenesis of human rickettsiosis is supported by the finding of elevated levels of immunoreactive TNF in plasma during the acute phase of Mediterranean spotted fever (MSF) and the correlation of this phenomenon with disease severity (13).

Besides its useful antirickettsial activity, TNF may also exert cytotoxic effects on host cells, with potentially deleterious consequences. Cell surface receptors for TNF and their soluble forms, which are released into the circulation, are important in mediating and regulating these divergent TNF effects (16). Two TNF receptors with molecular masses of 55 and 75 kDa have been cloned. The 55-kDa receptor mediates cytotoxic effects, upregulates adhesion molecules, and appears to be important for resistance to infection by the intracellular organisms *Listeria monocytogenes* and *Chlamydia* spp. (2, 10, 14, 18). The functions of the 75-kDa receptor are still being debated. It may contribute to cytotoxic TNF effects via the 55-kDa receptor (17). In their cleaved, soluble forms (sTNFR-p55 and sTNFR-p75), which are shed into the circulation, both receptors may bind and inactivate TNF. However, inactivation requires a large molar excess of sTNFRs, particularly of sTNFR-p75, over TNF (15, 21). In the present study, we measured the levels of both sTNFR fragments (sTNFR-p55 and sTNFR-p75) in plasma samples obtained from patients with MSF in the acute phase and during convalescence.

Twenty consecutive patients with serologically confirmed MSF were included in this study. Details on demographic, clinical, and laboratory data for these patients have been reported earlier (13). Five patients were considered to have severe MSF, with the development of shock, renal or liver failure, confusion and/or respiratory insufficiency. An arbitrary clinical severity scoring system, with scores ranging from 0 to 12, was constructed on the basis of laboratory data and major symptoms on admission. The score was calculated as the sum of different point values assigned to specific criteria: central nervous system involvement (1 point); severe myalgia (1 point); an elevated creatine kinase and/or aldolase level (1 point); radiographically documented pulmonary involvement (1 point); an elevated serum creatinine level of 1.5 to 3.5 mg/100 ml (1 point) or of >3.5 mg/100 ml (2 points); an elevated serum glutamic pyruvic transaminase level of 41 to 100 U/liter (1 point) or of >100 U/liter (2 points); thrombocytopenia with platelet counts of 100,000 to 149,000/μl (1 point), of 50,000 to 99,000/μl (2 points), or of <50,000/μl (3 points); and an elevated level of fibrinogen degradation products (1 point). The median score for the patients on admission was 3, with the scores ranging from 0 to 9 (mean ± standard deviation [SD], 3.0 ± 2.1). The five patients with severe MSF had a median score of 5 (mean ± SD, 5.0 ± 2.4), and the patients with nonsevere disease had a median score of 2 (mean ± SD, 2.3 ± 1.3). The score was independent of age, sex, and duration or height of fever.

Blood was collected in sterile EDTA-containing polystyrene tubes on admission and 4 to 6 weeks later during convalescence. The blood samples were immediately centrifuged and stored at −80°C for further analysis. TNF was measured in duplicate by a commercially available sandwich enzyme-linked immunosorbent assay (Chromogenix, Mölndal, Sweden) with a detection limit of 5 pg/ml. C-reactive protein (CRP) was measured with a nephelometer. sTNFR concentrations were determined in duplicate by an enzyme-linked immunobinding assay, kindly donated by H. Gallati, Basel, Switzerland, as

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TABLE 1. Levels of TNF, and sTNFR-p55, and sTNFR-p75 in plasma samples from 20 patients with MSF rickettsiosis

Substance	Median levels in plasma[a]			
	Acute stage			Convalescent stage
	Severe	Nonsevere	All cases	
TNF	121.7[b] (31.1–206.2)	31.0 (<5–64.0)	32.3[c]	8.4[d] (<5–41.5)
sTNFR-p55	5.8[b] (3.1–17.5)	2.0 (0.6–7.1)	3.1[c]	0.6[d] (0.3–4.4)
sTNFR-p75	11.0 (8.0–20.7)	6.9 (2.4–16.8)	7.8[c]	2.2[d] (1.1–10.4)

[a] TNF values are in picograms per milliliter, and sTNFR values are in nanograms per milliliter. The values in parentheses are ranges.
[b] Significantly higher than the value for patients with nonsevere disease; $P < 0.01$.
[c] Significantly higher than the value after convalescence; $P < 0.01$.
[d] Not significantly different from the value for 20 healthy controls.

