ORIGINAL ARTICLE

Decreased Cytokine Expression in Peripheral Blood Leukocytes of Patients With Severe Sepsis

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Background: High levels of tumor necrosis factor (TNF) α messenger RNAs and interleukin (IL) 8 have been reported in leukocytes of patients with sepsis.

Hypothesis: Assessment of leukocyte intracytoplasmic levels of proinflammatory and anti-inflammatory cytokines might be clinically more relevant to determine prognosis in patients with severe sepsis.

Design: Cohort study.

Setting: Surgical intensive care units of a university hospital.

Patients and Interventions: Leukocyte suspensions obtained from 16 patients, 6 during early sepsis or septic shock and 10 during late sepsis or septic shock, were incubated with anti-CD14 and anti-CD2 or anti-CD3 monoclonal antibodies and then with intracytoplasmic anticytokine antibodies staining for interferon-γ, TNF-α, IL-2, IL-6, IL-8, IL-10, and IL-12 and analyzed with a flow cytometer.

Main Outcome Measures: Mann-Whitney test and Spearman correlation test were used in statistical evaluations according to the 28-day all-cause mortality rates and multiple organ dysfunction and sepsis-related organ failure assessment scores.

Results: Higher serum IL-6, IL-8, C-reactive protein, and procalcitonin levels were found in patients with high multiple organ dysfunction and sepsis-related organ failure assessment scores (greater than or equal to the median values [8 and 11, respectively]), in contrast to decreased T-lymphocyte–associated IL-6 and TNF-α and monocyte-associated IL-10 and IL-12 proportions. Furthermore, in 28-day all-cause mortality analysis, there were higher levels of C-reactive protein and procalcitonin in nonsurvivors (n=9) than in survivors (n=7), while T-lymphocyte–associated IL-2, IL-6, IL-10, and TNF-α and monocyte-associated IL-10 and TNF-α proportions decreased in the nonsurvivors.

Conclusion: These results suggest that diminished lymphocyte- and monocyte-associated proinflammatory and anti-inflammatory cytokine levels are associated with worse prognosis in patients with severe sepsis.

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VARIOUS proinflammatory and anti-inflammatory cytokines have been implicated in the pathogenesis of the sepsis syndrome.[1-3] Increased production of proinflammatory tumor necrosis factor (TNF) α, interleukin (IL) 6, IL-8, IL-12, and anti-inflammatory IL-10 have been found to be correlated with increased Acute Physiology and Chronic Health Evaluation (APACHE) II and III scores and mortality, despite discrepancies in results of studies regarding the prognostic significance of IL-1β and interferon (IFN) γ levels.[4-7] However, the relationship between the absolute plasma levels of cytokines and sepsis appears to be inconsistent and variable. The short plasma half-life of these cytokines, such as IL-1β, with a rapid disappearance, and increased plasma levels of cytokine inhibitors such as soluble TNF receptors I and II, IL-1 receptor antagonists, or soluble IL-1 receptors may result in discrepancies among previous reports describing the incidence and kinetics of cytokine blood levels during sepsis.[8-11] The reticuloendothelial system, consisting of Kupffer cells

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(liver), alveolar macrophages (lung), etc, also plays an important role in sepsis as a potential source of cytokines.[12,13] As a consequence, plasma levels of cytokines represent the "tip of the iceberg," and investigations at the cellular level can provide more relevant information.[14]

Therefore, peripheral-blood leukocyte–associated cytokines have been in-

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vestigated in sepsis in the past decade, and contradictory results have been obtained in these studies. High levels of leukocyte-associated IL-8 were observed in septic patients,[15] while reduced TNF-α and IL-6 expression was reported in sepsis by Ertel et al.[16] However, the prognostic significance of these intracytoplasmic cytokine expressions remains to be answered. In the present study, we investigated the prognostic value of spontaneous expression of the monocyte-associated (TNF-α, IL-6, IL-8, and IL-12) and T-lymphocyte–associated (IL-2, IFN-γ, TNF-α, IL-6, and IL-10) proinflammatory and anti-inflammatory cytokines and also their serum levels in addition to procalcitonin (PCT) and C-reactive protein (CRP) levels as the main acute-phase proteins.

