

## Comparative Effects of Ciprofloxacin and Ceftazidime on Cytokine Production in Patients with Severe Sepsis Caused by Gram-Negative Bacteria

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**In the present study the effect of ciprofloxacin versus ceftazidime on concentrations of pro- and anti-inflammatory cytokines in the sera of patients with severe sepsis was evaluated. The study included 58 previously healthy patients suffering from severe sepsis caused by gram-negative bacteria, treated with either ciprofloxacin or ceftazidime after thorough clinical and microbiological evaluation and followed up for clinical outcome. Levels of the proinflammatory cytokines tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1b (IL-1b), IL-6, and IL-8 and of the anti-inflammatory cytokine IL-10, as well as of IL-1 receptor antagonist and soluble TNF receptors I and II, in serum were measured at baseline and 24 and 48 h after the first antimicrobial dose. Mean SAPS-II scores, development of septic shock, and mortality rates were similar in the two groups (43.2  $\pm$  9.2, 21.4%, and 14.3% in the ceftazidime group versus 49.8  $\pm$  11.3, 20%, and 13.3% in the ciprofloxacin group). Serum TNF- $\alpha$  and IL-6 levels at 24 and 48 h were significantly lower in the ciprofloxacin group, while the IL-10/TNF- $\alpha$  ratio was significantly higher, than those for the ceftazidime group. Among patients with high baseline TNF- $\alpha$  levels, there were significant increases in the IL-10/TNF- $\alpha$  ratio at both 24 and 48 h over that at admission for the ciprofloxacin group, while no differences were noted in the ceftazidime group. These results indicate that ciprofloxacin may have an immunomodulatory effect on septic patients by attenuating the proinflammatory response, while there is no evidence that differences in the cytokines measured have any impact on the final outcome.**

Sepsis is the systemic immune response to severe infections and is mediated through systemic release primarily of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), IL-6, and IL-8, and secondarily of anti-inflammatory molecules, such as IL-10, soluble TNF receptors I and II (sTNFR-I and -II), and IL-1 receptor antagonist (IL-1ra). Cytokine production in sepsis has been widely studied, and it seems that both pro- and anti-inflammatory cytokines are overproduced in sepsis syndrome. However, their clinical significance and prognostic value have not been elucidated (1, 6, 22). It seems that a complex network of interactions between different cytokines and possibly other components of the immune response takes place during severe infections. There are accumulating data suggesting that an equilibrium between the pro- and anti-inflammatory responses is important for the final outcome of patients with severe sepsis (11).

Pathogen-associated microbial patterns, such as endotoxin in gram-negative bacteria and lipoteichoic acid in gram-positive bacteria, have been recognized as major contributors to the pathogenesis of sepsis syndrome, and their clinical significance has been investigated in clinical studies for more than 20 years (13, 20). Endotoxin (lipopolysaccharide [LPS]) is a component of the outer membrane of gram-negative bacteria that stimulates macrophages to secrete a wide array of proinflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 (5).

Since the 1970s there has been growing interest in the effect of antimicrobial agents on endotoxin release. A number of in vitro studies indicate that exposure of gram-negative pathogens to antibacterial agents can result in both endotoxin and cytokine release related to the mode of antibacterial activity (14, 24). Antimicrobial agents with a high affinity for penicillin-binding protein-3 (PBP-3), such as cephalosporins, induce massive endotoxin release in vitro, while other classes of antimicrobial agents, such as imipenem or aminoglycosides, induce little endotoxin release (15).

Although theoretically antibiotic-induced endotoxin release and consequent cytokine synthesis may be deleterious for septic patients, as shown in vitro or in animal models, clinical studies have failed to describe the clinical relevance of these experimental observations. The purpose of the present study was to determine the concentrations of the proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 and of the anti-inflammatory cytokines IL-10, sTNFR-I, sTNFR-II, and IL-1ra in the sera of a homogenous group of patients with severe sepsis caused by gram-negative bacteria and to evaluate the effect of ceftazidime versus ciprofloxacin on cytokine production and the clinical course of the patients.

