INFECTION AND IMMUNITY, Nov. 1994, p. 4997–5002
0019-9567/94/$04.00+0

Vol. 62, No. 11

# Beneficial Effects of Interleukin-6 in Neonatal Mouse Models of Group B Streptococcal Disease

GIUSEPPE MANCUSO,[1] FRANCESCO TOMASELLO,[1] MARIA MIGLIARDO,[1] DEMETRIO DELFINO,[1] JOEL COCHRAN,[2] JAMES A. COOK,[3] AND GIUSEPPE TETI[1]*

*Istituto di Microbiologia, Facoltà di Medicina e Chirurgia, Università degli Studi di Messina, I-98122 Messina, Italy,[1] and Departments of Pediatrics[2] and Physiology,[3] Medical University of South Carolina, Charleston, South Carolina 29425*

Received 13 June 1994/Returned for modification 29 July 1994/Accepted 18 August 1994

**Previous studies have shown that tumor necrosis factor alpha (TNF-α) plays a pathophysiologic role in sepsis induced in rat pups by group B streptococci (GBS). In this model, TNF-α is also partially responsible for the induction of interleukin-6 (IL-6). The present study was undertaken to investigate the role of IL-6 in neonatal BALB/c mice infected with type III GBS. The effect of anti-IL-6 monoclonal antibodies and recombinant IL-6 on lethality and TNF-α production was investigated. In mouse pups infected with GBS strain COH1, plasma IL-6 reached levels of 3,067 ± 955 and 1,923 ± 891 U/ml when measured at 22 and 48 h, respectively ($P < 0.05$ compared with uninfected controls). Pretreatment with 25 μg of anti-IL-6 antibodies totally prevented the increase in circulating IL-6 bioactivity at both 22 and 48 h after infection ($P < 0.05$). Treatment with anti-IL-6 also induced a moderate decrease in survival time of mice infected with lethal doses of strains COH1 and COH31, as evidenced by increased lethality ($P < 0.05$) at 24 to 48 h but not at 96 h. Mouse recombinant IL-6 (12,500 U) given 6 h before challenge with strains COH1 and COH31 consistently increased survival time, as evidenced by decreased ($P < 0.05$) lethality at 48 to 72 h but not at 96 h. The effects of IL-6 pretreatment were dose dependent, since no protection was observed with doses lower than 12,500 U. In addition, no effects on lethality were noted when IL-6 was given at the time of challenge or at later times. TNF-α elevations ($P < 0.05$ compared with uninfected controls) were measured at 12, 22, and 48 h after challenge with strain COH1 (68 ± 28, 233 ± 98, and 98 ± 34 U, respectively). Pretreatment with IL-6 significantly ($P < 0.05$) decreased plasma TNF-α levels at 12 and 22 h, with 55 and 69% inhibitions, respectively. Anti-IL-6 had an opposite effect, as evidenced by a 145% increase ($P < 0.05$) in TNF-α levels at 48 h after challenge. Collectively, our data are compatible with the hypothesis that IL-6 is involved in negative feedback regulation of plasma TNF-α levels in experimental GBS sepsis. In this model, IL-6 pretreatment can increase survival time. Future studies will be needed to investigate the mechanisms underlying this effect.**

Interleukin 6 (IL-6) is one of the major mediators of the immune system's acute responses to any infectious or inflammatory stimulation (34). This cytokine is produced by both lymphoid and nonlymphoid cells and has been shown to regulate proliferation and differentiation of T (17, 30) and B (16) lymphocytes and hematopoietic stem cells (6, 41). In addition, IL-6 is a major mediator of the acute-phase response, mediating the synthesis and release of a variety of acute-phase proteins in the liver (11, 24).

The role of IL-6 in various infectious processes is not yet well understood. Elevated levels of IL-6 are present in serum and tissues during experimental infections of mice with *Listeria monocytogenes* (15), mycobacteria, and brucellae (28). Endotoxins are known to activate IL-6 synthesis (9, 23), and the levels of IL-6 and other cytokines such as tumor necrosis factor alpha (TNF-α) and IL-1 are elevated in septic adults (14) and infants (7, 13, 22).

