Anti-inflammatory response after infusion of p55 soluble tumor necrosis factor receptor fusion protein for severe sepsis

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ABSTRACT. Objectives: To investigate the effects of Lenercept®, a recombinant soluble TNF receptor p55 fused to an immunoglobulin heavy chain IgG_1, on the balance of pro- and anti-inflammatory mediators in sepsis.
Design: *Post hoc* analysis of a subgroup of patients enrolled in a multicenter phase III, prospective, double-blind, placebo-controlled, randomized study of Lenercept® in severe sepsis.
Setting: Surgical and medical intensive care units, and postoperative recovery room of a tertiary care teaching hospital.
Patients: A total of 57 patients were enrolled in the multicenter study in our center.
Intervention: Septic patients were randomly assigned to receive either Lenercept® 0.125 mg/kg or placebo. The patients were followed for up to 28 days after randomization.
Measurements and main results: Circulating levels of TNF-α, IL-6, $TNFsR_{75}$ and IL-1Ra were measured before and after treatment. The two groups were comparable with regard to age, gender and diagnosis distribution. The total level of TNF-α increased significantly in treated patients, compared to patients receiving placebo. The levels of the other inflammatory mediators did not differ between the two groups.
Conclusions: Lenercept®-treated patients experienced a protracted TNF-α half-life, leading to higher total TNF-α levels throughout the study. However, the treatment had no effects on anti-inflammatory mediators. Therefore, peripheral inflammatory processes might not have been significantly modified by the treatment. This might account for the lack of efficacy this treatment in septic patients.

Keywords: tumor necrosis factor, TNF-antagonist, cytokines, TNF receptor, sepsis

INTRODUCTION

Severe sepsis is associated with a significant degree of morbidity and mortality in intensive care units (ICU) [1, 2]. It can lead to organ failure along with hemodynamic instability, accounting for the potentially fatal outcome of a septic episode [3].

Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-6 seem to play a key role in the pathogenesis of sepsis. IL-6 is considered to be a marker of the severity, and its circulating levels correlate with the outcome of sepsis in human [4]. The production of pro-inflammatory mediators induces an anti-inflammatory response through increased production of TNF soluble receptors ($TNFsR_{55}$ and $TNFsR_{75}$) and IL-1 receptor antagonist (IL-1Ra) [5-9]. These inhibitors, and particularly IL-1Ra, which is an acute phase protein, are good indicators of the inflammatory processes [10]. Thus, the assessment of both pro- and anti-inflammatory mediators may give a better insight into the entire inflammatory state [5].

$TNFsR_{55}$ fused with the heavy chain of immunoglobulin IgG_1 (Lenercept®, Ro 45-2081) has been shown to protect and treat animals challenged with live bacteria [11]. This protection seemed to be conferred by the inhibition of the earliest pro-inflammatory mediator, i.e. TNF-α. A recent multicenter study investigated the effect of Lenercept®, administered to patients with severe sepsis [12]. In that report, Lenercept® induced an increase in serum levels of TNF-α without modification of IL-6 levels, suggesting that TNF-α was biologically inactive. This study was launched to confirm the lack of activity of TNF-α by measuring the effect of Lenercept® on various inflammatory cytokines and their specific antagonists.

METHODS

Subjects and study design

This study was conducted in a subgroup of patients enrolled at the Geneva University Hospital in a phase III multicenter clinical trial using Lenercept®. The ethical committees of the departments involved approved the study, and informed consent was obtained from the patients or next-of-kin.

The methodology has already been described [12]. Briefly, patients with severe sepsis were randomized into two groups to receive Lenercept® at a dose of 0.125 mg/kg, or placebo. All patients received standard care as required by their clinical condition during the 28 days of the study period. Before treatment (BT), immediately after treatment (day 1), the day after (day 2), as well as on days 3 and 7, the simplified acute physiology scores II (SAPS II) [13] were assessed. At the same time points, the serum levels of TNF-α, IL-6, IL-1Ra and $TNFsR_{75}$ were measured. TNF-α and IL-6 were determined by enzyme-linked immunosorbent assays (ELISA, Medgenix, Fleurus, Belgium and Chromogenix, Endotell, Allschwill, Switzerland, respectively). IL-1Ra and $TNFsR_{75}$ were measured by ELISAs (Quantikine R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions. Age, gender and body mass index (BMI = body weight/height^2 [kg/m^2]) were determined. The duration of mechanical ventilation, length of stay in ICU (LOS), as well as ICU and hospital mortality were recorded.