### MATERIALS AND METHODS

This open randomized trial was performed at the Patras University Hospital, a 700-bed secondary and tertiary referral hospital in southwestern Greece, for a period of 2 years. A total of 92 patients (53 men and 39 women) with signs of severe sepsis suspected to be due to gram-negative infections, based on clinical evaluation and localization of the infection, were included in the study. All patients were enrolled in the study upon diagnosis, before culture results were known. Eighteen patients were excluded from the study because of prior antimicrobial administration or protocol violation. Sixteen other patients were not

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evaluated because cultures revealed a resistant organism (7 patients) or a gram-positive or polymicrobial infection (9 patients), leaving 58 patients (32 men and 26 women; mean age,  $61.35 \pm 8.92$  years) assessable for the initial evaluation. Of these, 19 patients (32.75%) were referred to the hospital by their family physician or by the regional health centers, 8 patients (13.79%) were self-referred, 12 (20.68%) were transferred from other hospitals, and 19 (32.75%) developed sepsis during their hospitalization. In order to evaluate the immunomodulatory effect of the antimicrobial agents irrespective of susceptibility, we performed a complementary analysis including all patients ( $n = 34$ ) having positive blood cultures with both resistant and susceptible pathogens. The study was performed according to the European guidelines for good clinical practice and according to national rules and regulations and was approved by the local hospital ethics committee. Informed consent was obtained from all patients or their next of kin before randomization. Patients with severe underlying diseases (e.g., chronic renal failure, diabetes mellitus, chronic obstructive pulmonary disease, congestive heart failure, cirrhosis), allergy to quinolones or  $\beta$ -lactams, or prior or concomitant use of any kind of immunomodulating therapy were excluded. Ten healthy nonhospitalized volunteers with a mean age similar to that of our patients ( $60.63 \pm 7.84$ ) served as controls.

Sepsis was defined according to the criteria of the American College of Chest Physicians–Society of Critical Care Medicine Consensus Conference Committee (11) as the presence of confirmed infection and two or more of the following criteria: (a) a temperature of  $>38^\circ\text{C}$  or  $<36^\circ\text{C}$ , (b) a heart rate of  $\geq 90$  beats/min, (c) tachypnea, manifested by a respiratory rate of  $\geq 20$  breaths/min, or hyperventilation, indicated by a  $\text{PaCO}_2$  of  $\leq 32$  mm Hg, and (d) an altered white blood cell count of  $>12,000$  or  $<4,000$  cells/ $\text{mm}^3$  or the presence of  $>10\%$  immature forms. Severe sepsis was defined as the presence of sepsis plus at least one organ dysfunction indicated by the following: (a) hypotension (systolic blood pressure of  $\leq 90$  mm Hg or mean arterial pressure of  $\leq 65$  mm Hg corrected within 1 h by fluid replacement), (b) arterial hypoxemia ( $\text{PaO}_2$  of  $\leq 75$  mm Hg without evidence of primary respiratory tract disease), (c) metabolic acidosis ( $\text{pH} \leq 7.3$  or a base deficit of  $\geq 5$  meq/liter), (d) oliguria (urine output,  $\leq 30$  ml/h), (e) liver dysfunction, (f) acute alteration of mental status, or (g) recent coagulation abnormality (activated partial thromboplastin time,  $\geq 1.2$  times the upper normal limit plus  $\geq 500$  D-dimers or  $\leq 100,000$  thrombocytes/ $\mu\text{l}$ ). Septic shock was defined as severe hypotension lasting  $>1$  h, despite adequate fluid replacement and the use of vasopressor agents.

Intravenous antimicrobial treatment was initiated immediately following randomization. All patients were randomized to receive either ciprofloxacin (400 mg twice a day, group A, 30 patients) or ceftazidime (2 g three times a day, group B, 28 patients) in a 1:1 randomization procedure. Both antimicrobial agents were administered intravenously in 100 ml of 5% dextrose solution over 30 min. No other antimicrobial agents were administered during the 48-h study period, except for metronidazole for intra-abdominal infections (8 patients in the ciprofloxacin group and 9 in the ceftazidime group) and vancomycin when appropriate. All patients ( $n = 12$ ) who received concomitant vancomycin during the 48-h study period were excluded from the initial evaluation. However, in order to investigate the effect of vancomycin use on the final outcome and cytokine synthesis, we performed a complementary analysis including all patients with sepsis caused by gram-negative bacteria irrespective of the empirical use of vancomycin ( $n = 70$ ). Patients in the two treatment groups received similar concomitant adjunct interventions. All patients were under close monitoring during their hospital stay, and critically ill patients with septic shock were transferred to the intensive care unit. Forty-eight hours after the initiation of therapy, antimicrobial treatment was altered according to the susceptibility pattern and the clinical condition of the patient.

