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Soluble triggering receptor expressed on myeloid cells 1 as an anti-inflammatory mediator in sepsis

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Abstract Objective: To define the significance of soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) in the septic cascade by comparing its kinetics to those of other proinflammatory mediators and of interleukin (IL) 10. **Design:** Prospective study in a tertiary unit. **Patients:** Blood was sampled from 90 patients with septic syndrome due to ventilator-associated pneumonia for 7 days after the appearance of symptoms. Concentrations of tumor necrosis factor (TNF) α , IL-6, IL-8, IL-10, and sTREM-1 were determined by enzyme-linked immunosorbent assay. **Results:** Serum levels of TNF α , IL-6, IL-10, and sTREM-1 were higher in nonsurvivors than in survivors; similar differences were not found for IL-8. Positive correlations were found between the ratios IL-10/TNF α and sTREM-1/TNF α , between IL-10/IL-6

and sTREM-1/IL-6, and between IL-10/IL-8 and sTREM-1/IL-8. Median values of IL-10/TNF α upon presentation of sepsis, severe sepsis, and septic shock were 3.21, 2.16, and 2.86, respectively (NS). Respective values for sTREM-1/TNF α were 21.28, 7.33, and 27.78 ($p = 0.047$ between sepsis and severe sepsis, $p = 0.003$ between severe sepsis and septic shock). **Conclusions:** sTREM-1 follows the kinetics of IL-10 and should therefore be considered an anti-inflammatory mediator in sepsis. Decreased ratios of sTREM-1/TNF α might determine transition from sepsis to severe sepsis and from severe sepsis to septic shock.

Keywords Sepsis · Septic shock · sTREM-1 · Survival

Introduction

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a novel receptor highly expressed on neutrophils and monocytes in the event of sepsis and septic shock [1, 2]. Its soluble counterpart, sTREM-1, has recently been described [3]. The concentration of this novel molecule is substantially higher in the sera of patients with septic shock than in patients with sepsis or severe sepsis [4]. The exact role of sTREM-1 in the inflammatory cascade remains to be elucidated, but it has been proposed to act as an anti-inflammatory mediator [3]. Other authors have reported that transition from sepsis to severe sepsis and septic shock may result from changes in the

balance between proinflammatory and anti-inflammatory cytokines [5]; sTREM-1 may be implicated in this process.

In an attempt to evaluate the role of sTREM-1 in the course of the septic process, its daily kinetics in serum was compared to that of other pro- and anti-inflammatory mediators in a clinical study in which enrolled patients suffered the same underlying infection, i.e., ventilator-associated pneumonia (VAP). The need for uniformity in the underlying cause of sepsis permitted the minimization of any discrepancy occurring when a great variety of antigenic stimuli trigger the innate immune system thus offering clear connections between changes in pro- and anti-inflammatory mediators and the course of the septic cascade.

Patients and methods

Study design

A total of 90 patients were enrolled in a prospective study conducted from June 2004 to January 2005. Patients were hospitalized in the Department of Critical Care of the Evangelismos General Hospital and in the Second Department of Critical Care of the Attikon University Hospital of Athens. The study was approved by the ethics committee of each hospitals. Clinical characteristics of patients enrolled in the study are shown in Table 1. All enrolled patients were intubated for at least 48 h prior enrollment, and they were aged above 18 years. Written informed consent was provided by their relatives. Exclusion criteria were: (a) neutropenia (≤ 500 neutrophils/mm³), (b) HIV infection, and (c) oral intake of corticosteroids at a dose equal to or higher than 1 mg/kg equivalent prednisone for a period greater than 1 month. Inclusion criteria were the concomitant presence of (a) VAP and (b) sepsis ($n = 27$), severe sepsis ($n = 27$), or septic shock ($n = 36$). Diagnosis of VAP was established in any patient presenting with the following signs: (a) core temperature higher than 38 °C or lower than 36 °C, (b) new or persistent consolidation in lung radiography, (c) purulent trancheobronchial secretions (TBS), and (d) clinical pulmonary infection score

(CPIS) equal to or more than 6 [6, 7, 8, 9, 10]. Diagnosis of sepsis was based on the presence of at least two of the following [11]: (a) core temperature higher than 38 °C or lower than 36 °C, (b) PCO₂ below 32 mmHg, (c) pulse rate above 90/min, and (d) white blood cell count greater than 12,000/ μ l or lower than $< 4,000/\mu$ l or less than 10% of bands.