In some gram-positive and gram-negative bacterial sepsis models, lethality and pathophysiologic changes are mediated by TNF-α (10, 33) and IL-1 (2, 25). Less is known about the role of IL-6 in septic shock models. Administration of anti-IL-6 monoclonal antibody produced divergent effects in two gram-negative shock models. Anti-IL-6 protected mice against a lethal *Escherichia coli* challenge, suggesting a pathophysiologic role for this cytokine (29). However, anti-IL-6 enhanced endotoxin-induced lethality in galactosamine-sensitized mice when TNF-α activity was partially blocked by limited doses of anti-TNF-α (3).

We have previously documented that TNF-α plays a pathophysiologic role in sepsis induced in rat pups by group B streptococci (GBS), a major human neonatal pathogen (19, 31). In this model, plasma levels of TNF-α and other cytokines, including IL-6, correlate with sepsis severity. In addition, TNF-α is at least partially responsible for the induction of IL-6, as evidenced by a reduction in circulating IL-6 bioactivity following neutralization of TNF-α by specific antibodies (31). The present study was undertaken to investigate the role of IL-6 in experimental GBS sepsis. The effect of anti-IL-6 antibodies and recombinant IL-6 (rIL-6) on lethality and TNF-α production in septic neonatal mice was investigated. Neonatal mice rather than rats were used because of the wider availability of mouse-specific reagents and the opportunity to use smaller quantities of these reagents. Previous studies have shown that GBS infections in neonatal mice and rats have a similar clinical course, histopathologic features, (26), and cytokine release (32).

## MATERIALS AND METHODS

**Neonatal mice.** Neonatal (24 to 48 h old) BALB/c mice were used. Parental mice were obtained from Charles River Italia

* Corresponding author. Mailing address: Istituto di Microbiologia, Piazza XX Settembre, 4, I-98122 Messina, Italy. Phone: 39-90-712110/672508. Fax: 39-90-719910.

(Calco, Italy). Pups from each litter were randomly assigned to control or experimental groups, marked, and kept with the mother.

**Bacteria.** GBS strains COH1 and COH31, originally isolated from septic neonates, were kindly provided by Craigh Rubens, University of Washington, Seattle (20). Strain COH1 produces higher amounts of type III capsular polysaccharide and is more virulent than strain COH31 in animals (19, 20, 32). Bacteria were grown to the late logarithmic phase in Todd-Hewitt broth (Difco, Diagnostic International Distribution, Milano, Italy) and diluted to the appropriate concentration in phosphate-buffered saline (PBS; 0.01 M phosphate, 0.15 M NaCl [pH 7.2]) before inoculation into neonatal mice.

**Sepsis model.** Mouse pups received subcutaneous injections of viable GBS resuspended in 25 μl of PBS. Inocula were adjusted to give bacterial numbers corresponding to one or five times the 90% lethal dose ($LD_{90}$). The $LD_{90}$s were $1.5 \times 10^2$ and $1 \times 10^5$ CFU, respectively, for strains COH1 and COH31, confirming the higher virulence of the former strain. Mortality was assessed every 12 h. With this model, deaths rarely occurred after 96 h. GBS were confirmed as the cause of death by reisolating the bacteria from blood.

**Administration of rIL-6 and anticytokine antibodies.** Mouse rIL-6 (specific activity, 100 U/ng), rat anti-mouse IL-6 monoclonal antibodies (MAb), and rabbit anti-mouse TNF-α serum were purchased from Genzyme (Cinisello Balsamo, Italy). IL-6 and anti-IL-6 were given neat or diluted in PBS supplemented with 0.1% bovine serum albumin intraperitoneally in 25- or 50-μl volumes at different times before challenge with viable or killed bacteria. Control animals received an equal amount of vehicle. In some experiments, anti-TNF-α (Genzyme) or normal rabbit serum (25 μl) was given intraperitoneally 6 h before challenge.