Blood cultures or other appropriate microbiological samples were collected before the initiation of antimicrobial therapy and during follow-up, where indicated. All clinical isolates were tested for susceptibility to both ceftazidime and ciprofloxacin by standard laboratory methods. Specific diagnostic procedures, such as ultrasound, computed tomography, or gallium scanning, were performed to identify the infection site. Vital signs and simplified acute physiology scoring (SAPS-II), as a tool for assessment of severity of illness (12), were recorded before and 48 h after the initiation of antimicrobial treatment. Patient survival was defined as being alive after 28 days of meeting criteria for severe sepsis.

Sera for cytokine determination from all patients were obtained from peripheral blood clotted for 30 min at  $37^\circ\text{C}$  and stored at  $-70^\circ\text{C}$  until measurement. Serum cytokine levels were measured before, and at 24 and 48 h after, initiation of antimicrobial treatment, by using an enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems, Minneapolis, Minn.). The baseline blood sampling was performed just before the first dose of antibiotics in both study arms. We measured the levels of the proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 and the anti-inflammatory cytokine IL-10, as well as IL-1ra and sTNFR-I

and -II, in serum. All measurements for patients and healthy controls were performed simultaneously.

Normal distribution of data was checked for each variable. Results are expressed as means  $\pm$  standard errors of the means (SEM). Data obtained for patients were compared by the unpaired Student's  $t$  test or its nonparametric equivalent, the Mann-Whitney U test. For comparisons of data from the same group of patients, but from different times, we used repeated-measures analysis of variance. Categorical data were analyzed by the  $\chi^2$  test. A  $P$  value of  $\leq 0.05$  was considered statistically significant.

## RESULTS

**Clinical outcome.** All 58 patients entered in the study suffered from severe sepsis, including at least one organ dysfunction, as defined previously. On randomization, 20 patients (34.48%) had hypotension, 17 patients (29.31%) were oliguric, 18 patients (31.03%) had coagulation disorders, 14 patients (24.14%) had hypoxemia, 10 patients (17.24%) had liver dysfunction, 9 patients (15.52%) had central nervous system dysfunction, and 7 patients 12.07% had lactic acidosis.

Eighteen patients (31.03%) had urinary tract infections, 17 (29.31%) had intra-abdominal infections, 11 (18.97%) had respiratory tract infections, 3 (5.17%) had infections due to intravenous lines, and 9 (15.52%) had bacteremia of unknown origin. Twenty-eight patients (46.55%) had gram-negative bacteremia, 20 (34.48%) had cultures from other sites, and for the remaining 10 patients (17.24%), the diagnosis of infection was based on clinical grounds. Pathogens isolated were *Escherichia coli* for 20 patients, *Pseudomonas aeruginosa* for 12 patients, *Proteus* spp. for 9 patients, and *Klebsiella pneumoniae* for 7 patients.

Twelve patients (20.69%) developed septic shock, and 5 of them (41.67%) died. A total of eight patients (13.79%) died during their hospital stay. One patient succumbed from an acute pulmonary embolism 30 h after the initiation of treatment, while the rest died after the completion of the 48-h study period. The prevalences of septic shock and death were similar in the two study groups (21.4 and 14.3% in the ceftazidime group and 20.0 and 13.3% in the ciprofloxacin group, respectively). There were no differences in the surgical management of patients with intra-abdominal infections between the ciprofloxacin and the ceftazidime group. Neither the presence of bacteremia nor the site of infection had an impact on the final outcome, while changes in cytokine levels or the IL-10/TNF ratio from baseline to 24 h did not bear any relationship to survival. Finally, no differences in clinical outcome were noted between the two groups, irrespective of the concomitant administration of vancomycin.

There were no differences in demographic characteristics, clinical data, or SAPS-II scores between the two study groups. The clinical and bacteriological data of the patients are shown in Table 1.

**Immunological assessment.** All septic patients had detectable concentrations of all cytokines measured, while in healthy individuals serum cytokine levels were very low or undetectable, as shown in Table 2.