Severe sepsis was determined as the acute dysfunction of at least one organ, i.e., the acute presentation of at least one of the following [11]: (a) acute respiratory distress syndrome (ARDS), as any value of the pO₂/FIO₂ ratio below 200 with the presence of diffuse shadows on lung radiography, (b) acute renal failure, as the production of less than 0.5 ml/kg body weight per hour of urine for at least 2 h provided that the negative fluid balance of the patient was corrected, (c) metabolic acidosis as any pH greater than 7.30 or any base deficit greater than 5 mEq/l and serum lactate at least more than twice the normal value, and (d) acute coagulopathy as any platelet count higher than 100,000/ μ l or INR higher than 1.5. Septic shock was considered as any value of systolic pressure below 90 mmHg requiring the administration of inotropic agents [11]. Upon enrollment in the study quantitative TBS cultures were performed; TBS were collected after insertion of a sterile catheter in the intubation tube or in the tracheotomy connected to a negative pressure device.

Table 1 Clinical characteristics of patients with ventilator-associated pneumonia enrolled in the study (APACHE Acute Physiology and Chronic Health Evaluation, SOFA Sequential Organ Failure Assessment, CPIS clinical pulmonary infection score)

	Sepsis ($n = 27$)	Severe sepsis ($n = 27$)	Septic shock ($n = 36$)
Age (years)	52.6 \pm 20.8	67.7 \pm 13.6	58.0 \pm 18.1
Sex: M/F	20/7	18/9	27/9
APACHE II score	15.63 \pm 6.06	17.88 \pm 4.19	19.40 \pm 7.03
SOFA score	5.78 \pm 2.82	7.21 \pm 2.83	9.54 \pm 4.10
CPIS score	7.54 \pm 1.32	7.70 \pm 1.54	7.47 \pm 1.29
White blood cells (μ l)	12.132 \pm 5.060	13.850 \pm 7.027	14.206 \pm 8.683
Underlying conditions			
Multiple injuries	8 (29.62%)	1 (3.70%)	9 (25.00%)
Brain hemorrhage	6 (22.22%)	7 (25.92%)	3 (8.33%)
Chronic obstructive pulmonary disease	6 (22.22%)	5 (18.51%)	6 (16.66%)
Lower respiratory tract infection	2 (7.41%)	3 (11.11%)	5 (13.88%)
Intraabdominal infection	1 (3.70%)	2 (7.41%)	4 (11.11%)
Celiac aortic aneurysm replacement	–	2 (7.41%)	3 (8.33%)
Others	4 (14.81%)	7 (25.92%)	6 (16.66%)
Predisposing factors			
Diabetes mellitus 2	4 (14.81%)	4 (14.81%)	6 (16.66%)
Coronary heart disease	3 (11.11%)	8 (29.62%)	4 (11.11%)
Hypertension	2 (7.41%)	6 (22.22%)	4 (11.11%)
Others	6 (22.22%)	–	8 (22.22%)
Pathogens			
<i>Acinetobacter baumannii</i>	10 (37.03%)	11 (40.74%)	16 (44.44%)
<i>Pseudomonas aeruginosa</i>	5 (18.51%)	7 (25.92%)	4 (11.11%)
Others	2 (7.41%)	3 (11.11%)	2 (5.55%)
Bacteremia	3 (11.11%)	3 (11.11%)	5 (13.88%)
Resolution of VAP	19 (70.4%)	17 (62.96%)	13 (36.11%)*
Case fatality	6 (22.2%)	9 (33.3%)	18 (50.0%)*
Survival (days)	25.48 \pm 1.19	23.38 \pm 1.58	20.19 \pm 1.67**

* $p < 0.05$ vs. patients with sepsis or severe sepsis

** $p = 0.019$ sepsis vs. septic shock

Enrolled patients were followed-up on a daily basis for a total of 28 days; evaluation comprised lung radiography, calculation of the pO_2/FIO_2 ratio, and administration of the Acute Physiology and Chronic Health Evaluation II and Sequential Organ Failure Assessment instruments. Patients died as a result of multiple-organ dysfunction.