Statistical analysis

In some cases, insufficient serum was available to perform all the necessary dilutions for cytokine measurement. For statistical analysis, the upper limit of the assay was used. Statistical analysis was performed with Instat 3.01 (GraphPad Software, San Diego, CA, USA) and SPSS 6.0 (Niles Software/ISI, Berkeley, CA, USA). Based on the results of a normality test (Kolmogorov-Smirnov), we used the Mann-Whitney *U*-test to compare the two groups unless normally distributed, or Welch's corrected *t*-test if the distribution was Gaussian. In case of normally distributed, matched groups, the Wilcoxon matched pairs test was used. Fisher's exact test was used to analyze contingency tables. All tests were two-sided. Differences between the two groups were considered significant at $p < 0.05$. Results are expressed as mean ± standard deviation in the case of normal distribution and median (lower extreme-upper extreme) otherwise.

RESULTS

Among the 57 patients enrolled in the Lenercept® study in our center, 28 received placebo and 29 Lenercept®. The patients' characteristics were similar between the two groups (Table 1). The overall mortality at day 28 did not differ. There were no differences between the placebo and the treated group regarding the co-morbidities, the types and sites of infection, incidence and duration of organ dysfunction.

Before treatment, a wide range of TNF-α levels was found, without a significant difference between the two groups; median circulating TNF-α level amounted to 84 pg/ml (5-2830) in placebo patients, compared to 74 pg/ml (2-1270) in treated patients ($p = 0.70$) (Figure 1A). In treated patients, the total TNF-α level started to rise on day 2 and remained high ($p < 0.001$) until day 7, whereas in placebo patients there was an overall decrease ($p < 0.05$). The levels of soluble $TNFsR_{75}$ remained unchanged and were similar in placebo and treated patients before treatment (placebo: 7 ng/ml (1.9-10) *versus* treated 6.4 ng/ml (1.6-10)) and throughout the observation period (Figure 1B). The TNF/$TNFsR_{75}$ ratio increased significantly in the treated patients, matching the rise in total TNF-α levels, contrasting with the stable ratio in placebo patients.

IL-6 serum levels were similar between placebo and treated patients before treatment (Figure 1C) and decreased in both groups.

IL-1Ra measurements showed strikingly elevated levels before treatment but to a similar extent in both groups (placebo (n = 25) 19580 pg/ml (1500 – 60000) *versus* treated (n = 26) 22802 (850-60000), $p > 0.05$). These levels decreased significantly during the subsequent days of the study (Figure 1D).

When stratified according to the outcome of the disease at day 28, patients who died had significantly higher levels of TNFsR75 before treatment compared to those who survived, while the TNF-α/TNFsR75 ratio was similar. The levels of the other cytokines did not differ statistically, although IL-6 levels tended to be higher in the patients who subsequently died (Table 2).

DISCUSSION

Our results confirm the results of the multicenter study [12] regarding the lack of activity of the increased levels of the immunologically reactive TNF-α in Lenercept® - treated patients. We extend their observation showing that the drug did not induce any modification of the levels of natural inhibitors of cytokines such as IL-1Ra and $TNFsR_{75}$. In addition, we showed the evolution of TNF-α,

Table 1
Characteristics of the patients

	Placebo n = 28	*Treated* n = 29	p
Age (years)	61 ± 18	61 ± 16	NS
M/F ratio n (%)	15/13 (54/46)	18/11 (62/38)	NS
BMI (kg/m^2)	25 ± 4	26 ± 5	NS
Severe sepsis/septic shock (%)	17/11 (61/39)	21/8 (72/28)	NS
SAPSII at admission	52 ± 14	50 ± 16	NS
Mechanical ventilation (days)	4 ± 6	3 ± 4	NS
Length of stay (days)	10 ± 7	6 ± 7	< 0.01
Mortality n (%)	8 (29)	7 (24)	NS