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described previously (6). Briefly, this assay uses mouse monoclonal antibodies against the functionally active receptor fragments sTNFR-p55 (clone *htr*-20) and sTNFR-p75 (clone *utr*-4); after the diluted samples are incubated with the antibodies, the plasma is removed, and free TNF binding sites are detected after incubating the samples with ^{125}I-TNF, washing them, and measuring the residual radioactivity. The detection limit of each of these two sTNFR assays is 0.1 ng/ml. When pooled serum and plasma samples with normal and increased values were used, the interassay variation was 15% as determined after repeated tests in this laboratory. In 20 healthy controls, the mean (±SD) plasma sTNFR-p55 concentration was 1.3 ± 0.3 ng/ml, the mean (±SD) sTNFR-p75 concentration was 2.8 ± 0.5 ng/ml, and the mean TNF concentration was <15 pg/ml.

Spearman rank correlation coefficients were used to describe the association of CRP, TNF, the sTNFRs, and the sTNFR/TNF ratios with clinical severity. For TNF concentrations below the detection limit (<5 pg/ml), a value of 4 was entered into the calculation of the sTNFR/TNF ratios. For the comparison of numerical data, Wilcoxon's exact rank sum test was used.

The levels of TNF and both fragments of sTNFR were elevated in the plasma samples from patients with acute disease (Table 1), as was the concentration of CRP. The median values were 3.1 and 7.8 ng/ml for sTNFR-p55 and sTNFR-p75, respectively. The sTNFR values correlated significantly with those of both TNF (Fig. 1) (sTNFR-p55, $r = 0.91$ and $P = 0.0001$; sTNFR-p75, $r = 0.82$ and $P = 0.001$) and CRP (sTNFR-p55, $r = 0.7$ and $P = 0.0007$; sTNFR-p75, $r = 0.61$ and $P = 0.004$). In most patients, the levels of the sTNFRs in plasma during convalescence had returned to levels comparable to those of healthy controls, with median values of 0.6 ng/ml for sTNFR-p55 and 2.2 ng/ml for sTNFR-p75 (Table 1).

There was no significant difference between the sTNFR values in the acute phase for patients with or without purpuric rash, severe myalgia, or high fever (>39°C). Also, plasma sTNFR values did not correlate with the duration of illness prior to admission; with levels of sodium, aldolase, creatine kinase, or glutamic pyruvic transaminase in serum; or with leukocyte counts.

Patients with severe MSF had higher levels of both receptor fragments than patients with nonsevere disease (Table 1). The differences were significant for sTNFR-p55 (median, 5.8 versus 2.0 ng/ml; $P = 0.008$) but not for sTNFR-p75 (median, 11.0 versus 6.9 ng/ml; $P = 0.06$). Given the proportionately higher values for both TNF and sTNFR-p55 in patients with severe MSF, the sTNFR-p55/TNF ratios did not differ significantly for the two patient subgroups ($P = 0.5$), while the sTNFR-p75/TNF ratios became significantly different ($P = 0.01$), with disproportionately lower values for sTNFR-p75 relative to TNF in patients with severe disease. Similar results were obtained when we used the clinical severity score: TNF ($P = 0.02$) and, inversely, the sTNFR-p75/TNF ratio ($P = 0.04$) correlated significantly with the score, but the sTNFR-p55/TNF ratio ($P = 0.14$) and CRP levels ($P = 0.07$) did not.