CPIS was determined after individual scoring for each of the following parameters [12], as follows: (a) core temperature $36.5\text{--}38.4\text{ }^\circ\text{C} = 0$ points; $38.5\text{--}38.9\text{ }^\circ\text{C} = 1$ point; $36\text{ }^\circ\text{C}$ or below or $39\text{ }^\circ\text{C}$ or above = 2 points; (b) white blood cells $4,000\text{--}11,000/\mu\text{l} = 0$ points; fewer than 4,000 or more than $11,000/\mu\text{l} = 1$ point; more than $11,000/\mu\text{l}$ and more than 10% bands = 2 points; (c) pO_2/FIO_2 ratio of at least 240 or the presence of ARDS = 0 points; pO_2/FIO_2 ratio less than 240 in the absence of ARDS = 2 points; (d) diffuse shadows on lung radiography = 1 point; localized shadow on lung radiography = 2 points; (e) purulent TBS = 2 points; and (f) TBS cultures yielding a pathogen at or above 10^6 cfu/ml with negative Gram stain = 1 point; TBS cultures yielding a pathogen at or above 10^6 cfu/ml with positive Gram stain = 2 points. Patients were divided as cases of early or late VAP as to whether the period from the advent of VAP since the first blood sampling was less or more than 24 h. For the measurement of cytokines and sTREM-1 in patients' sera, 5 ml blood was sampled after venipuncture of a peripheral vein under sterile conditions. Blood was drawn daily for 7 days; it was collected into sterile tubes. After centrifugation the serum was kept at $-70\text{ }^\circ\text{C}$ until assayed.

Laboratory techniques

Quantitative TBS cultures were performed immediately after collection; 0.5 ml TBS was added to a sterile tube with 2 ml Mueller-Hinton broth and diluted five times at 1:10. Volumes of 0.1 ml of each dilution were plated onto McConkey, blood and Saboureaux agar (Becton Dickinson). Dishes were incubated for 5 days at $37\text{ }^\circ\text{C}$ or $42\text{ }^\circ\text{C}$ for Saboureaux plates, and their count was determined after multiplying with the appropriate dilution factor. Cultures yielding a pathogen at a count of 1×10^6 cfu/ml or higher were considered positive [13, 14]. Flasks with blood were incubated for 7 days. Identification of pathogens was performed by the API20E and the API20NE systems (bioMérieux, Paris, France). Concentrations of tumor necrosis factor (TNF) α , interleukin (IL) 6, IL-8, and IL-10 in sera were estimated in duplicate by an enzyme immunosorbent assay (Diacclone, Paris, France). Lowest limits of detection were 0.5 pg/ml for TNF α , 6.25 pg/ml for IL-6, 62.5 pg/ml for IL-8, and 15.12 pg/ml for IL-10. sTREM-1 was measured by a home-made enzyme immunosorbent assay. Capture antibody of sTREM-1 (R&D, Minneapolis, Minn., USA) was diluted to 4000 ng/ml and distributed to a 96-well plate at a volume of 0.1 ml

per well. After overnight incubation at $25\text{ }^\circ\text{C}$, wells were thoroughly washed with a 0.05% solution of Tween in PBS (Merck) (pH 7.2–7.4). Then 0.1 ml of standard concentrations of sTREM-1 (15.1–4000 pg/ml, R&D) or serum was added in wells. After incubation for 2 h wells were washed three times, and 0.1 ml of one 400 ng/ml dilution of sTREM-1 detection antibody (R&D) was added per well. The plate was then incubated for 2 h, and attached antibodies were marked by streptavidin. Concentrations of sTREM-1 in each well were determined by the optical density detected at 450 nm after addition of one 1:1 solution of H_2O_2 : tetramethylbenzidine as a substrate (R&D). All determinations were performed in duplicate; the interday variation of the assay was 5.23%. The prescribed antimicrobial regimens did not alter within the first 3 days after inclusion in the study of all patients enrolled.

Statistical analysis

Results were expressed as medians \pm 95% confidence intervals (CI). Ratios of IL-10 or sTREM-1 to proinflammatory cytokines were determined. Comparisons were performed by the Mann-Whitney U test after Bonferroni's correction. Statistical correlations were calculated by Spearman's nonparametric coefficient (r_s). Survival time was estimated after Kaplan-Meier analysis; groups with sepsis, severe sepsis, or septic shock were compared by log-rank tests. Differences with a p value below 0.05 were considered statistically significant. Cox regression analysis was performed to define the prognostic role of any of the estimated parameters. Variables included estimated concentrations of IL-10, sTREM-1, and proinflammatory cytokines on day 1 and of ratios of IL-10 or sTREM-1 to proinflammatory cytokines of the same day. Relative risks (RR) and CIs were defined.