PATIENTS AND METHODS

PATIENT SELECTION

Between November 1, 1998, and December 31, 1999, 16 patients with sepsis syndrome, severe sepsis, or septic shock in the surgical intensive care units of the Departments of Anesthesiology and General Surgery, Istanbul Faculty of Medicine, University of Istanbul, Istanbul, Turkey, were eligible for this study, according to criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference.[17] Sepsis syndrome was defined by fever or hypothermia (temperature >38°C or <36°C, respectively), tachycardia (>90 beats per minute in the absence of β-blockade), and tachypnea (respiratory rate >20 breaths per minute or the requirement of mechanical ventilation). Severe sepsis or septic shock was defined as clinical diagnosis of sepsis syndrome plus organ dysfunction, hypoperfusion, or hypotension (systolic blood pressure <90 mm Hg or a 40–mm Hg decrease below baseline systolic blood pressure), or the use of vasopressor drugs to maintain blood pressure; and/or by clinical signs of altered organ perfusion resulting in mental disorientation, oliguria, or elevated lactate levels. Patients who were younger than 18 years or older than 70 years, and with any immunologic disorder, pregnancy, any organ transplantation, or any immunosupressive therapy (eg, use of corticosteroids) were excluded.

Six patients were considered to have early sepsis or septic shock and 10 patients to have late sepsis or septic shock, with early and late sepsis or septic shock defined as symptoms earlier or later than 72 hours. All patients underwent mechanical ventilation for respiratory problems and were also invasively monitored and followed up to study completion at day 28 or death. A blood sample was obtained from each patient on day 1, 2, or 3 after admission to the intensive care unit. Patients were categorized as having low or high multiple organ dysfunction (MOD) and Sepsis-related Organ Failure Assessment (SOFA) scores,[18,19] with low scores defined as those less than the median value and high scores as those equal to or more than the median value, and as survivors and nonsurvivors according to the 28-day all-cause mortality.

BLOOD AND CULTURE SAMPLES

After assessment of the APACHE II,[20] MOD, and SOFA scores, 20 mL of peripheral blood was collected for immunologic, hematologic, and biochemical analysis. Blood samples for determination of the specific cytokine, CRP, and PCT levels were spun at 3000 rpm for 10 minutes, and serum was frozen at –80°C. Blood, tracheal, and urine cultures and cultures from the suspected source of infection (eg, incisions and abdominal drains) were also obtained.

FLOW CYTOMETRIC ANALYSIS

Peripheral-blood mononuclear cells were isolated by standard Ficoll-Hypaque density gradient centrifugation and washed twice in phosphate-buffered saline. A cell fixation/permeabilization kit (Cytofix/Cytoperm; Pharmingen, San Diego, Calif) was used in the intracytoplasmic cytokine determination. Aliquots of the cell suspensions (100 µL) were first incubated with 5 µL of anti–CD2–fluorescein isothiocyanate (FITC) (DAKO Diagnostika GmbH, Hamburg, Germany) and anti–CD14-FITC (DAKO Diagnostika GmbH) monoclonal antibodies (mAbs) for the phycoerythrin-labeled cytokines and anti–CD3-phycoerythrin (DAKO Diagnostika GmbH) and anti–CD14-FITC mAbs (DAKO Diagnostika GmbH) for the FITC-labeled cytokines or corresponding isotypic control antibodies (DAKO Diagnostika GmbH) for 30 minutes at room temperature in the dark. After being washed twice in staining buffer, the cells were incubated in 100 µL of Permeafix (Pharmingen) (phosphate buffered saline containing 4% paraformaldehyde and 0.1% saponin) for 20 minutes at 4°C and washed 2 times in Permwash solution (Pharmingen) (phosphate buffered saline containing 0.1% saponin). The pellets were suspended in 50 µL of Permwash solution and incubated with pretitrated amounts of cytokine-specific mAbs and corresponding control antibodies for 20 minutes at 4°C (anti–IFN-γ, anti–IL-6, anti–IL-8, and anti–IL-12 mAbs were FITC-labeled and anti–IL-10, anti–TNF-α, and anti–IL-2 mAbs were phycoerythrin-labeled [all antibodies were purchased from Pharmingen]). The cells were washed twice in Permwash solution and stored after suspension in staining buffer containing 2% paraformaldehyde at 4°C in the dark before analysis.