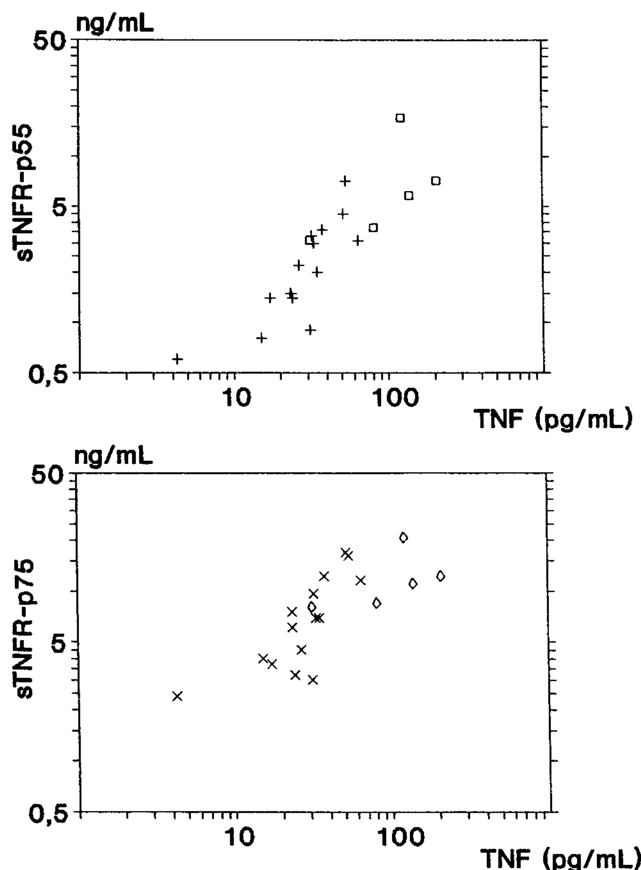

FIG. 1. Correlation of concentrations of sTNFR-p55 and sTNFR-p75 in plasma with plasma TNF levels in 20 patients with MSF. Shown are the values measured in blood samples obtained on admission. Open squares and diamonds indicate values obtained for patients with severe disease, while plus signs and exes indicate values from patients with nonsevere disease. The sTNFR-p55/TNF ratios did not differ for patients with or without severe disease, while the ratio of sTNFR-p75 to TNF was significantly lower in patients with severe disease. Both sTNFR-p55 values ($r = 0.91$, $P = 0.0001$) and sTNFR-p75 values ($r = 0.82$, $P = 0.001$) correlated significantly with those of TNF.

These results provide additional evidence of a role for TNF in the pathogenesis of human infection due to *R. conorii*. As in a variety of other infections and endotoxinemia (5–7, 9, 15, 19–21), sTNFRs were released early during rickettsial infection, and the amount of receptors shed correlated highly with TNF levels. In molar terms, the concentrations of the sTNFRs exceeded those of TNF by ≈41-fold (sTNFR-p55) and ≈92-fold (sTNFR-p75).

The prognostic significance of sTNFR levels in MSF, as in other infections, however, remains unclear. Nonacute infections such as bacterial endocarditis and progressive human immunodeficiency virus infection have been associated with increased sTNFR and low or undetectable TNF levels, resulting in high sTNFR/TNF ratios (1, 8). As one might expect, sTNFR/TNF ratios in acute infections such as *Plasmodium falciparum* malaria or meningococcal sepsis are comparatively lower (5, 6, 8, 20), and in one previous study, low ratios of sTNFRs to circulating TNF in patients with acute meningococcemia appeared to have prognostic value (5). Significantly lower levels of sTNFR relative to TNF were observed in nonsurvivors. In the same study, however, TNF and sTNFR values changed rapidly; 6 h after admission, sTNFR/TNF ratios were no longer different for survivors and nonsurvivors (5). In complicated malaria, sTNFRs/TNF ratios were higher, not lower than in uncomplicated disease (6).

In the present study, sTNFR/TNF ratios in MSF did not prove to discriminate better between severe and nonsevere disease than did acute-stage TNF levels alone. In contrast to the sTNFR-p55/TNF ratio, there was an indication that lower sTNFR-p75/TNF ratios correlated with more severe disease. This might be related to the higher TNF levels in severe disease as well as the less rapid clearance of sTNFR-p75 from the bloodstream in comparison with sTNFR-p55 (6, 15, 19–21). A role for relatively larger increases of sTNFR-p75 (relative to TNF) in preventing the development of severe disease via reduced toxic TNF effects cannot be excluded. Such an effect appears unlikely, however, in view of previous observations that a >300-fold molar excess of sTNFR-p75 over TNF is required to reduce TNF toxicity by >50% (15, 21). The disproportionately lower increase of sTNFR-p75 in our patients with severe MSF than in those with nonsevere MSF appears minor (in molar terms, ≈43-fold versus ≈109-fold), and for none of our patients were excesses of >300-fold documented.

In summary, these observations show that sTNFRs are released into the circulation during rickettsial infection in humans and indicate an important role for TNF-TNF receptor system interplay in the pathogenesis of this infection. The plasma sTNFR concentration, like the plasma TNF concentration, offers information relevant to gauging disease severity. Given the ready availability of other indicators of disease severity, such as CRP levels, platelet counts, creatinine levels, and other variables besides the clinical status, it is unclear, however, whether the measurement of sTNFR levels is important and of any major advantage in clinical practice.

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