Results are expressed as mean ± standard deviation

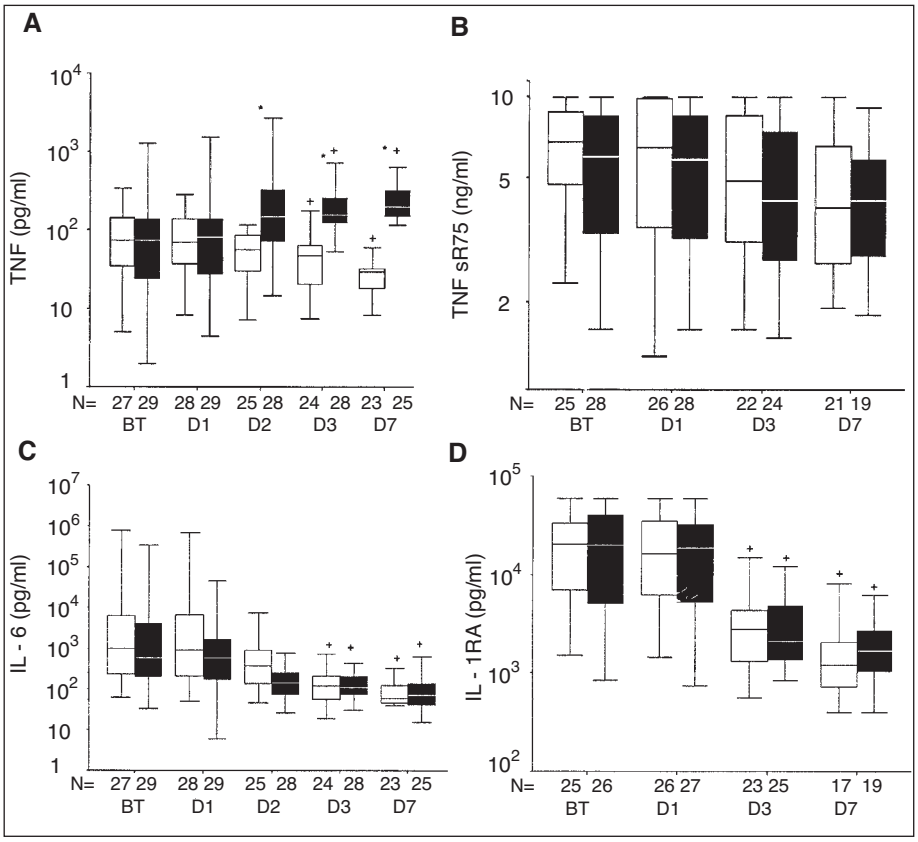

Figure 1
Evolution of cytokine levels in patients treated with Lenercept®, expressed in logarithmic values. Open boxes represent placebo patients and solid boxes treated patients. Box plots indicate the interquartile range, the horizontal line, the median value, and the vertical bars the 10 and 90 percentiles. + $p < 0.05$ before treatment *versus* D1-7 *$p < 0.05$ between the 2 groups. N represents the number of patients in each group. A) Total TNF-α levels in patients. B) $TNFsR_{75}$ C) IL-6 levels D) IL-1Ra. BT: before treatment, D1: day 1, day of treatment, D2-D7: day 2 to 7.

IL-6, IL-1Ra and $TNFsR_{75}$ over the time up to 7 days after treatment. However, the small number of patients who could be enrolled locally is a limitation of our study.

Our initial hypothesis was that inhibiting TNF-α may lead to a decrease in the subsequent pro- and anti-inflammatory cascade, as has been reported for anti-TNF-α therapy elsewhere [14-17].