Results

Concentrations of TNF α , IL-6, and IL-8 in relation to final outcome are shown in Fig. 1. Serum levels of TNF α were lower in survivors than in nonsurvivors on days 5 and 7, and those of IL-6 on days 1, 3, 4, 5, 6, and 7. No differences in IL-8 were found between survivors and nonsurvivors on any day of follow-up. Positive correlations between sTREM-1 and IL-10 were found on day 1 ($r_s = 0.244$, $p = 0.032$), on day 2 ($r_s = 0.209$, $p = 0.049$), and on day 6 ($r_s = 0.271$, $p = 0.020$) but not on days 3, 4, 5, or 7. Concentrations of sTREM-1 and IL-10 in relation to final outcome are shown in Fig. 2. Serum levels of IL-10 were higher in nonsurvivors than in survivors on days 1, 4, 5, 6, and 7; those of sTREM-1 were higher in nonsurvivors than in survivors on days 1–7.

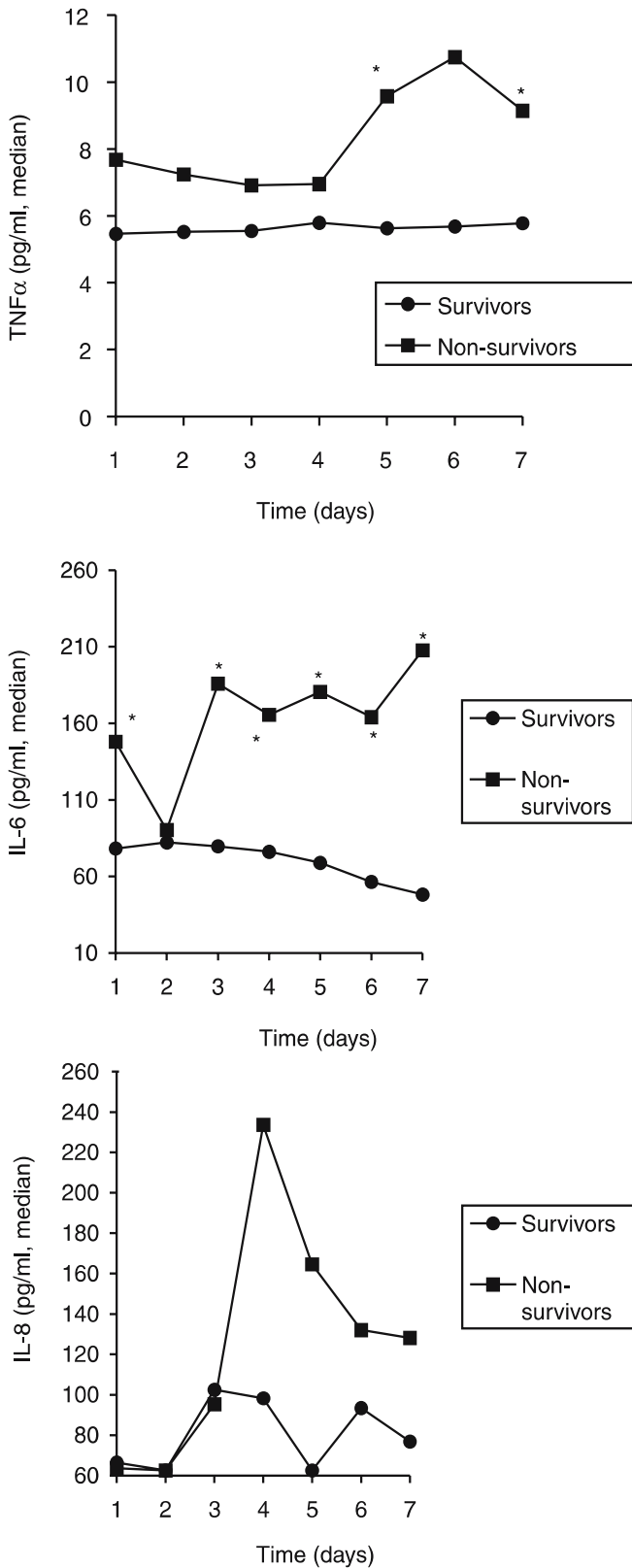


Fig. 1 Concentrations of TNF α , IL-6, IL-6, and IL-8 during follow-up of septic patients in relation to their final outcome. * $p < 0.05$ survivors vs. non-survivors on the same day of follow-up