List mode data were acquired on a flow cytometer (FACS Calibur; Becton Dickinson Immunocytometry Systems, San Jose, Calif) and analyzed by means of CellQuest software (Becton Dickinson Immunocytometry Systems). After appropriate instrument settings and spectral compensations were achieved, the instrument settings were not changed and a minimum of 10 000 events were acquired in the gating window computed in list mode by means of log-amplified fluorescence signals and linearly amplified side scatter and forward scatter signals. Two-parameter dot plots showing cytokine staining were created according to lymphocyte and monocyte gates, respectively, and quadrants were then placed according to the staining of negative controls. Estimated median values were presented as positive net percentages in the figures.

CYTOKINE CONCENTRATIONS

Commercially available enzyme-linked immunosorbent assay kits were used for the measurement of IFN-γ, TNF-α, and IL-2 (ELISA kits; Immunotech, Marseille, France); IL-10 and IL-12 (Medgenix Combo IL-10/IL-12 kit, BioSource Europe SA, Nivelles, Belgium); and IL-6 and IL-8 (CLB PeliKine Compact Human ELISA kits; CLB, Amsterdam, the Netherlands) cytokine levels. The sensitivity, defined as the lowest concentration significantly different from the zero standard with a probability of 95%, was 0.08 IU/mL for IFN-γ and 5 pg/mL for TNF-α and IL-2, whereas the sensitivity of IL-6, IL-8, and IL-10, defined as the mean calculated zero signal + 3 SDs, was 0.2 pg/mL for IL-6 and 1 pg/mL for both IL-8 and IL-10. Furthermore, the minimum detectable concentration, defined as the IL-10 and IL-12 concentrations corresponding to the average optic density of 20 replicates of the zero standard + 2 SDs, was estimated as 3 pg/mL for both IL-10 and IL-12.

CRP AND PCT LEVELS

For the determination of CRP levels, we used a kit (Dade Behring, Marburg, Germany) with a lower detection limit of 2.5

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Table 1. Patient Characteristics and Clinical Course*

Patient No./ Sex/Age, y	APACHE II Score	MOD Score	SOFA Score	Septic Focus	28-Day Outcome
1/M/57	20	7	7	Whipple operation + aspiration pneumonia related to upper gastrointestinal tract bleeding	NS
2/F/70	23	11	12	Left hemicolectomy + Hartmann colostomy related to rectal tumor	NS
3/F/60	28	10	12	Drainage of the intra-abdominal abscess	NS
4/F/50	15	8	10	Whipple operation	S
5/M/33	27	9	13	Left hemicolectomy + Hartmann colostomy related to left colon perforation	NS
6/M/55	14	4	4	Debridement after necrotizing fasciitis	S
7/M/61	31	14	16	Intra-abdominal multitrauma + pneumonia after severe thoracic injury	NS
8/M/57	22	10	14	Peritonitis after sigmoid perforation	S
9/F/41	18	3	4	Leakage after subtotal gastrectomy	S
10/M/33	17	7	8	Necrotizing pancreatitis	NS
11/M/62	19	10	13	Pneumonia after right upper lobectomy due to lung cancer	NS
12/F/70	21	6	9	Loop colostomy due to colon perforation	S
13/M/63	11	3	4	Leakage after neocystostomy	S
14/F/65	23	8	11	Left colonic fistula	NS
15/M/69	21	9	11	Radical prostatectomy	S
16/M/57	18	6	6	Necrotizing pancreatitis	S

*APACHE indicates Acute Physiology and Chronic Health Evaluation; MOD, multiple organ dysfunction; SOFA, Sepsis-related Organ Failure Assessment; NS, nonsurvivor; and S, survivor.

mg/L. Levels of PCT were determined by an immunoluminometric assay (LUMI test PCT, BRAHMS Diagnostica GmbH, Berlin, Germany). The PCT sequence consists of 3 segments: katacalcin, calcitonin, and an *N*-terminal residue. In this test, the PCT molecule is sandwiched between 2 mAbs, 1 of which has a luminescent label. The test ultimately produces a luminescence signal directly proportional to the PCT concentration of the serum. The functional assay sensitivity (ie, the lowest value measured with a precision of 20% maximum interassay variation) is approximately 0.3 ng/mL. The analytic assay sensitivity (which is the value that can be discriminated from 0 with a 95% confidence interval) is 0.1 ng/mL.

STATISTICAL ANALYSIS

The SPSS 10.1 software package (SPSS Inc, Chicago, Ill) was used for statistical analysis. The Mann-Whitney test was used to evaluate differences between groups according to the low and high MOD and SOFA scores and the 28-day all-cause mortality. Furthermore, the relationships between the variables were assessed by Spearman correlation test. All analyses are 2-tailed, and $P \leq .05$ was considered significant.