No differences in baseline serum levels of either pro- or anti-inflammatory cytokines were noted between patients with positive versus negative blood cultures (Table 3). In the ciprofloxacin-treated group, a significant decrease in serum IL-6 levels at 48 h was detected ( $P < 0.01$ ). Although levels of all

TABLE 1. Clinical and bacteriological data of the patients

Variable	Value for group	
	Ciprofloxacin (n = 30)	Ceftazidime (n = 28)
Age (yr)	63.54 ± 13.24	59.16 ± 8.75
Sex (male/female)	14/16	18/10
SAPS-II score	49.8 ± 11.3	43.2 ± 9.2
Bacteriology		
Bacteremia	15	13
Other microbiologically documented infection	9	11
Clinically diagnosed infection	6	4
Pathogens		
<i>E. coli</i>	11	9
<i>P. aeruginosa</i>	5	7
<i>Proteus</i> spp.	4	5
<i>K. pneumoniae</i>	4	3
Source of infection		
Urinary tract	10	8
Intra-abdominal	8	9
Respiratory tract	6	5
Intravenous catheter	2	1
Unidentified	4	5
Septic shock	6 (20%)	6 (21.4%)
Outcome (survivors/nonsurvivors [mortality])	26/4 (13.3%)	24/4 (14.3%)

proinflammatory cytokines were decreased at 24 and 48 h after treatment in this group, the difference was not statistically significant. With regard to the anti-inflammatory molecules, we detected a significant decrease in IL-10 levels from 24 to 48 h, while levels of all anti-inflammatory cytokines were rather increased at 24 h after treatment and then decreased to below-baseline levels. Interestingly, although neither the decrease in serum TNF-α levels nor the increase in serum IL-10 levels at 24 h was statistically significant, they resulted in a significant increase in the IL-10/TNF-α ratio, from 2.28 ± 0.21 at admission to 3.17 ± 0.36 at 24 h (*P* < 0.01). The ratio returned to baseline levels (2.36 ± 0.11) at 48 h after treatment initiation.

In the ceftazidime-treated group, although the levels of the proinflammatory cytokines were modestly increased, while those of the anti-inflammatory molecules were slightly decreased or unchanged at 24 and 48 h, no statistically significant differences were noted in the cytokine levels or the IL-10/TNF-α ratio.

When we compared the two groups at all time points, we found significantly higher serum TNF-α levels at 24 h in the ceftazidime group than in the ciprofloxacin group (114.6 ± 10.3 versus 61.9 ± 9.7 pg/ml; *P* < 0.01). We also detected significantly higher serum IL-6 levels in the ceftazidime group than in the ciprofloxacin group at both 24 and 48 h (144.3 ± 37.7 versus 85.2 ± 17.8 pg/ml at 24 h and 164.3 ± 21.2 versus 52.9 ± 9.4 pg/ml at 48 h; *P* < 0.01). Significantly lower IL-10/TNF-α ratios were observed in the ceftazidime group than in the ciprofloxacin group at both 24 h (1.83 ± 0.22 versus 3.17 ± 0.36; *P* < 0.001) and 48 h (1.58 ± 0.13 versus 2.36 ± 0.11; *P* < 0.01). Serum cytokine levels and IL-10/TNF-α ratios at all time points are shown in Table 2. No differences were noted between septic patients with positive and negative blood cultures at any time point (data not shown).

In a complementary analysis, which included all patients with positive blood culture who received ciprofloxacin or cefta-

zidime irrespective of susceptibility, we also detected significantly lower IL-10/TNF-α ratios in the ceftazidime group at both 24 h (1.92 ± 0.24 versus 2.99 ± 0.26; *P* < 0.01) and 48 h (1.65 ± 0.30 versus 2.62 ± 0.31; *P* < 0.01).

We then divided our patients according to baseline serum TNF-α levels. The low baseline TNF-α group had baseline TNF-α levels of <100 pg/ml (22 patients under ciprofloxacin and 19 patients under ceftazidime), and the high baseline TNF-α group had baseline TNF-α levels of >100 pg/ml (8 patients under ciprofloxacin and 9 patients under ceftazidime). We set a high level (>100 pg/ml) of TNF-α as a threshold in order to investigate the immunomodulatory effect of ciprofloxacin in a subpopulation of high-risk septic patients who experience an uncontrolled proinflammatory response. There is evidence in the literature that for such patients, mean serum TNF-α levels are high, usually >100 pg/ml (11, 26). Baseline

TABLE 2. Levels of pro- and anti-inflammatory cytokines at admission and 48 h after initiation of antimicrobial treatment