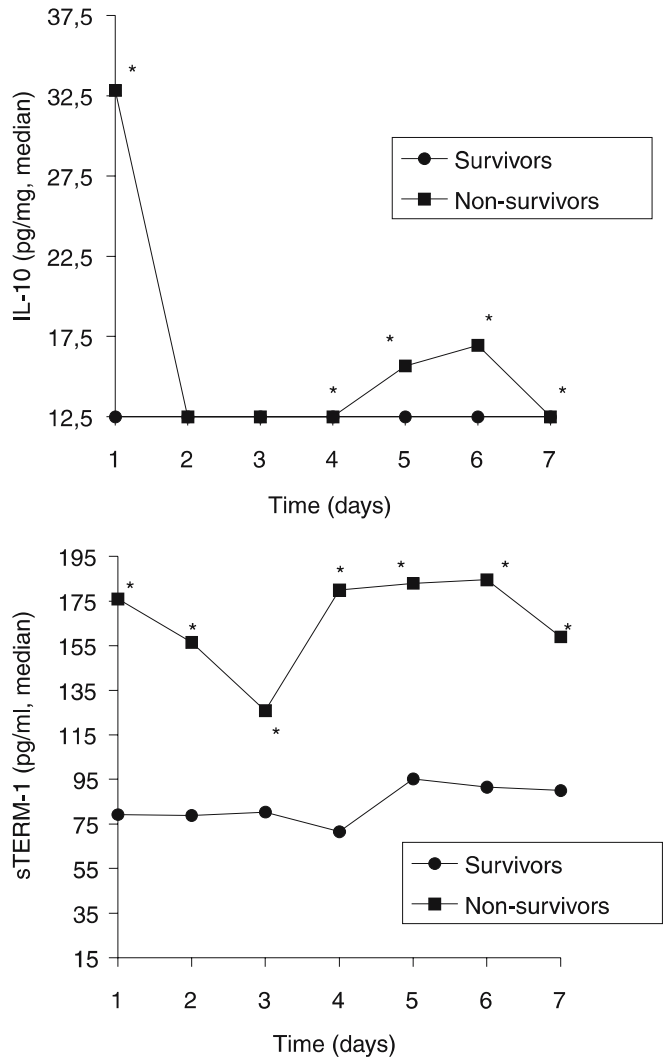


Fig. 2 Concentrations of sTREM-1 and IL-10 during follow-up of septic patients in relation to their final outcome. * $p < 0.05$ survivors vs. nonsurvivors on the same day of follow-up

Median sTREM-1 was 80.55 pg/ml in patients with microbiologically documented VAP and 138.48 pg/ml in those without (NS); 75.94 pg/ml in patients with early VAP and 138.48 pg/ml in those with late VAP ($p = 0.031$); and 76.93 pg/ml in patients with *Pseudomonas aeruginosa* and 97.53 pg/ml in those with *Acinetobacter baumannii* (NS). Median TNF α was 6.69 pg/ml in patients with multiple trauma on day 1 and 5.92 pg/ml in those without (NS). Respective values for IL-6 were 73.35 and 90.18 pg/ml, for IL-8 62.5 and 66.93 pg/ml, for IL-10 12.5 and 12.5 pg/ml, and for sTREM-1 113.68 and 88.43 pg/ml (all NS).

Figure 3 presents the statistical correlations of the ratios of IL-10 and sTREM-1 to pro-inflammatory cytokines on the first day. Positive correlations were found between IL-10/TNF α and sTREM-1/TNF α ($r_s = 0.468$, $p < 0.0001$), between IL-10/IL-6 and sTREM-1/IL-6