RESULTS

The median age of the patients was 58.5 years (range, 33-70 years); 10 patients (62%) were male. Patient characteristics and clinical outcome are shown in **Table 1**. The 28-day all-cause mortality rate was 56.3%.

CHANGES IN CYTOKINE, CRP, AND PCT LEVELS ACCORDING TO 28-DAY ALL-CAUSE MORTALITY ANALYSIS

Nonsurvivors were found to have higher CRP and PCT levels than survivors ($P = .048$ and $P = .05$, respectively). In contrast, nonsurvivors had decreased percentages of lymphocyte-associated IL-2, IL-6, IL-10, and TNF-α ($P = .05$, $P = .02$, $P = .03$, and $P = .009$, respectively) and monocyte-associated IL-10 and TNF-α ($P = .048$ and $P = .03$, respectively) compared with the survivors, as shown in **Figure 1**.

CHANGES IN CYTOKINE, CRP, AND PCT LEVELS ACCORDING TO MOD AND SOFA SCORES

The median APACHE II score of the patients was 21 (range, 11-31), whereas the median MOD and SOFA scores were 8 (3-14) and 10.5 (4-16), respectively. Accordingly, all of the immunologic measures were analyzed according to low (<8) and high (≥ 8) MOD scores and low (<11) and high (≥ 11) SOFA scores. In patients with high MOD and SOFA scores, serum levels of CRP ($P = .18$ and $P = .009$, respectively), PCT ($P = .06$ and $P = .10$, respectively), IL-6 ($P = .009$ and $P = .007$, respectively), and IL-8 ($P = .002$ and $P = .02$, respectively) were found to be increased. However, lymphocyte-associated IL-6 ($P = .03$ and $P = .08$, respectively) and TNF-α ($P = .04$ and $P = .03$, respectively) and monocyte-associated IL-10 ($P = .04$ and $P = .13$, respectively) and IL-12 ($P = .10$ and $P = .03$, respectively) proportions were determined to be decreased, as shown in **Figure 2** and **Figure 3**.

CORRELATION ANALYSIS

The statistically significant Spearman correlation coefficients are presented in **Table 2** to show the relationship of serum levels of acute-phase proteins, cytokines, and leukocyte-associated cytokine percentages with MOD and SOFA scores. Serum PCT, IL-6, and IL-8 levels positively correlated with MOD and SOFA scores by different *P* values, while a reverse correlation was found between MOD and SOFA scores and both lymphocyte-associated IL-6 and TNF-α and monocyte-associated IL-10, IL-12, and TNF-α percentages.

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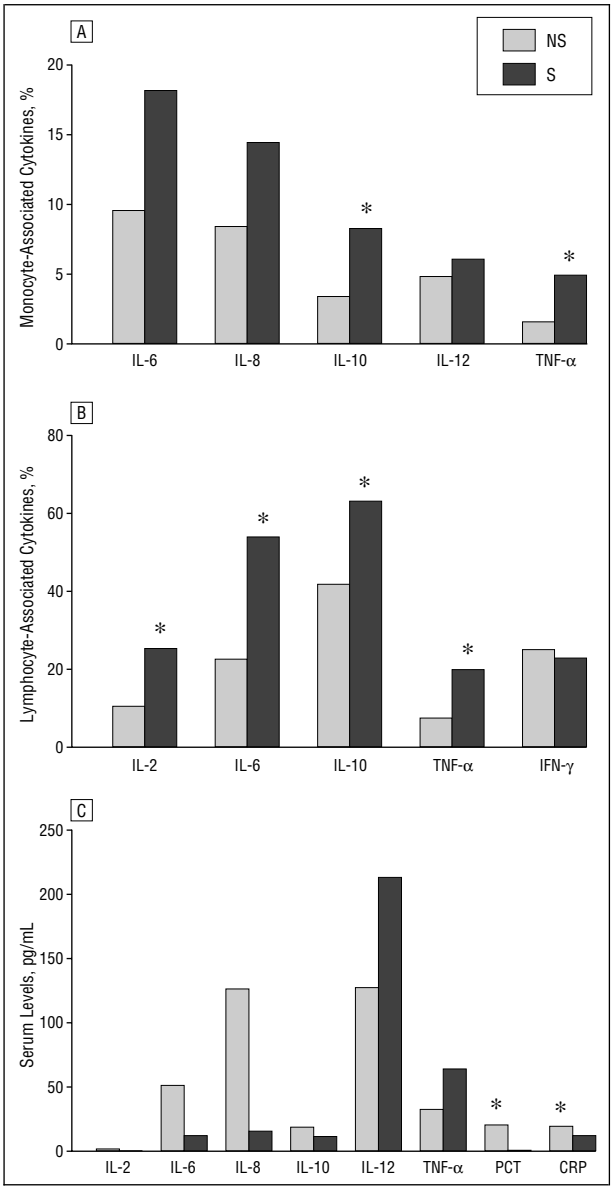