Variable	Value for group (mean ± SEM)		
	Healthy controls (n = 10)	Ciprofloxacin (n = 30)	Ceftazidime (n = 28)
<b>Proinflammatory cytokines (pg/ml)</b>			
<b>TNF-α</b>			
On admission	≤5.35	73.6 ± 11.3	83.3 ± 13.1
24 h		61.9 ± 9.7	114.6 ± 10.3 <sup>a</sup>
48 h		48.4 ± 7.8	91.5 ± 9.4 <sup>a</sup>
<b>IL-6</b>			
On admission	≤9.2	179.3 ± 33.9	158.1 ± 21.6
24 h		85.2 ± 17.8 <sup>b</sup>	144.3 ± 37.7
48 h		52.9 ± 9.4 <sup>c</sup>	164.3 ± 21.2 <sup>a</sup>
<b>IL-1β</b>			
On admission	≤14.8	18.9 ± 5.4	21.3 ± 6.2
24 h		16.3 ± 3.9	24.2 ± 8.2
48 h		12.7 ± 2.9	19.3 ± 5.5
<b>IL-8</b>			
On admission		167.5 ± 51.8	144.9 ± 26.2
24 h		186.1 ± 53.6	165.3 ± 28.9
48 h		118.7 ± 19.4	127.7 ± 31.7
<b>Anti-inflammatory cytokines (pg/ml)</b>			
<b>IL-10</b>			
On admission	≤9.2	174.9 ± 36.8	211.3 ± 32.1
24 h		192.7 ± 14.4	167.3 ± 24.9
48 h		114.5 ± 11.8 <sup>d</sup>	148.6 ± 17.3
<b>IL-1ra</b>			
On admission	892.3 ± 44.1	5,743.7 ± 521.9	4,810.4 ± 798.2
24 h		7,986.5 ± 482.2	5,329.6 ± 891.3
48 h		4,762.4 ± 379.8	4,228.3 ± 662.1
<b>sTNFR-I</b>			
On admission	522.7 ± 58.2	2,561.8 ± 353.4	3,120.7 ± 321.8
24 h		3,167.9 ± 431.2	3,849.6 ± 482.9
48 h		1,983.5 ± 277.5	2,939.6 ± 338.1
<b>sTNFR-II</b>			
On admission	1,188.9 ± 128.4	5,296.4 ± 607.2	6,302.5 ± 772.9
24 h		7,753.7 ± 557.1	6,967.8 ± 536.6
48 h		3,391.5 ± 429.7 <sup>d</sup>	4,982.1 ± 558.2
<b>IL-10/TNF-α ratio</b>			
On admission		2.28 ± 0.21	2.43 ± 0.28
24 h		3.17 ± 0.36 <sup>b</sup>	1.83 ± 0.22 <sup>a</sup>
48 h		2.36 ± 0.11	1.58 ± 0.13 <sup>a</sup>

<sup>a</sup> *P* < 0.01 for the ceftazidime versus the ciprofloxacin group.

<sup>b</sup> *P* < 0.05 for ciprofloxacin on admission versus 24 h.

<sup>c</sup> *P* < 0.01 for ciprofloxacin on admission versus 48 h.

<sup>d</sup> *P* < 0.01 for ciprofloxacin at 24 versus 48 h.

TABLE 3. Baseline levels of pro- and anti-inflammatory cytokines upon admission in patients with positive or negative blood cultures

Patient group	Mean level of pro-inflammatory cytokine $\pm$ SEM (pg/ml)				Mean level of anti-inflammatory cytokine $\pm$ SEM (pg/ml)				Mean IL-10/TNF ratio $\pm$ SEM
	TNF- $\alpha$	IL-6	IL-1 $\beta$	IL-8	IL-10	IL-1ra	sTNFR-I	sTNFR-II	
Positive cultures	79.9 $\pm$ 10.8	187.6 $\pm$ 25.2	17.8 $\pm$ 4.0	149.8 $\pm$ 50.2	201.7 $\pm$ 27.8	5,352.8 $\pm$ 467.7	2,964.4 $\pm$ 361.2	5,458.4 $\pm$ 468.2	2.29 $\pm$ 0.29
Negative cultures	74.7 $\pm$ 11.2	175.4 $\pm$ 19.3	22.5 $\pm$ 5.4	156.3 $\pm$ 44.6	185.2 $\pm$ 31.4	5,667.4 $\pm$ 601.1	2,648.6 $\pm$ 322.3	6,069.5 $\pm$ 593.9	2.38 $\pm$ 0.27