**TNF-α and IL-6 measurements.** To measure circulating levels of TNF-α and IL-6, groups of 10 animals were killed by decapitation under ether anesthesia at different times after challenge with viable bacteria. Mixed venous-arterial blood was collected in heparinized containers and centrifuged after saving 10 μl for colony counts. These were performed by standard pour plate methods. Pooled plasma from two animals (50 to 70 μl) was stored at −70°C until assayed for TNF-α and IL-6 activity by cytotoxicity with L929 murine fibroblasts, and B9 proliferation assays, respectively, as previously described (31).

TNF-α activity was expressed in units per milliliter, 1 U being defined as the amount of cytokine causing 50% lysis of L929 cells. Six serial twofold dilutions (final dilutions, 1:20 to 1:640) were tested in duplicate for each sample. The assay was calibrated by using murine recombinant-TNF-α (specific activity, 20 U/ng; Genzyme) as a standard. TNF-α activity in selected plasma samples was totally inhibited by a 1:100 dilution of rabbit anti-murine TNF-α serum but not by normal rabbit serum.

IL-6 activity was also expressed in units per milliliter, 1 U being defined as the amount of IL-6 causing 50% maximal proliferation of B9 cells. Nine serial twofold dilutions (from 1:20 to 1:5,120) were tested in duplicate for each sample. The assay was calibrated by using murine rIL-6 as a standard.

**Data expression and statistical analysis.** TNF and IL-6 levels are expressed as means ± standard deviations for five independent observations. Each observation was conducted on pooled samples from two animals. To calculate mean values, results below the detection level (20 U/ml) were assigned a theoretical value of 10 U/ml. Differences in plasma cytokine levels were assessed by one-way analysis of variance and the Student-Newman-Keuls test. Differences in lethality were assessed by the two-tailed Fisher exact test. With both tests, differences were considered significant when $P$ values were $<0.05$.

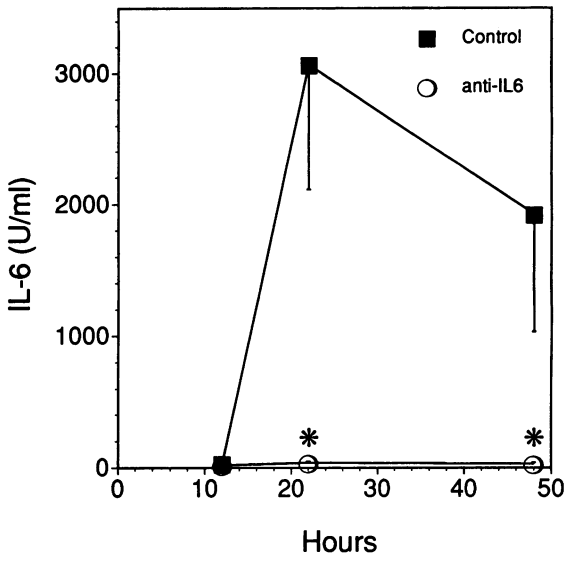


FIG. 1. Circulating IL-6 levels in neonatal mice infected with 5 $LD_{90}$s of type III GBS strain COH1. Plasma was collected at various times after challenge from pups (10 per group) pretreated intraperitoneally with vehicle (■) or 25 μg of anti-mouse IL-6 MAb (○) at 6 h before challenge. Points and bars represent means ± standard deviations for five observations. Each observation was conducted on pooled plasma samples from two pups. *, significantly ($P < 0.05$) different from vehicle controls by one-way analysis of variance and the Student-Newman-Keuls test.