Figure 1. Intracytoplasmic cytokine profile of unstimulated monocytes (A) and lymphocytes (B) and serum levels of cytokines, procalcitonin (PCT), and C-reactive protein (CRP) (C) from survivors (S; n=7) and nonsurvivors (NS; n=9) according to the 28-day all-cause mortality analysis. Results showing the median values were compared with the Mann-Whitney test. Asterisk indicates statistically significant changes ($P<.05$). Procalcitonin values are in nanograms per milliliter. IL indicates interleukin; TNF, tumor necrosis factor; and IFN, interferon.

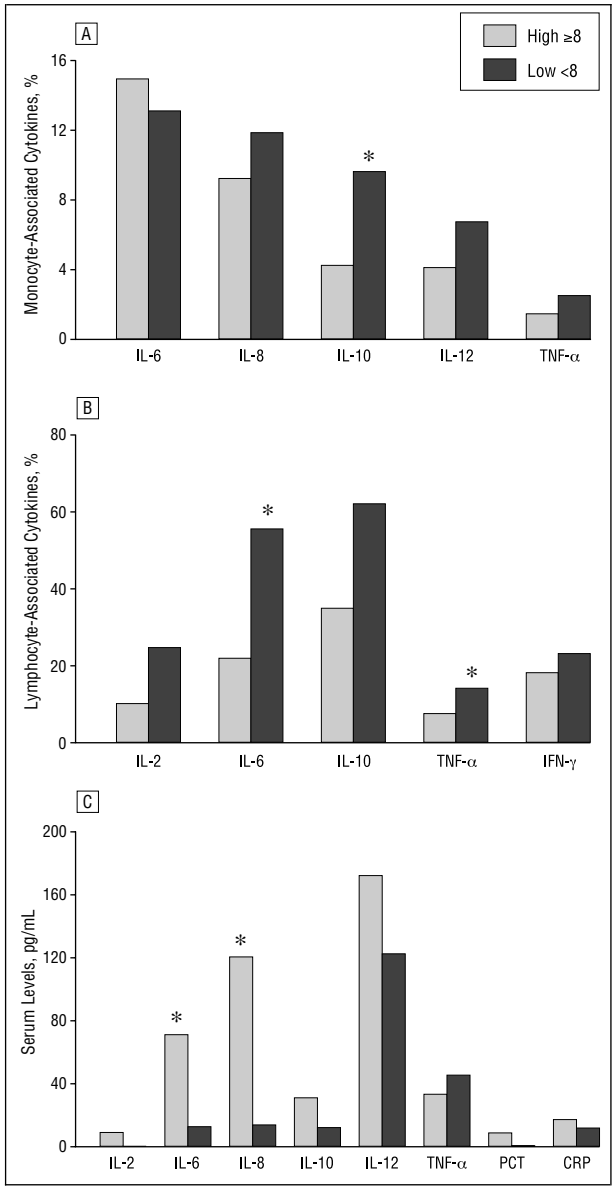

Figure 2. Intracytoplasmic cytokine profile of unstimulated monocytes (A) and lymphocytes (B) and serum levels of cytokines, procalcitonin (PCT), and C-reactive protein (CRP) (C) of patients with severe sepsis (n=16) according to low (n=7) and high (n=9) multiple organ dysfunction scores. Results showing the median values were compared with the Mann-Whitney test. Asterisk indicates statistically significant changes ($P<.05$). Procalcitonin values are in nanograms per milliliter. IL indicates interleukin; TNF, tumor necrosis factor; and IFN, interferon.