IL-10/TNF- $\alpha$  ratios were significantly lower ( $P < 0.01$ ) in the high baseline TNF- $\alpha$  group. No statistically significant differences in the IL-10/TNF- $\alpha$  ratio were observed in the low baseline TNF- $\alpha$  group at any time point. In contrast, a very significant increase was noted for patients in the high TNF- $\alpha$  group who were treated with ciprofloxacin compared to those treated with ceftazidime at 24 h ( $4.98 \pm 0.38$  versus  $0.92 \pm 0.07$  pg/ml;  $P < 0.001$ ), due to a significant ( $P < 0.01$ ) decrease in serum TNF- $\alpha$  levels and an increase ( $P < 0.05$ ) in serum IL-10 levels in the ciprofloxacin group (Table 4 and Fig. 1). This is of potential interest, because a future, rapid determination of TNF- $\alpha$  levels may assist in the choice of optimum antimicrobial therapy or other immunomodulating agents in the treatment of severe sepsis. Although we found that the IL-10 or TNF- $\alpha$  levels make no difference in outcome for our patients, further studies including large numbers of patients in different sepsis stages (e.g., sepsis, severe sepsis, septic shock, or organ failure) are needed to confirm any clinical impact of cytokine levels.

Finally, multivariate analysis including age, sex, SAPS-II score, antibiotic use, requirement for alpha-agonist, and baseline serum pro- and anti-inflammatory cytokine levels with respect to the IL-10/TNF- $\alpha$  ratio at 24 and 48 h revealed low baseline serum TNF- $\alpha$  levels (relative risk [RR], 1.62; 95% confidence interval [95% CI], 1.38 to 1.99;  $P < 0.01$ ) and IL-6 levels (RR, 1.48; 95% CI, 1.32 to 1.94;  $P < 0.05$ ) and use of ciprofloxacin (RR, 2.31; 95% CI, 1.65 to 2.87;  $P < 0.01$ ) as the only independent risk factors for a decrease in the IL-10/TNF- $\alpha$  ratio at 24 h but not at 48 h.

## DISCUSSION

It is widely accepted that the sepsis syndrome has a bimodal nature and is characterized by a primary, rather brief proinflammatory phase, described as the "systemic inflammatory response syndrome" (SIRS), followed by a sustained anti-inflammatory phase, termed the "compensatory anti-inflammatory response syndrome" (CARS). SIRS is presumed to be driven by the release of proinflammatory cytokines, mainly TNF- $\alpha$ , IL-6, and IL-1, while many of the components of CARS may be attributed to the biological effects of the anti-inflammatory cytokines, such as IL-10, IL-1ra, sTNFR-I, and sTNFR-II (2). High levels of TNF- $\alpha$ , IL-6, IL-8, and IL-1 $\beta$  have been associated with a compromised prognosis for septic patients, while other investigators have demonstrated that persistently high levels in serum rather than the absolute value are most important. Subsequent studies indicated that anti-inflammatory molecules were produced in even greater amounts during the sepsis syndrome and that their levels in serum could also be related to the severity of the disease and to the final outcome (3, 29). It seems that the balance between pro- and counterinflammatory agents in the septic state determines the severity of the systemic response to infection and that the excessive production of both may exhibit deleterious effects. The ratio of serum IL-10 levels to serum TNF- $\alpha$  levels has been studied as an indicator of the inflammatory response in sepsis syndrome and of the severity of the disease (22, 29).

The effects of antimicrobial agents on endotoxin release and subsequently on the release of inflammatory agents, mainly

TABLE 4. TNF- $\alpha$  and IL-10 levels and IL-10/TNF- $\alpha$  ratio at admission and 48 h after initiation of antimicrobial treatment