Table 2
Levels of cytokines before treatment, stratified according to outcome

Mediator	Alive n = 41	Deceased n = 15	p
TNF-α (pg/ml)	89 (2-2 830)	75 (18-1 270)	NS
$TNFsR_{75}$ (ng/ml)	5.4 (1.6-10)	9 (3.8-10) (n = 12)	0.0055
TNF-α/$TNFsR_{75}$	0.01 (0.001-0.44)	0.01 (0.004-0.28) (n = 12)	NS
IL-6 (pg/ml)	638 (33-119 740)	1 480 (115-765 000)	NS
IL-1Ra (ng/ml)	19 246 (850-60 000) (n = 38)	31 689 (1 906-60 000) (n = 13)	NS

Results are expressed as median and range (minimum-maximum)

The levels of TNF-α started to increase on day 2 in patients who received Lenercept® and continued up to day 7, whilst the initial high levels of IL-6 and IL-1Ra decreased regardless of the treatment in both groups of patients. The levels of $TNFsR_{75}$ were only moderately elevated in both groups and did not vary significantly throughout the study. The increase in total TNF-α levels has already been reported in Phase III of the Lenercept® study, and is most probably due to a protracted half-life of the TNF-α molecule bound to the fusion protein [12]. Owing to the immunological method used in our study, only the total amount of circulating TNF-α was determined, without distinguishing between active and inactive forms. The bioactivity of the molecule may depend on the equilibrium between the free TNF-α form and that bound to the soluble and membrane receptors as well as to the fusion protein [18]. A number of plasma proteins, such as α_2-macroglobulin or acute-phase proteins are able to complex the molecule and modulate its bioactivity [19]. While Abraham suggested that circulating TNF-α was bioinactive, the effect of Lenercept® at the tissue level in septic patients is not known. Tissue inflammation may play a major role in inflammatory regulation since systemic inflammation might depend on local production of cytokines [20, 21]. Since we did not assess the buffer capacity or factors influencing its variability, it is very difficult to achieve a definite understanding of the kinetics of TNF-α and its antagonist measured during this study.

Nevertheless, our results suggest that the amount of Lenercept® remaining at Day 7 could still sufficiently buffer TNF-α.

While IL-6 is known to be a very good indicator of the severity of the global inflammatory response [4], IL-1Ra and $TNFsR_{75}$ represent the homeostatic, anti-inflammatory response paralleling the release of pro-inflammatory mediators in sepsis [5, 8, 22]. The plasma levels of $TNFsR_{75}$ do not depend only on the levels of circulating TNF-α [16]. The circulating levels of IL-1Ra reflect both its production by hepatocytes, as part of the acute phase reaction, and mononuclear cell activation [10]. Our findings for IL-1Ra and $TNFsR_{75}$ suggest that Lenercept® did not influence their production at any level.

Another interesting finding was that patients who eventually died had increased levels of $TNFsR_{75}$ before treatment. Previous studies have already shown higher $TNFsR_{75}$ in deceased patients [6], yet the biological significance of this finding is difficult to establish. This study was not powered as to allow any outcome conclusion.

Numerous reasons have been evoked to account for the failure of anti-mediator trials in septic patients [23]. A recent study has even shown Lenercept® to have deleterious effects on patients with multiple sclerosis [24]. Our results demonstrate that the tested drug did not affect the pro- and anti-inflammatory balance in severe sepsis. It may be advisable to gain more insight into the effects of new therapeutics on the kinetics of both pro- and anti-inflammatory mediators before launching large-scale clinical trials. Also, a better understanding of drug action at tissue levels appears to be critical to ensure that the right inhibition occurs at the right place.

This study was approved by Ph. Van den Auwera and E. Abraham (for the "Lenercept® in sepsis Study Steering Committee").

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APPENDIX

Members of the Geneva Sepsis Network involved in the present study were:

D. Pittet (chair), V. Butty, J.-C. Chevrolet, J.-M. Dayer, J. Garbino, S. Harbarth, Ph. Jolliet, D. Lew, J. Pugin, B. Ricou, J.A. Romand, P.M. Suter.

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Erratum 1

There has been a mistake in the last issue of ECN 4 2002, in the article on page 425, titled "Plasma levels of interleukin-12 (IL-12), interleukin-18 (IL-18) and transforming growth factor beta (TGF-beta) in Plasmodium falciparum malaria" by L. Malaguarnera *et al.* The affiliation of authors is the following:

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Moreover in the discussion on page 429, line 11, figure 2 was mistaken because is referred to figure 1.