COMMENT

Activation of inflammatory pathways, including the cytokine network, is considered to play a major role in the pathogenesis of sepsis. The particular type of immune response is determined by the differentiation of naive helper T (T_H0) cells into T_H1 and T_H2 cells, which can be induced to express the T_H1 phenotype by exposure to the cytokine IL-12, whereas exposure to IL-4 induces the T_H2 phenotype.[3] Interleukin 12 is produced predominantly by monocytes and macrophages. The T_H1 cells predominantly produce IFN-γ, IL-2, and TNF-β, and are responsible for both humoral and cell-mediated responses. The T_H2 cells produce predominantly IL-4, as well as IL-5, IL-6, IL-10, and IL-13, and provide help for humoral responses, including IgE isotypes and mucosal immunity. Several other proteins are secreted both by T_H1 and T_H2 cells, including IL-3, TNF-α, and granulocyte-macrophage colony-stimulating factor. Furthermore, IL-6, IL-10, and TNF-α are also major monocyte products. The cytokine network produced by these effector cells is proinflammatory, which are mainly TNF-α, IL-1, IL-12, IFN-γ, or IL-6 with both proinflammatory and anti-inflammatory properties, or anti-inflammatory, which are IL-4, IL-10, and IL-13. Both types of T_H cells regulate the immune

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responses through these different cytokines with antagonistic interactions in septic patients.

Numerous studies[1-11] have been published on serum or plasma concentrations of cytokines in patients with sepsis. In general, proinflammatory cytokines can be detected only in a subset of patients, whereas anti-inflammatory cytokines and soluble inhibitors can be found in the vast majority of patients with sepsis. However, the results regarding the relationship between circulating cytokines and APACHE II and III scores and mortality are showing great variability. In comparison with other cytokines, IL-6 (a mixed proinflammatory and anti-inflammatory cytokine) was most consistently reported in the circulation of septic patients, showing a positive correlation with mortality.[21] These discrepancies may be due to patients having different stages or severity of sepsis syndrome, timing of samples relative to the onset of sepsis or septic shock, high interindividual variation, and methods.[22] Our results also did not show any difference in the circulating cytokine levels in terms of 28-day all-cause mortality analysis, while CRP and PCT levels were found to be significantly elevated in patients with poor prognosis. However, there was a significant positive correlation between MOD and SOFA scores and serum IL-6, IL-8, and PCT levels in concordance with some reports,[23-25] while CRP levels did not show any significant correlation. Rau et al[26] also reported higher levels of IL-8 and PCT in patients with infected pancreatic necrosis than in those with sterile necrosis, whereas there was no difference in levels of CRP. Furthermore, PCT was found to be increased earlier and returned to the normal range more quickly than was CRP in children with infectious disease, and also was a more specific and sensitive marker of IL-6 and TNF-α secretion than was CRP in septic patients.[27,28] However, additional large studies are needed to determine whether PCT is superior to CRP in differentiating between inflammation of infectious and noninfectious origin and also in evaluating the severity of sepsis or multiple organ dysfunction.

Measuring cytokine levels in the circulation should be viewed as an inadequate exploration of the tip of the iceberg. Short plasma half-life of circulating cytokines, soluble cytokine receptors or inhibitors, and peripheral-blood cells such as erythrocytes or leukocytes trapping excessive exogenous cytokines via their specific receptors can cause a reduction of these individual cytokines, resulting in misleading results. Erythrocytes were shown to bind to IL-8 and other chemokines via the Duffy antigen.[29,30] It was also observed that a larger amount of IL-8 could be found in cell-associated form in comparison with its plasma levels after activation of leukocytes including monocytes and polymorphonuclear neutrophils of septic patients, as determined in cell lysates by enzyme-linked immunosorbent assay technique.[31-33]

Taking these findings into consideration, there has been a tendency to investigate leukocyte intracytoplasmic cytokines separately from their corresponding serum levels. With a recently developed technology for intracellular detection of cytokine synthesis on a single-cell level, a powerful diagnostic tool has become available to discriminate a shift of functionality in T-cell subsets via their individual cytokine profiles by performing flow cytometric analysis.[34] In this study, we used this flow cytometric approach, which allows for the determination of the functional potential of T cells and also monocytes for mediator synthesis to evaluate the participation of these subsets in the immune response of patients with severe sepsis in terms of MOD and SOFA scores and survival analysis. In the study design, we aimed to investigate the unstimulated spontaneous cytokine expression in the peripheral-blood lymphocytes and monocytes under the influence of septic conditions, comparing the results with their corresponding serum levels and also CRP and PCT. It was previously reported that intracytoplasmic cytokines were almost undetectable within unstimulated cells in healthy volunteers.[35] However, in the present study, we demon-

