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Interleukin-6, Gamma Interferon, and Tumor Necrosis Factor Receptors in Typhoid Fever Related to Outcome of Antimicrobial Therapy

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To study mechanisms of antibiotic effects in typhoid fever, levels of interleukin-6 (IL-6), gamma interferon (IFN-γ), and cytokine receptors (tumor necrosis factor receptor [TNF-R] p55 and TNF-R p75) were measured in the plasma of 29 adult Nepalese with culture-positive typhoid fever before therapy and on days 4 and 15 after start of therapy with either ceftriaxone at 2 g/day for 3 days or chloramphenicol at 50 mg/kg of body weight per day for 14 days. Bacteriologic cure was defined as blood cultures testing negative on days 4 and 15 after start of therapy; clinical cure was defined as symptomatic improvement within 5 days after start of therapy and absence of relapse. Clinical and bacteriologic cures occurred in 24 patients. There were two clinical failures, two patients who failed to complete therapy because of leukopenia, and one relapse. Mean levels before therapy were elevated compared with those in healthy controls (IL-6, 11.4 pg/ml; IFN-γ, 1.3 ng/ml; TNF-R p55, 3.8 ng/ml; and TNF-R p75, 6.1 ng/ml) and fell progressively during and after therapy. For six patients (three in each treatment group) who showed prolonged fever (>5 days) or relapse, mean levels of IL-6 and TNF-R p55 before therapy (29.5 pg/ml and 6.1 ng/ml, respectively) and on day 4 (17.7 pg/ml and 4.0 ng/ml) were significantly greater than corresponding means for 23 patients who showed early defervescence (on admission, 6.7 pg/ml and 3.3 ng/ml, and on day 4, 1.8 pg/ml and 2.7 ng/ml, $P < .05$). These results indicate that the concentrations of plasma cytokines and their receptors are elevated in typhoid fever and that these concentrations can be useful in predicting outcome.

Chloramphenicol has remained the drug of choice for typhoid fever for more than 35 years because no newer antimicrobial drug has been shown to give better or more consistent clinical improvement at a comparable cost. In vitro resistance to chloramphenicol in *Salmonella typhi* occurs (6, 14) but has not become prevalent in most areas where this organism is endemic (7). Chloramphenicol, on the other hand, is not an ideal treatment for typhoid fever because (i) treatment does not prevent fecal carriage of *S. typhi*, relapses after the end of therapy, or the complications of intestinal perforation and bleeding; (ii) residual mortality occurs during therapy; (iii) reversible bone marrow suppression develops and aplastic anemia, though rare, is a risk; and (iv) a long (14-day) course of treatment requires dosing four times a day. Shorter courses of chloramphenicol are not advised because relapses occur in 10 to 20% of treated cases 1 to 2 weeks after the end of therapy.

Ceftriaxone is a newer cephalosporin antibiotic with good in vitro activity as shown by MICs of 0.05 μg/ml against most tested strains of *Salmonella* (15). Its prolonged serum half-life of 8 h and biliary excretion permit less frequent dosing of enteric infections (2, 17). The use of ceftriaxone in mouse typhoid showed a good therapeutic effect (1), and an open trial in 14 patients with typhoid fever in Singapore produced cures in 13 patients (18). Randomized ceftriaxone trials in Bangladesh (ceftriaxone once daily for 7 days) (11) and in the Philippines (ceftriaxone once daily for 3 days) (13) showed results comparable to those with chloramphenicol. In Taiwan, ceftriaxone was used for only 2 to 3 days and showed satisfactory results (5).

In clinical trials, drugs have been compared for rates of clinical cure and bacteriologic cure. Clinical cures are based on resolution of fever and other symptoms; bacteriologic cures are based on negative blood cultures during or after therapy. Levels of the cytokines tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6 are elevated in the blood of patients with typhoid fever (16). Because disease severity in meningococcemia (19) and gram-negative sepsis (20) correlates with concentrations of IL-6 in blood, measurement of cytokines may offer a new dimension in the assessment of the clinical efficacy of antimicrobial therapy.

MATERIALS AND METHODS

Patient selection and randomization. Adults, both males and females, ≥18 years old with fever for ≥4 days were screened for inclusion. The presence of two or more of the following features was required for inclusion: a temperature of ≥38°C, diarrhea, splenomegaly, hepatomegaly, and rose spots. Exclusion criteria were effective antimicrobial therapy administered during the preceding 2 weeks, allergy to chloramphenicol or cephalosporins, or the presence of complicated disease such as shock, coma, rectal bleeding, or suspected intestinal perforation. After patients gave written informed consent, they were randomized to either ceftriaxone or chloramphenicol therapy by opening a sealed envelope containing the name of the treatment drug, which was assigned from a table of random numbers. Only data for

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patients with blood or stool cultures positive for *S. typhi* or *Salmonella paratyphi* A were utilized in data analysis.