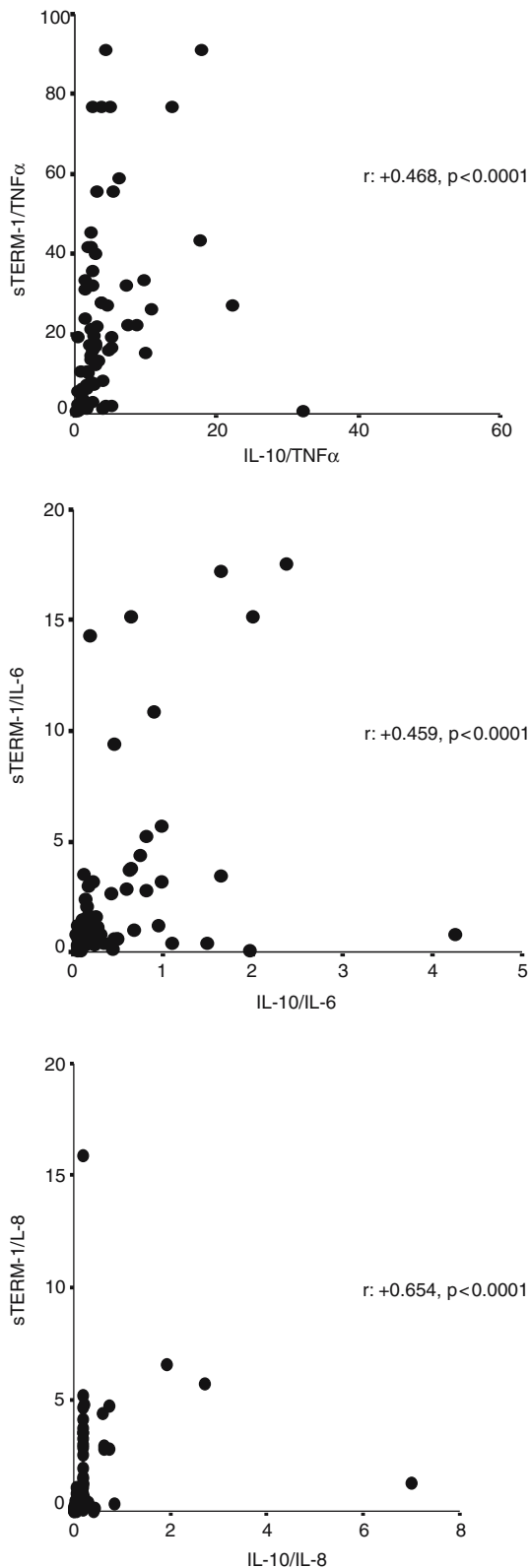


Fig. 3 Correlations of the ratio of IL-10 to proinflammatory cytokines (TNF α , IL-6, IL-8) and of sTREM-1 to proinflammatory cytokines of the sera on the first day of patients with ventilator-associated pneumonia and septic syndrome

($r_s = 0.459$, $p < 0.0001$), and between IL-10/IL-8 and sTREM-1/IL-8 ($r_s = 0.654$, $p < 0.0001$). Figure 4 compares the TNF α /IL-10 and of TNF α /sTREM-1 ratios on the first day between patients with sepsis, severe sepsis, and septic shock. Median values on day 1 of IL-10/TNF α of patients with sepsis, severe sepsis, and septic shock were 3.21, 2.16, and 2.86, respectively (NS between patients). Respective values for sTREM-1/TNF α were 21.28, 7.33, and 27.78 ($p = 0.047$ between sepsis and severe sepsis; $p = 0.003$ between severe sepsis and septic shock).

Cox regression analysis revealed that the more significant prognostic factors of survival were TNF α on day 1 (RR 1.02, 95% CI 1.01–1.03, $p = 0.001$); IL-6 on day 1 (RR 1.01, 95% CI 1.00–1.02, $p = 0.007$); IL-10/IL-6 ratio on day 1 (RR 29.65, 95% CI 1.83–481.63, $p = 0.017$), sTREM-1/IL-6 ratio on day 1 (RR 0.69, 95% CI 0.48–1.00, $p = 0.049$), and IL-10/IL-6 ratio on day 1 (RR 0.11, 95% CI 0.02–0.51, $p = 0.005$).

Discussion

sTREM-1 is a novel molecule whose role in the septic cascade remains to be elucidated. It is found to be elevated in blood in the event of endotoxemia and septic shock [4, 13]. It is considered to represent the soluble counterpart of the TREM-1 receptor that is highly expressed on neutrophil cell membranes in patients with sepsis [1, 2]. The present study examined the role of sTREM-1 in the sequence of events taking place in sepsis. Based on the daily kinetics of the proinflammatory cytokines TNF α , IL-6, and IL-8 and of the anti-inflammatory cytokine IL-10, the correlation to the kinetics of sTREM-1 was evaluated.

The entire population enrolled in the present study became septic because of the same underlying infection, i.e., VAP. This is a striking difference to other studies involving patients with sepsis, and it was based on the need to elaborate an entire study population conferring an antigenic stimulus to the immune system that did not differ considerably within patients. Patients were infected by Gram-negative bacterial species conferring similar stimuli to the innate immune system (Table 1). sTREM-1 on day 1 did not differ between patients infected by *P. aeruginosa* or by *A. baumannii*; we also observed no differences in sTREM-1 levels between patients with or without microbiological documentation of VAP.

