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Pattern of Soluble TNF Receptors I and II in Sepsis

Summary: The serum levels of soluble TNF receptors I (sTNFR I) and sTNFR II were measured frequently in 14 patients with sepsis to evaluate the pattern of these TNF antagonists in relation to TNF alpha. Soluble TNFR I and II could be detected in all samples with significantly higher levels (p<0.001) compared to healthy controls. The concentration of sTNFR I as well as sTNFR II was significantly higher in nonsurvivors compared to survivors during the first 36 h of sepsis (p<0.001). Levels remained elevated throughout the evaluation with maximal values in patients who died. A positive correlation exists between both receptors and between soluble receptors and simultaneously obtained sepsis score (p<0.01) while TNF immunoreactivity detected in 80% of all samples did not correlate to soluble receptor levels or sepsis score. Soluble receptors were constantly found in the circulation representing the inflammatory state throughout the evaluation even when TNF activity was undetectable.

Introduction

Tumor necrosis factor alpha (TNF) is a cytokine that mediates a wide range of immunological, inflammatory and cytotoxic effects [1]. In sepsis TNF is thought to be a major factor contributing to multiple organ failure related to a high mortality rate in humans [2]. The strong association of TNF activity and clinical effects of shock or sepsis in animal models could inconsistently be reproduced in critically ill ICU patients [3]. TNF detectable in the circulation may represent excessive local activity and therefore does not reflect the bioactivity of this cytokine [4]. TNF exerts its pleiotropic effects by linking two high affinity TNF receptors (TNFRs) of 55 and 75 kDa on a variety of cells [5-8]. Soluble fragments of the human 55 kDa TNF receptor (soluble TNFR I) and the 75 kDa receptor (sTNFR II) are identical to the extracellular domain of both receptors [9-11]. Soluble receptors apparently are shed from the cell surface into the circulation in patients with sepsis [12,13], meningococemia [14] and in response to endotoxemia [15]. Released receptors inhibit TNF activity by binding to TNF and preventing binding of the ligand to TNF cell receptors [16]. This regulatory process may modulate TNF activity in response to inflammation [15]. The mechanisms of soluble TNF receptor production as well as the kinetics of release are not precisely known. To evaluate the pattern of soluble TNF receptors I and II in surgical patients with sepsis we frequently measured the ligand inhibitors related to the ligand TNF.

Patients and Methods

Patients: Fourteen patients in an operative ICU were studied prospectively with the diagnosis of sepsis. Inclusion criteria were used according to the definition of Bone [17]. Patients received standard intensive care treatment including adequate fluid replacement, vasoactive agents, cardiovascular monitoring with Swan-Ganz catheter, mechanical ventilation, antibiotics and surgery if necessary. The severity of sepsis was classified according to the sepsis score of Elebute and Stoner [18].

Blood analysis: All patients or their relatives gave informed consent to draw blood for analysis. Serial blood samples were obtained within 12 h after diagnosis of sepsis, every 6 h for the first 48 h and every 12 h thereafter until patients died or recovered. Samples of blood were taken for measurement of serum concentrations of TNF and soluble TNF receptors I and II. The samples were centrifuged immediately and stored at -70°C. Tumor necrosis factor alpha: TNF immunoreactivity [19] was measured in a sandwich ELISA (T Cell Science, Cambridge, Massachusetts, USA). The assay measuring total (free and receptor bound) TNF with a detection limit of 10 pg per ml was carried out according to the manufacturer’s instructions and optical densities at 490 nm were read on a 96-well microtiter plate ELISA-reader (MR 700, Dynatech Laboratories).

Soluble TNF receptors: Soluble TNFR I and sTNFR II were assayed by an enzyme-linked immunological binding assay [14]. Briefly, 96-well microtiter plates were coated with monoclonal antibodies to sTNFR I (clone htr 20) or to sTNFR II (clone utr 4), then saturated with BSA (Sigma). Microtiter plates were washed and 100 μl of standard (human recombinant sTNFR I and sTNFR II, provided by Hoffmann-La Roche Ltd.) or diluted samples were dispensed onto the plates. Peroxidase-conjugated human recombinant TNF (Hoffmann-La Roche) was added to the wells, and plates were incubated overnight at room temperature. After washing, tetramethyl-benzidine/H₂O₂ was added and incubated for 15–30 min.

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The reaction was stopped with H₂SO₄ and read at 450 nm. The results for TNF-ELISA, therefore do not bind to the antibodies, whereas free soluble receptors do.