Clinical assessment of patients and bacteriologic investigations. Histories were taken and physical examinations were performed before the start of therapy. Oral temperatures, pulse rates, and blood pressures were recorded at 8-h intervals. Changes in symptoms and signs were recorded at least twice daily until the end of 14 days in the hospital. On admission, blood, stool, and urine samples were cultured by standard methods. Identification was based on colony appearances and reactions in MacConkey agar, triple sugar iron slant, and motility medium and agglutinations with *Salmonella* O antiserum for factor 9 (group D) and group A (Difco Laboratories, Detroit, Mich.). Strains of *S. typhi* and *S. paratyphi* A were tested for antimicrobial susceptibility by agar diffusion. Ceftriaxone discs (F. Hoffmann-La Roche and Co. Ltd., Basel, Switzerland) containing 30 μg of antibiotic were used, and a clear zone >16 mm in diameter indicated susceptibility. Blood cultures were repeated on hospital days 4 and 15. Stool cultures were repeated on days 7, 15, and 21 after the start of therapy. On day 21, patients returned to be examined for evidence of relapse.

Assessment of treatment. Patients were considered clinically cured if they became afebrile without a major complication (perforation or bleeding) and without the need to change antibiotic therapy and if they did not experience relapse after discharge. A bacteriologic cure was defined as blood cultures testing negative for *S. typhi* or *S. paratyphi* A on days 14 and 15 after the start of therapy. The first afebrile day was defined as the first day the patient's oral temperature fell to <38.0°C and remained there for at least 48 h. Relapse was defined as return of symptoms with isolation of *S. typhi* or *S. paratyphi* A in the blood within 2 months after the start of therapy. Patients were instructed to report to the hospital if fever recurred after discharge from the hospital.

Therapy. Patients randomized to receive ceftriaxone were given 2 g of ceftriaxone by intravenous infusion over 30 min once daily for 3 consecutive days. Patients randomized to receive chloramphenicol were given capsules (Parke-Davis) orally at a dosage of 50 mg/kg of body weight (divided into four doses) per day for 14 days. Results of the complete clinical trial will be reported separately (9a).

Cytokines and cytokine receptors. Venous blood samples were collected into tubes containing sodium EDTA and aprotinin (final concentration, 0.5 trypsin inhibitor unit/ml) before treatment and on days 4 and 15 after start of treatment. After thorough mixing, the blood was immediately centrifuged at 4°C and the plasma was frozen at −20°C. Plasma was transported on dry ice to Basel, Switzerland, for assays. IL-6 and gamma interferon (IFN-γ) levels were measured by enzyme-linked immunoassay. The detection limit was 5 pg/ml for IL-6 and 0.05 ng/ml for IFN-γ. The soluble TNF receptor (TNF-R) assays to measure TNF-R p55 and TNF-R p75 were designed to detect only functionally active receptor fragments by using enzyme-labeled recombinant human TNF as the revealing agent. The simultaneous presence of <10 ng of TNF per ml of plasma did not influence the results, indicating that free as well as reversibly occupied soluble TNF-R molecules were detected. Microtiter plates (Immuno Plate, Maxisorp F 96; Nunc, Roskilde, Denmark) were coated with monoclonal mouse antibodies, clone utr-4 against TNF-R p55 and clone htr-20 against TNF-R p75. For determination of levels of soluble TNF-Rs, the samples and standards (0 to 5 ng/ml) were incubated in the coated microtiter plates (immunologic binding). At the same time, recombinant TNF-horseradish peroxidase conjugate was added to the test solution (biologic binding). After a washing, the amount of peroxidase bound to the microtiter plate was measured enzymatically. The detection limit is 0.1 ng/ml for both soluble TNF-Rs (12).

TABLE 1. Characteristics of patients before treatment in two treatment groups

Characteristic	Value for group	
	Ceftriaxone (n = 15)	Chloramphenicol (n = 14)
Age (yr, mean ± SD)	23.4 ± 5.9	24.2 ± 7.6
No. of males/no. of females	15/0	13/1
Duration of fever before admission (days, mean ± SD)	9.7 ± 10.4	10.4 ± 8.8
Temperature (°C, mean ± SD)	39.0 ± 0.8	39.5 ± 0.5
No. of cases with blood cultures positive for:		
S. typhi	9	14
S. paratyphi A	6	0
No. of blood isolates susceptible to:		
Ceftriaxone	15	14
Chloramphenicol	14	14

RESULTS

Patients. After randomization to the two treatment groups, the patients were comparable in regard to age, male/female ratio, duration of fever before admission, and temperature (Table 1). All patients treated with chloramphenicol were infected with *S. typhi*, whereas 6 of 15 patients treated with ceftriaxone were infected with *S. paratyphi* A. All isolates were susceptible in vitro to the antibiotic initially used. One isolate from patients treated with ceftriaxone was resistant to chloramphenicol.