Daily measurements of proinflammatory cytokines and IL-10 (Figs. 1, 2) showed greater concentrations of TNF α , IL-6, and IL-10 in nonsurvivors than in survivors. Similar differences were not found for IL-8. Other authors have reported higher serum concentrations of IL-10 in septic patients who eventually died than in those who survived [15, 16]. In the present study sTREM-1 followed the kinetics of IL-10. Our levels of sTREM-1 differed from those recently reported by other authors [17] who found higher

levels of sTREM-1 in survivors than in nonsurvivors on the first day after admission. However, there is agreement that sTREM-1 in nonsurvivors is higher than that in survivors over follow-up. The observed discrepancy may be due to (a) the enrollment of patients in the present study with the same underlying infection, i.e., VAP, and (b) the significantly lower levels of sTREM-1 in patients with early VAP than in those with late VAP.

It has been considered that the ratios of anti- to proinflammatory cytokines are indicative of the immune status of the septic host [18]. Strong statistical correlations were found on the first day of the presentation of symptoms between the ratios of IL-10 to proinflammatory mediators and of sTREM-1 to proinflammatory mediators (Fig. 3). The latter positive correlations and the similar kinetics of sTREM-1 to the anti-inflammatory cytokine IL-10 provide evidence that sTREM-1 can behave as an anti-inflammatory mediator. The probable anti-inflammatory role of sTREM-1 is further supported by the favorable prognostic role of the ratio of sTREM-1/IL-6 for the outcome of the septic host being similar to the role of the ratio of IL-10/IL-6.

The ratio of IL-10/TNF α has been considered to provide evidence of the existence or not of an immune disequilibrium of the septic host [5, 18]. This ratio was similar in our patients presenting either with sepsis, severe sepsis, or septic shock (Fig. 4). The ratio of sTREM-1/TNF α was significantly lower in patients with severe sepsis than in those with either sepsis or septic shock, leading thus to the probable hypothesis that changes in this ratio may determine transition from sepsis to severe sepsis and then from severe sepsis to septic shock. This hypothesis is further strengthened by the fact that no differences in sTREM-1 were found between trauma and nontrauma patients, since trauma is a situation accompanied by severe immunosuppression [19] that may act as a confounding factor.

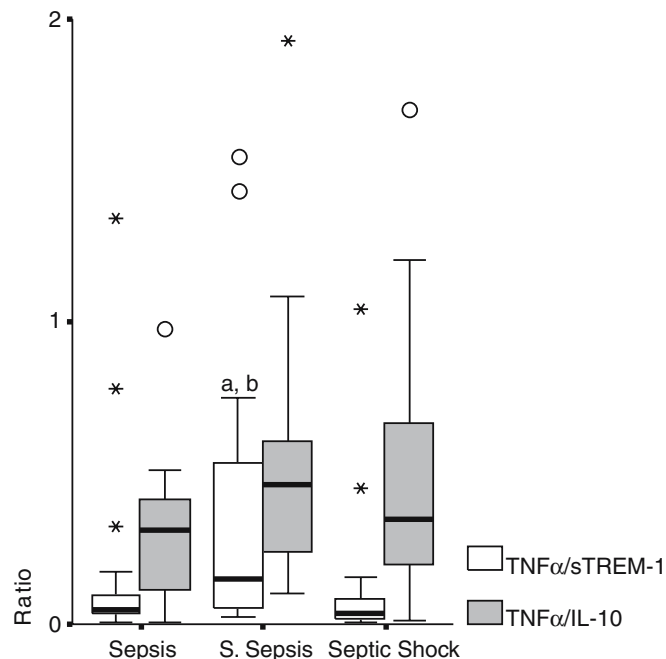


Fig. 4 Comparative values of the ratio of IL-10 to TNF α and of sTREM-1 to TNF α on the first day of symptoms of patients with sepsis, severe sepsis, and septic shock in the field of ventilator associated pneumonia. Circles Outliers; asterisks extremes. ^a $p = 0.047$ sepsis vs. severe sepsis, ^b $p = 0.003$ severe sepsis vs. septic shock

In conclusion, this is the first study to report that sTREM-1 possesses an anti-inflammatory role in the septic process. The evidence is based on the similar kinetics of sTREM-1 and IL-10. Decreased ratios of sTREM-1/TNF α are characterized by a significant pathophysiological hallmark determining transition from sepsis to severe sepsis and then from severe sepsis to septic shock.