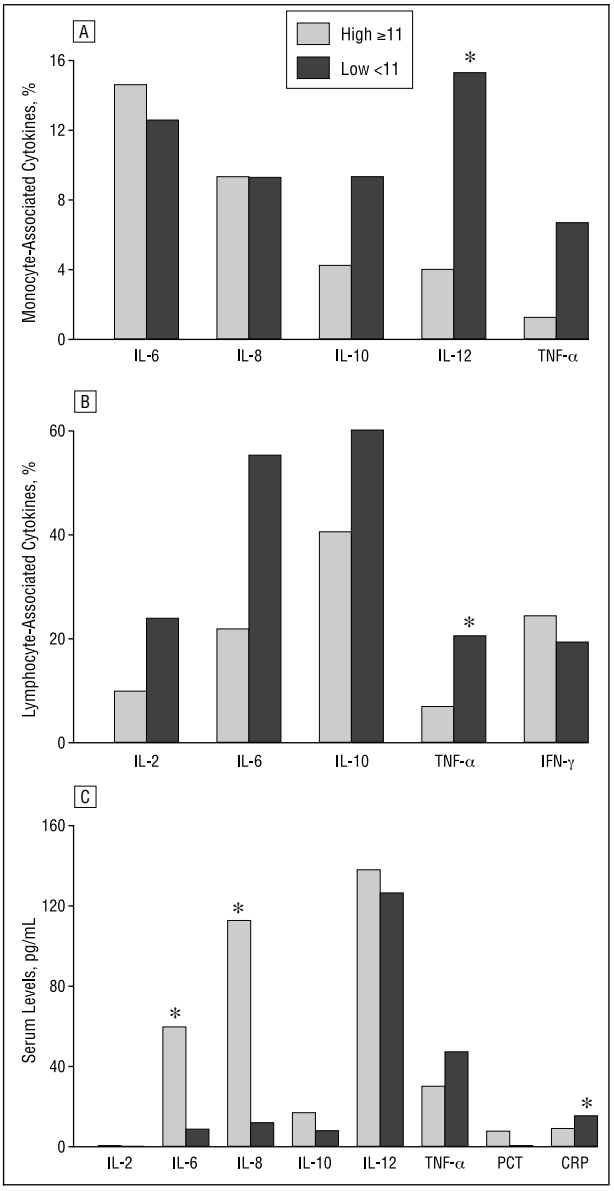

Figure 3. Intracytoplasmic cytokine profile of unstimulated monocytes (A) and lymphocytes (B) and serum levels of cytokines, procalcitonin (PCT), and C-reactive protein (CRP) (C) of patients with severe sepsis (n=16) according to low (n=7) and high (n=9) sepsis-related organ failure assessment scores. Results showing the median values were compared with the Mann-Whitney test. Asterisk indicates statistically significant changes ($P<.05$). Procalcitonin values are in nanograms per milliliter. IL indicates interleukin; TNF, tumor necrosis factor; and IFN, interferon.

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Table 2. Correlations Between Immunologic Variables and MOD and SOFA Scores*

Variable	MOD Score		SOFA Score	
	P	*r*	*P*	*r*
C-reactive protein, pg/mL	.1	0.45	.12	0.44
Procalcitonin, ng/mL	.02	0.6	.03	0.59
IL-6, pg/mL	.02	0.57	.03	0.54
IL-8, pg/mL	.01	0.62	.006	0.65
LA IL-6, %	.006	−0.66	.04	−0.51
LA TNF-α, %	.002	−0.72	.03	−0.54
MA IL-10, %	.01	−0.62	.07	−0.46
MA IL-12, %	.02	−0.56	.05	−0.49
MA TNF-α, %	.04	−0.52	.12	−0.4

*MOD indicates multiple organ dysfunction; SOFA, sepsis-related organ failure assessment; IL, interleukin; LA, lymphocyte-associated; TNF, tumor necrosis factor; and MA, monocyte-associated. All *P* values are 2-tailed and significant if ≤.05.

strated higher percentages of lymphocyte-associated TNF-α, IL-2, IL-6, and IL-10 and monocyte-associated TNF-α, IL-10, and IL-12 in septic patients with a better prognosis. Our results suggest that both T_H1- and T_H2-cell responses are necessary for the survival of the septic patients, most of who have intra-abdominal sepsis after major visceral surgery. However, diminished or absent T_H1 and T_H2 responses characterized by immunologic anergy resulted in poor clinical outcome.