Outcome of therapy. Clinical cures without relapse or the need for change of treatment occurred in 12 patients (80%) in the group treated with ceftriaxone and in 12 patients (86%) in the group treated with chloramphenicol ($P > 0.05$ by the Fisher exact test). In patients treated with ceftriaxone, two showed no improvement in 4 to 5 days and were subsequently treated with chloramphenicol or amoxicillin. One patient infected with *S. paratyphi* A and treated with ceftriaxone responded well with defervescence on day 7 and had negative blood cultures on days 4 and 15 but experienced a relapse on day 21 with return of fever and bacteremia. In the group treated with chloramphenicol, treatment for two patients was changed to amoxicillin, one on day 5 and one on day 9 after start of therapy, because leukopenia was detected. When blood cultures were repeated on days 4 and 15 after start of therapy, all were negative for growth. Stool cultures for *Salmonella* organisms were positive before therapy in only one case, and all stool cultures obtained on days 15 and 21 after start of therapy gave negative results.

Cytokines and cytokine receptors in serum. Mean concentrations of IL-6, IFN-γ, TNF-R p55, and TNF-R p75 in plasma were elevated above the normal ranges in these patients before therapy (Table 2). Repeated measurements on day 4 after start of therapy showed that mean values had fallen, with a significant decrease occurring in the mean concentration of TNF-R p55 ($P < 0.05$). Measurements on day 15 after start of therapy showed further decreases in mean values to near the normal ranges. The changes in mean concentrations of all the cytokines and receptors from the

TABLE 2. Concentrations of cytokines and cytokine receptors in plasma in 29 patients with typhoid fever[a]

Type of value	IL-6 (pg/ml)	IFN-γ (ng/ml)	TNF-R p55 (ng/ml)	TNF-R p75 (ng/ml)
Normal	<5	0–0.5	1.2–2.0	0.2–1.4
Obtained before treatment	11.4 ± 18.0	1.3 ± 1.0	3.8 ± 1.7	6.1 ± 2.8
Obtained on day 4 after start of treatment	5.1 ± 8.8	0.9 ± 0.9	2.9 ± 1.3*	5.2 ± 3.3
Obtained on day 15 after start of treatment	0.5 ± 2.4*	0.6 ± 0.6*	1.8 ± 0.7*	2.7 ± 1.4*

[a] Values are means ± standard deviations. *, mean is significantly lower than that obtained before treatment ($P < 0.05$).

mean values before therapy were statistically significant ($P < 0.05$).

A comparison of mean levels of cytokines and cytokine receptors between the groups of patients treated with ceftriaxone and chloramphenicol was undertaken. At the sampling time before therapy and on days 4 and 15 after start of therapy, there were no significant differences between respective mean values in the two treatment groups ($P > 0.05$, data not shown).

To examine whether concentrations of cytokines and cytokine receptors in plasma might correlate with clinical responses to antibiotic treatment, measurements in samples from 23 patients who were cured with prompt defervescence (within 5 days) were compared with measurements in samples from 6 patients (3 in each treatment group) who failed to improve (with fever lasting beyond 5 days) or who had a relapse (Table 3). Before therapy, mean concentrations of IL-6 and TNF-R p55 were significantly higher in the six patients with prolonged fevers or relapse ($P < 0.05$). Measurements made on day 4 after start of therapy showed higher mean concentrations of IL-6, TNF-R p55, and TNF-R p75 in the six patients with prolonged fevers or relapse ($P < 0.05$).

DISCUSSION

In this comparison of chloramphenicol and ceftriaxone for the treatment of culture-proven typhoid fever, both antibiotics produced clinical and bacteriologic cures in most patients. Rates of clinical failure, including relapse, and of drug toxicity requiring change of therapy were low in both groups without statistically significant differences between the treatment groups ($P > 0.05$). The short course of once-daily ceftriaxone for 3 days used in this trial confirms the results of Chi-kin et al. (5) and Lasserre et al. (13) that short courses of ceftriaxone are clinically effective against typhoid fever. Other studies of typhoid fever in Pakistan (14) and South Africa (6) also indicated that cephalosporins are useful alternatives to chloramphenicol when patients are infected with bacterial strains resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole.