## RESULTS

**Effects of anti-IL-6 antibodies.** Figure 1 depicts plasma IL-6 levels in neonatal mice pretreated with 25 μg of anti-IL-6 MAb and in vehicle-treated controls at 12, 22, and 48 h after challenge with 5 $LD_{90}$s of strain COH1. Plasma IL-6 values were 19 ± 9 U/ml ($n = 10$) in uninfected controls (not shown). In infected controls, IL-6 levels were significantly increased to 3,067 ± 955 and 1,923 ± 891 U/ml ($P < 0.05$ compared with uninfected controls) when measured at 22 and 48 hours, respectively, as observed previously in neonatal rats (31). Pretreatment with anti-IL-6 MAb totally prevented the increase in circulating IL-6 bioactivity at both 22 and 48 h after infection ($P < 0.05$) (Fig. 1).

In further experiments, the effect on lethality of the same anti-IL-6 treatment was investigated. To verify whether consistent results would be obtained using different bacterial numbers as a challenge, mouse pups were infected with 1 and 5 $LD_{90}$s of two strains with different degrees of virulence (see Materials and Methods). Anti-IL-6 MAb induced a moderate decrease in survival time, as evidenced by increased lethality ($P < 0.05$) at 24 to 48 h but not at 96 h. No significant effect was observed with the 1 $LD_{90}$ dose of the less virulent strain (Fig. 2, top right).

**Effects of rIL-6 on lethality.** Since anti-IL-6 tended to accelerate lethality in this model, we investigated whether administration of rIL-6 would be beneficial. Mouse pups were treated with various doses of mouse rIL-6 at 6 h before challenge with 5 $LD_{90}$s of GBS strain COH1 (Table 1). Pretreatment with 12,500 or 25,000 U per pup increased

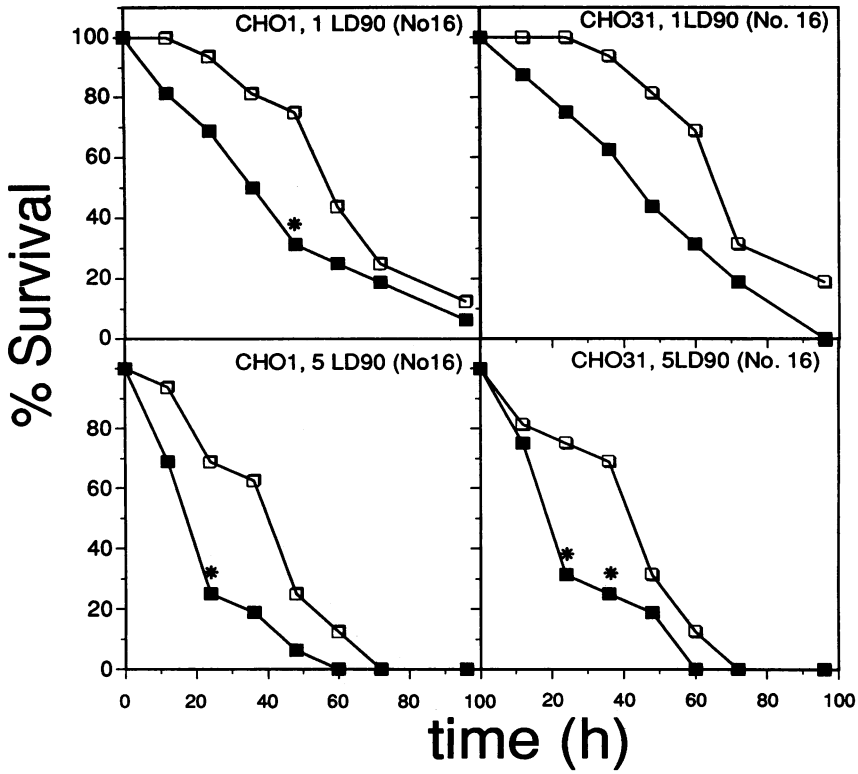


FIG. 2. Effect of anti-mouse IL-6 antibodies on survival of GBS-infected neonatal mice. Pups (16 per group) received vehicle (□) or 25 μg of anti-mouse IL-6 MAb (■) at 6 h before challenge with 1 or 5 $LD_{90}$s of type III GBS strains COH1 and COH31. *, significantly ($P < 0.05$) different from vehicle controls by Fisher exact test.

survival time, as evidenced by decreased ($P < 0.05$) lethality at 48 and 72 h. However, this effect was no longer significant at 96 h after infection. The effects of rIL-6 were dose dependent, since no protection was observed with doses lower than 12,500 U per pup (Table 1). Similar results were obtained when strain COH31 was used as the challenge strain (data not shown).