Group <sup>a</sup> and time	Mean cytokine level $\pm$ SEM (pg/ml)				IL-10/TNF- $\alpha$ ratio (mean $\pm$ SEM)	
	TNF- $\alpha$		IL-10		Ciprofloxacin group	Ceftazidime group
	Ciprofloxacin group	Ceftazidime group	Ciprofloxacin group	Ceftazidime group		
<b>Low-baseline TNF-<math>\alpha</math> group</b>						
On admission	57.9 $\pm$ 6.3	66.5 $\pm$ 8.6	123.2 $\pm$ 27.4	158.5 $\pm$ 20.8	2.15 $\pm$ 0.44 <sup>b</sup>	2.48 $\pm$ 0.30 <sup>b</sup>
24 h	41.5 $\pm$ 4.8	71.9 $\pm$ 7.2	94.8 $\pm$ 14.2	149.4 $\pm$ 19.3	2.29 $\pm$ 0.51	2.04 $\pm$ 0.28
48 h	24.7 $\pm$ 3.8	49.2 $\pm$ 5.1 <sup>c</sup>	49.3 $\pm$ 9.7 <sup>d</sup>	82.6 $\pm$ 13.4 <sup>e</sup>	2.17 $\pm$ 0.36	1.73 $\pm$ 0.22
<b>High-baseline TNF-<math>\alpha</math> group</b>						
On admission	124.7 $\pm$ 16.8	131.4 $\pm$ 20.5	157.6 $\pm$ 17.9	164.7 $\pm$ 21.2	1.26 $\pm$ 0.11	1.19 $\pm$ 0.10
24 h	49.8 $\pm$ 6.4 <sup>d</sup>	159.7 $\pm$ 23.2 <sup>e</sup>	248.3 $\pm$ 14.6 <sup>f</sup>	141.6 $\pm$ 16.9 <sup>e</sup>	4.98 $\pm$ 0.38 <sup>g</sup>	0.92 $\pm$ 0.07 <sup>h</sup>
48 h	37.8 $\pm$ 4.9 <sup>d</sup>	85.9 $\pm$ 12.6 <sup>e</sup>	88.7 $\pm$ 12.3	138.2 $\pm$ 15.1	2.29 $\pm$ 0.26	1.66 $\pm$ 0.17

<sup>a</sup> Low baseline,  $<100$  pg of TNF- $\alpha$ /ml; high baseline,  $>100$  pg of TNF- $\alpha$ /ml.

<sup>b</sup>  $P < 0.01$  for the low- versus the high-baseline TNF- $\alpha$  group.

<sup>c</sup>  $P < 0.01$  for the ceftazidime versus the ciprofloxacin group.

<sup>d</sup>  $P < 0.01$  for ciprofloxacin on admission versus 24 h.

<sup>e</sup>  $P < 0.05$  for ceftazidime versus ciprofloxacin.

<sup>f</sup>  $P < 0.05$  for ciprofloxacin on admission versus 48 h.

<sup>g</sup>  $P < 0.001$  for ciprofloxacin on admission versus 24 h.

<sup>h</sup>  $P < 0.001$  for ceftazidime versus ciprofloxacin.

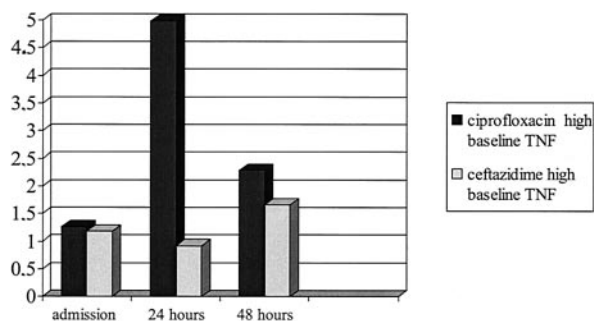


FIG. 1. IL-10/TNF- $\alpha$  ratios in the two treatment groups of patients whose baseline serum TNF- $\alpha$  concentrations were high (>100 pg/ml).

cytokines, have been evaluated in diverse *in vivo* and *in vitro* experimental models (9, 27). Antibiotics may have different effects on the release of LPS and the production of cytokines by human peripheral blood monocytes and lymphocytes. The *in vitro* effect of an antibiotic on endotoxin release may depend on the class of the antibiotic, the type of organism, the site of antibiotic action, and the Gram stain. Among the  $\beta$ -lactam antibiotics, PBP-2-specific drugs such as imipenem induce a much lower level of endotoxin release than PBP-3-specific cephalosporins such as ceftazidime. Since 1975 it has been known that antibiotic administration may induce the release of endotoxin from bacteria retained on intravenous inline filters (23). Changes in endotoxin concentrations as a result of antimicrobial treatment have been examined mainly in animal models of sepsis or meningitis (19), but this phenomenon has been investigated only in a limited number of clinical trials. Increased concentrations of endotoxin have been observed in patients with septic shock after different antibiotic regimens, such as imipenem, cefotaxime, cefuroxime, or aztreonam (8), while other investigators have failed to demonstrate antibiotic-induced endotoxemia in sepsis caused by gram-negative bacteria (18). In a recent study, ceftazidime was shown to induce significantly higher systemic endotoxin concentrations in patients with severe melioidosis than imipenem (25), but this finding was not confirmed by other studies (21).