Monoclonal antibodies to soluble receptors have no TNF neutraizing capacity. Complexes of soluble receptors with TNF, measured in the TNF-ELISA, therefore do not bind to the antibodies, whereas free soluble receptors do.

Normal values of both soluble receptors were measured in eight healthy volunteers.

**Statistical analysis:** Significance of differences between survivors and nonsurvivors was assessed by the Mann Whitney U test. Numeric data of TNF and sTNFR I and II are presented by median and interquartile (lower quartile - upper quartile). Correlation of soluble TNF-receptors, TNF and sepsis score were evaluated by linear regression analysis. We considered p values < 0.05 to be significant.

**Results**

The study population consisted of seven women and seven men with a mean age of 51 (35–74) years. The underlying conditions of sepsis were peritonitis (n=8), necrotizing pancreatitis (n=2), hemorrhagic shock (n=3) and pneumonia (n=1).

The isolated microorganisms included gram-negative bacilli in six patients, gram-negative and gram-positive mixed populations in two patients, gram-positive bacteria and fungal infection in one patient each and no evidence of infection in four patients (Table 1). Three patients died within 72 h while the other three died within 10 days, all due to multiple organ dysfunction resulting in an overall mortality of 43%.

The severity of sepsis was assessed simultaneously with serum levels of soluble TNFR I, II and TNF. Median of the sepsis score according to *Elebute* and *Stoner* [19] was 22 (16–29) in nonsurvivors and 16 (11–20) in survivors. A significant correlation was found between the sepsis score and sTNFR I (r=0.24; p<0.01) and sTNFR II (r=0.21; p<0.01; n=173).

There was no significant difference between patients who survived and those who died regarding age, sex, shock and serum creatinine at diagnosis (Table 1). A significant correlation between concentrations of soluble TNF receptors and renal function (serum creatinine) could be demonstrated throughout the evaluation period (r=0.23 for sTNFR I, p<0.01 and r=0.17 for sTNFR II, p<0.05; n=173). Serum for determination of TNF and soluble receptors was frequently assayed, resulting in 2–25 samples for each patient (Total: 173 samples). Soluble TNFR I and sTNFR II could be detected in all samples (100%) with a significantly higher level (p<0.001) compared to healthy controls (n=8; normal value: sTNFR I: 1.5 ng/ml; sTNFR II: 2.3 ng/ml).

Levels of soluble receptors remained elevated during the course of 36 h (Figure 1 and 2). The median serum concentration of soluble TNFR I was significantly higher in nonsurvivors compared to survivors (13.4 [10.8–18.2] vs. 9.2 [6.5–13.2]; n=40 vs. n=38; p<0.001) as well as the median concentration of sTNFR II (12.9 [11.4–24.0] vs. 9.6 [7.2–12.5]; p<0.001).

Soluble TNF receptor I correlated significantly with sTNF receptor II (r=0.57, p<0.001; n=173; Figure 3).

During the course concentrations of soluble receptors in survivors remained persistently elevated and decreased when patients recovered (demonstrated for sTNFR I in Figure 4). Maximal values of sTNFR I (59 ng/ml) and II (62 ng/ml) were detected only in patients who died. In all nonsurvivors an additional increase of elevated soluble receptors preceded fatal outcome (demonstrated for sTNFR I in Figure 5). The same pattern was found for sTNFR II in survivors and nonsurvivors (not shown).

TNF immunoreactivity was detected with a wide range in ten out of 14 patients and in 80% of the 173 samples. The levels of TNF were not significantly different in patients who died during the first 36 h compared to those patients who survived (Table 2). A positive correlation of both receptors with TNF could not be found (r=0.001 for sTNFR I; r=0.002 for sTNFR II; n=173) and no significant correlation existed between TNF levels and the simultaneously obtained sepsis score (r=0.05; n=173).

**Discussion**

Sepsis characterized by multiple organ dysfunction and a high mortality rate remains a serious problem in intensive care units. Although the etiology of sepsis is multifactorial the systemic inflammatory response is characterized by release of different cytokines with tumor necrosis factor alpha (TNF) considered the central mediator [20–22]. Trauma, shock, endotoxin as well as exotoxins of gram-positive bacilli and fungal cell wall compounds are known to induce the release of TNF as well as IL 1 and IL 6 predominantly by macrophages [2]. Most probably, endotoxin is the stimulator in the majority of patients with gram-negative infections in this study. Nevertheless, elevated levels of TNF are detectable in gram-positive and fungal infections as well. The clinical significance of TNF detection in critically ill patients varies in different series, which may be due to the short half-life of TNF with 15 to 17 min,