The time of IL-6 pretreatment relative to challenge was also critical (Table 2). No effects were noted when IL-6 was given at the time of challenge or at later times, while the cytokine was effective when given prophylactically at 6 or 18 h before challenge.

**Effect of IL-6 and anti-IL-6 on plasma TNF-α levels and CFU.** Because IL-6 can modulate TNF-α production (1), the effects of IL-6 or anti-IL-6 on circulating TNF-α levels in septic pups were investigated. Plasma TNF-α concentrations were below the detection limit in uninfected and baseline controls ($n = 10$; not shown). Figure 3 shows TNF-α levels in control and treated mice at various times after infection with 5 $LD_{90}$s of strain COH1. TNF-α elevations ($P < 0.05$ compared with baseline controls) were measured at 12, 22, and 48 h after GBS challenge in untreated animals (68 ± 28, 233 ± 98, and 98 ± 34 U/ml, respectively). Pretreatment with IL-6 significantly ($P < 0.05$) decreased TNF-α levels at 12 and 22 h by 55 and

TABLE 1. Effects of various doses of IL-6 on GBS-induced lethality[a]

| Expt no. | IL-6 dose (U/mouse) | No. (%) of mice dead | | | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h |
| 1 | 0 (vehicle) | 3 (21) | 10 (71) | 14 (100) | 14 (100) |
| | 6,250 | 2 (14) | 4 (29) | 10 (71) | 14 (100) |
| | 12,500 | 1 (7) | 2 (14)[b] | 6 (43)[b] | 11 (79) |
| 2 | 0 (vehicle) | 4 (29) | 9 (64) | 14 (100) | 14 (100) |
| | 3,125 | 3 (21) | 11 (79) | 12 (86) | 14 (100) |
| | 781 | 2 (14) | 8 (57) | 14 (100) | 14 (100) |
| 3 | 0 (vehicle) | 2 (14) | 10 (71) | 12 (86) | 14 (100) |
| | 12,500 | 0 (0) | 1 (7)[b] | 3 (21)[b] | 9 (64) |
| | 25,000 | 0 (0) | 3 (21)[b] | 3 (21)[b] | 10 (71) |

[a] Mouse pups (14 per group) were challenged with 5 $LD_{90}$s of strain COH1.
[b] $P < 0.05$ by Fisher exact test, compared with the respective vehicle controls.

TABLE 2. Effect of time of administration of IL-6 on GBS-induced lethality[a]

| Time of administration[b] (h) | No. (%) of mice dead | | | |
|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h |
| Expt 1 | | | | |
| —(vehicle) | 4 (29) | 9 (64) | 13 (93) | 14 (100) |
| −18 | 1 (7) | 2 (14)[c] | 2 (14)[c] | 11 (79) |
| −6 h | 0 (0) | 2 (14)[c] | 4 (29)[c] | 10 (71) |
| Expt 2 | | | | |
| —(vehicle) | 2 (14) | 8 (57) | 12 (86) | 14 (100) |
| 0 | 3 (21) | 6 (43) | 10 (71) | 12 (86) |
| +24 | 2 (14) | 7 (50) | 9 (64) | 13 (93) |

[a] Mouse pups (14 per group) were challenged with 5 $LD_{90}$s of strain COH1.
[b] Relative to time of challenge.
[c] $P < 0.05$ by Fisher exact test, compared with the respective vehicle controls.