The term *anergy* is defined as a state of nonresponsiveness to antigen, and it is used in the clinical literature to describe a condition characterized by the lack of delayed-type hypersensitivity response to skin-test antigens. In concordance with our results, Puyana et al[36] reported a higher mortality rate in anergic patients after trauma, whereas a significant inverse correlation was found between T-cell proliferation and APACHE III or MOD scores. There was a global reduction of T-cell lymphokine production, including IL-2, IFN-γ, IL-10, and IL-4 secreted by T_H1 and T_H2 cells, and a significant direct correlation between depressed IL-4 and IFN-γ levels in anergic patients. These findings are also in concordance with previous studies reporting strongly reduced TNF-α and IL-6 messenger RNA expression in peripheral-blood mononuclear cells harvested from lipopolysaccharide-stimulated whole blood of patients with severe sepsis[16] and decreased T-cell proliferation and production of IL-2 and TNF in patients with lethal intra-abdominal infection as compared with survivors and healthy controls.[37] In contrast, persisting levels of unstimulated monocyte-associated TNF-α were determined in surviving septic patients.[38] In addition to "T-cell anergy," monocytic deactivation was found to be associated with a higher mortality.[39] These monocytes were characterized by a markedly reduced HLA-DR expression, a loss of antigen-presenting capacity, and a profound reduction of their ability to produce lipopolysaccharide-induced TNF-α, IL-10, and IL-12 in vitro.[39-41] Nevertheless, a recent study demonstrated that postoperative sepsis was associated with immediate monocyte defects that affected both proinflammatory and anti-inflammatory cytokine secretion, and sepsis survival correlated with the recovery of the proinflammatory, but not anti-inflammatory, response.[42] Our findings regarding the inverse correlation between MOD and SOFA scores and monocyte-associated higher percentages of proinflammatory and anti-inflammatory cytokines, including IL-10, IL-12, and TNF-α, support the necessity of adequate monocytic capacity with a predominance of proinflammatory cytokine expression for immunologic surveillance in sepsis. Furthermore, IL-12 therapy significantly increased survival in mice with intra-abdominal sepsis induced by cecal ligation and puncture by enhancing TNF-α and IFN-γ production.[41]

In conclusion, the development of both types of T_H-cell response with a predominance of proinflammatory cytokine expression, especially by monocytes, is suggested to play a major role in survival of severely septic patients. Nevertheless, both T-cell and monocyte anergy characterized by low intracytoplasmic cytokine expression in this study is found to be a poor prognostic factor. Immunomodulatory agents like IL-12 that might correct this immunologic dysfunction are to be further studied in severe sepsis.

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Invited Critique

Modern surgical therapy can restore function to most patients, including many with disabling maladies. Yet, what is there about the presence of infection in these patients that occasionally leads to multiple organ failure and poor outcome?

Once patients become severely infected, their demographics for adverse outcome (age, sex, comorbidities, etc) are reasonably well captured by severity scoring systems. The report by Cabioglu et al supports this concept. Their article also reiterates the theme that outcome prediction can be enhanced (almost in real time) by the analysis of immune cell phenotype. Although their results are at some variance with those of previous reports in this journal,[1,2] Cabioglu and colleagues surmise that adverse clinical outcome correlates with an attenuation of immune cell proinflammatory mediator influence (T_H1) compared with the T_H2 phenotype. Unfortunately, the authors neither address the methodological and data variances from previous reports nor provide much in the way of mechanistic discussion as to how this immunological "anergy" (their term) develops or resolves.

They observed that this anergic state was noted at the very outset of severe infection, and no ex-vivo stimulation was required to detect the phenotype. Here again, the relationship to antecedent stresses and morbidities begs consideration, especially since this basal phenotype is seldom observed in the noninfectious, stressed state. Is acute stress a necessary prelude to the adverse phenotype of infection? What confounding genetic or metabolic influences promote this early determination of immune cell function? Finally, and perhaps most important, can those influences be manipulated to circumvent the therapeutic nihilism implied by the current observations? Only time will tell whether the observations of Cabioglu and colleagues will provide useful mechanistic insights or new diagnostic opportunities.

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