The cytokines, including TNF and IL-6, are important mediators of signs and symptoms of infectious diseases (8, 12). In our patients with typhoid fever, mean concentrations of cytokines (IL-6 and IFN-γ) and cytokine receptors (TNF-R p55 and TNF-R p75) in plasma were elevated before therapy and fell during and after therapy. Levels of the cytokines TNF, IL-1, and IL-6 were previously reported by Roine et al. (16) to be significantly elevated in Chilean children with typhoid fever. The mean concentration of IL-6 reported by Roine et al. (16) of 2,392 pg/ml was much greater than the mean concentration of 11.4 pg/ml in our patients. The reason for this disparity could be the fact that all our patients were adults, the lesser clinical severity of our cases, or differences in laboratory methodology.

We reasoned that measurement of cytokines on day 4 after start of therapy, when most patients were clinically improved, might be more discriminating than body temperature measurements for identifying patients with clinical failure and for comparing effectiveness of antimicrobial therapy. No significant differences in mean levels of cytokines and cytokine receptors between the two antibiotic treatments were detected before therapy or on day 4 or day 15 after start of therapy. However, when data for the two groups of patients were combined, mean concentrations of plasma cytokines and cytokine receptors were significantly greater before treatment and on day 4 after start of treatment in six patients with fevers lasting longer than 5 days or with relapse than in patients who showed earlier defervescence. Thus, cytokines and their receptors may have predictive value for clinical outcome of treatment in typhoid fever.

When serum IL-6 concentrations in our patients infected with *S. typhi* and *S. paratyphi* A were compared with results of Waage et al. (19) obtained with patients with meningococcemia, our levels of IL-6 were much lower. None of our patients had >100 pg of IL-6 per ml, whereas Waage et al. (19) reported means of 200 pg/ml in patients with meningitis and 189 ng/ml in patients with shock. Similarly, in gram-negative sepsis, Wortel et al. (20) reported that most patients had >100 pg of IL-6 per ml and that concentrations of >100 pg/ml were associated with high mortality. None of our patients died. The lower IL-6 concentrations in our patients are consistent with an overall mortality in typhoid that is less than 5% (3). Patients with typhoid fever complicated by coma, shock, intestinal perforation, or pneumonia show case fatality rates greater than 10% (3, 10) and might be expected

TABLE 3. Concentrations[a] of cytokines and cytokine receptors in plasma in typhoid fever patients

Patient group[b]	IL-6 (pg/ml)		IFN-γ (ng/ml)		TNF-R p55 (ng/ml)		TNF-R p75 (ng/ml)	
	Before treatment	On day 4	Before treatment	On day 4	Before treatment	On day 4	Before treatment	On day 4
Afebrile ($n = 23$)	6.7 ± 7.8	1.8 ± 4.8	1.2 ± 1.0	0.9 ± 1.0	3.3 ± 1.0	2.7 ± 0.8	5.7 ± 2.4	4.5 ± 1.8
Febrile ($n = 6$)	29.5 ± 32.4*	17.7 ± 9.7*	1.8 ± 0.9	0.9 ± 0.5	6.1 ± 4.2*	4.0 ± 2.4*	7.9 ± 3.9	8.0 ± 5.8*

[a] All values are means ± standard deviations. *, mean is significantly greater than corresponding value for afebrile patients ($P < 0.05$).
[b] Afebrile patients were those with no fever by day 5 and no relapse. Febrile patients were those with prolonged fever (beyond day 5), including one relapse.

to show higher concentrations of IL-6 in serum than our patients.

Concentrations of gamma interferon (IFN-γ) in plasma in our patients were higher than normal but did not show the same correlation with prolonged fever and relapse that IL-6 did. In studies with patients with meningococcemia, Girardin et al. (9) reported higher levels of IFN-γ in patients who died, but in studies with patients with septic shock reported by Calandra et al. (4), there was no significant correlation between serum IFN-γ concentration and outcome.

Concentrations of TNF-Rs in plasma were elevated in our patients with typhoid fever, and an increased mean concentration of TNF-R p55 in plasma before therapy and at 4 days after start of therapy was associated with prolonged fever or relapse. Thus, our patients with typhoid fever resemble malaria patients, in whom higher concentrations of TNF-Rs in serum were associated with severe disease (12). The concentrations of TNF-Rs in our patients were comparable to those found in patients with uncomplicated malaria (12). Although the role of TNF-Rs in disease remains unclear, they are capable of binding TNF and may protect patients against the harmful effects of TNF. Thus, a future therapeutic role for recombinant TNF-Rs in typhoid fever should be considered.

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