References

1. Bouchon A, Facchetti F, Welgand MA, Colonna M (2001) TREM-1 amplifies inflammation and is a crucial mediator of septic shock. *Nature* 410:1103–1107
2. Colonna M, Facchetti F (2003) TREM-1 (triggering receptor expressed on myeloid cells): a new player in acute inflammatory responses. *J Infect Dis* 187 [Suppl 2]:S397–S401
3. Gibot S, Kolopp-Sarda MN, Béné MC, Bollaert PE, Lozniewski A, Maory F, Levy B, Faure GC (2004) A soluble form of the triggering receptor expressed on myeloid cells-1 modulates the inflammatory response in murine sepsis. *J Exp Med* 200:1419–1426
4. Routsis C, Giamarellos-Bourboulis EJ, Antonopoulou A, Kollias S, Siasiakou S, Koronaios A, Zakynthinos S, Armanigidis A, Giamarellou H, Roussos A (2005) Does soluble triggering receptor expressed on myeloid cells-1 play any role in the pathogenesis of septic shock? *Clin Exp Immunol* 142:62–67
5. Gogos CA, Drosou E, Bassaris HP, Skoutelis A (2000) Pro- versus anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. *J Infect Dis* 181:176–180
6. Michel F, Franceschini B, Berger P, Arnal JM, Gainnier M, Sainty JM, Papazian L (2005) Early antibiotic treatment for BAL-confirmed ventilator-associated pneumonia. A role for routine endotracheal aspirate cultures. *Chest* 127:589–597
7. Chastre J, Fagon JY (2002) Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 165:867–903

8. Rello J, Paiva JA, Baraibar J, Barcenilla F, Bodi M, Castander D, Correa H, Diaz E, Garnacho J, Llorio M, Rios M, Rodriguez A, Sole-Violan J (2001) International conference for the development of consensus on the diagnosis and treatment of ventilator-associated pneumonia. *Chest* 120:955–970
9. Baughman RP (2003) Diagnosis of ventilator-associated pneumonia. *Curr Opin Crit Care* 9:397–402
10. Vincent JL (2004) Ventilator-associated pneumonia. *J Hosp Infect* 57:272–280
11. Levy M, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G (2003) 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. *Crit Care Med* 31:1250–1256
12. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM (1991) Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic “blind” bronchoalveolar lavage fluid. *Am Rev Respir Dis* 143:1121–1129
13. Camargo LFA, De Marco FV, Barbas CSV, Hoelz C, Bueno MAS, Rodrigues Jr M, Amado VM, Caserta R, Dalla Valle Martino M, Pasternak J, Knobel E (2004) Ventilator associated pneumonia: comparison between quantitative and qualitative cultures of tracheal aspirates. *Crit Care* 8:R422–R430
14. Knapp S, Gibot S, de Vos A, Versteeg HH, Colonna M, van der Poll T (2004) Cutting edge: expression patterns of surface and soluble receptor expressed on myeloid cells-1 in human endotoxemia. *J Immunol* 173:7131–7134
15. Loisa P, Rinne T, Laine S, Hurme M, Kaukinen S (2003) Anti-inflammatory cytokine response and the development of multiple organ failure in severe sepsis. *Acta Anaesthesiol Scand* 47:319–325
16. Stanilova SA, Karakolev ZT, Dimov GS, Dobrova ZG, Miteva LD, Slavov ES, Stefanov CS, Stanilov NS (2005) High interleukin 12 and low interleukin 10 production after in vitro stimulation detected in sepsis survivors. *Intensive Care Med* 31:401–407
17. Gibot S, Cravoisy A, Kolopp-Sarda MN, Béné MC, Faure G, Bollaert PE (2005) Time-course of sTREM (soluble triggering receptor expressed on myeloid cells)-1, procalcitonin, and C-reactive protein plasma concentrations during sepsis. *Crit Care Med* 33:792–796
18. Monneret G, Finck ME, Venet F, Debard AL, Bohé J, Bienvenu J, Lepape A (2004) The anti-inflammatory response dominates after septic shock: association of low monocyte HLA-DR expression and high IL-10 concentration. *Immunol Lett* 95:193–198
19. Jiang JX, Tian KL, Chen HS, Zhu PF, Wang ZG (1997) Plasma cytokines and endotoxin levels in patients with severe injury and their relationship with organ damage. *Injury* 28:509–513