Several studies have shown that some antibiotics may induce a differential release of cytokines, with the theoretical potential to either accelerate or down-regulate cytokine-induced organ dysfunction or septic shock. Ampicillin-sulbactam and cefamandole seem to induce gamma interferon production, clindamycin seems to induce TNF- $\alpha$  and IL-6 release, lincomycin induces IL-4 release, and teicoplanin induces TNF- $\alpha$ , IL-1 $\alpha$ , and IL-6 release (10, 12, 28). Conversely, erythromycin, roxithromycin, and vancomycin have been shown to down-regulate TNF- $\alpha$  production (13). In a recent study, conducted with patients with infections caused by gram-negative bacteria, antibiotic-induced endotoxemia was detectable in 9 of 27 patients, while both ceftazidime- and imipenem-treated individuals showed increases in TNF- $\alpha$  and IL-6 levels (4). In another study including patients with nosocomial pneumonia, it was shown that although patients had high concentrations of LPS, IL-6, and TNF- $\alpha$  in plasma, antimicrobial therapy with either ceftazidime or imipenem did not significantly modify these concentrations (17).

While *in vitro* studies on the effect of ciprofloxacin on en-

dotoxin release are conflicting (13), such studies have clearly demonstrated that the fluoroquinolones exhibit several immunomodulatory actions, mainly affecting cellular and humoral immunity by attenuating cytokine response. In general, although they seem to induce *in vitro* IL-2 synthesis, they inhibit the synthesis of IL-1, IL-6, TNF- $\alpha$ , and IL-12 and significantly enhance the synthesis of IL-10 and colony-stimulating factors. Mechanisms explaining their immunomodulatory effects include the following: (a) an effect on intracellular cyclic adenosine-3'-5'-monophosphate and phosphodiesterases, (b) an effect on NF- $\kappa$ B, and (c) a triggering effect on the eukaryotic equivalent of the bacterial SOS response (7). More specifically, ciprofloxacin has been found to protect mice from LPS-induced death by decreasing TNF- $\alpha$  and IL-12 concentrations and by increasing IL-10 concentrations (16). The immunomodulatory effects of quinolones seem to be triggered by several stimulants, including LPS, irradiation, cytotoxic agents, and phytohemagglutinin. Therefore, besides their antimicrobial effect, they may have an additional immunomodulating effect in sepsis caused by gram-negative bacteria. However, the pathogenesis of the septic syndrome is rather complex, involving a great variety of inflammatory molecules and coagulation factors, and therefore, the correlation between antimicrobial agent-induced release of cytokines and outcome is not clear.

In order to evaluate the differential effects of ciprofloxacin and ceftazidime on the pro- and anti-inflammatory cytokine profile, we organized our study so as to enroll a homogenous patient population, including previously healthy patients with severe sepsis due to gram-negative infections. In the whole population of our patients, a slight increase in the proinflammatory response and a parallel decrease in the anti-inflammatory response was observed in the ciprofloxacin group, as characterized by an increase in the IL-10/TNF- $\alpha$  ratio 24 h after the initiation of antimicrobial therapy. No such differences were noted in the ceftazidime group. The observed anti-inflammatory effect of ciprofloxacin appeared rather early: it was observed at 24 but not at 48 h after treatment initiation. This can be further emphasized by the persistence of this observation irrespective of the presence of bacteremia with resistant pathogens.

Most importantly, when we studied our patients according to baseline serum TNF- $\alpha$  levels, we detected a very significant increase in the IL-10/TNF- $\alpha$  ratio in patients with high (>100 pg/ml) baseline TNF- $\alpha$  levels, due to a significant decrease in serum TNF- $\alpha$  levels and an increase in serum IL-10 levels at 24 h after the initiation of treatment with ciprofloxacin. These changes were corrected at 48 h of treatment. In contrast, in the ceftazidime group, the IL-10/TNF- $\alpha$  ratio and the cytokine levels were unchanged at all time points, while serum TNF- $\alpha$  levels were significantly higher, and serum IL-10 levels and the IL-10/TNF- $\alpha$  ratio were significantly lower, than those in the ciprofloxacin group at 24 h. It seems, therefore, that there is an early (at 24 h of therapy) immunomodulating effect of ciprofloxacin in severe sepsis, especially if there is massive production of TNF- $\alpha$ , which is rapidly reversed. Although this effect does not seem to be clinically relevant, since the outcomes of our patients in the two groups were similar, further studies including large number of patients with severe sepsis are required to exploit fully the potential of the immunomodulatory

effect of fluoroquinolones in the case of sepsis syndrome, especially during the proinflammatory phase of the disease.

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