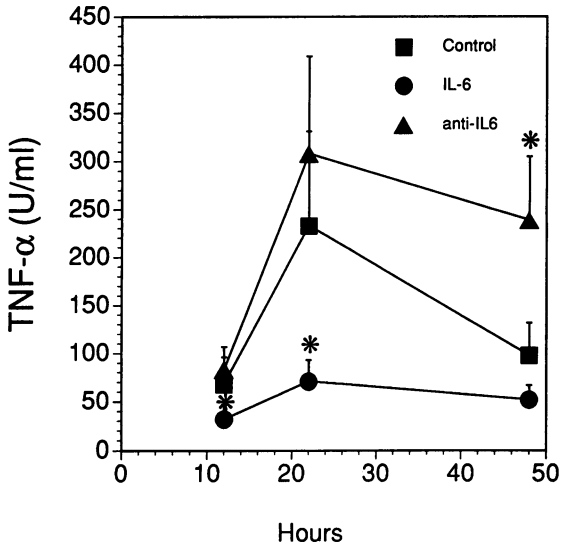


FIG. 3. Plasma TNF-α levels in neonatal mice infected with 5 $LD_{90}$s of type III GBS strain COH1. Plasma was collected at the indicated times after challenge from pups (10 per group) pretreated with vehicle (■), 12,500 U of rIL-6 (●), or 25 μg of anti-mouse IL-6 MAb (▲) at 6 h before challenge. Points and bars represent means ± standard deviations for five observations. Each observation was conducted on pooled plasma samples from two pups. *, significantly ($P < 0.05$) different from vehicle controls by one-way analysis of variance and the Student-Newman-Keuls test.

69%, respectively. Anti-IL-6 had an opposite effect, as evidenced by a 145% increase ($P < 0.05$) in TNF-α levels at 48 h after challenge.

Since experimental abrogation of TNF-α activity was beneficial in septic rat pups (31), the effects on lethality of IL-6 were compared with those of anti-TNF-α. Table 3 shows that rIL-6 and anti-TNF-α had similar effects in increasing survival at 48 and 72 h ($P < 0.05$). However, combined treatment with these agents did not afford any further protection over treatment with IL-6 or anti-TNF-α alone (Table 3).

CFU counts performed on blood samples obtained from animals treated with IL-6 (12,500 U) or anti-IL-6 (25 μg) 6 h before challenge with 5 $LD_{90}$s of strain COH1 did not reveal significant differences among the experimental groups (data not shown).

TABLE 3. Effect of combined treatment with IL-6 and anti-TNF-α on GBS-induced lethality[a]

| Pretreatment | No. (%) of mice dead | | | |
|---|---|---|---|---|
| | 24 h | 48 h | 72 h | 96 h |
| Expt 1 | | | | |
| Normal rabbit serum | 3 (21) | 9 (64) | 14 (100) | 14 (100) |
| Anti-TNF-α | 0 (0) | 1 (7)[b] | 5 (36)[b] | 10 (71) |
| Anti-TNF-α + IL-6 | 0 (0) | 0 (0)[b] | 6 (43)[b] | 9 (64) |
| Expt 2 | | | | |
| Vehicle | 4 (29) | 8 (57) | 13 (93) | 14 (100) |
| IL-6 | 1 (7) | 2 (14)[b] | 4 (29)[b] | 12 (86) |
| Anti-TNF-α + IL-6 | 0 (0) | 1 (7)[b] | 3 (21)[b] | 9 (64) |

[a] Mouse pups (14 per group) received treatments 6 h before challenge with 5 $LD_{90}$s of strain COH1.

[b] $P < 0.05$ by Fisher exact test, compared with the respective vehicle controls.

## DISCUSSION

Cytokine mediators may play major roles in the pathophysiology of septic shock induced by either gram-negative or gram-positive bacteria. While TNF-α has a protective role in localized infections and in diseases caused by intracellular bacteria, high circulating TNF-α and IL-1 levels can produce detrimental inflammatory and hemodynamic responses, leading to multiorgan failure and death (36). The role of IL-6 in septic shock is highly controversial. Because IL-6 decreases TNF-α production (1) and the expression of TNF receptors in macrophages (4), it can be considered mainly anti-inflammatory. In addition, IL-6 is the main inducer of hepatic acute-phase proteins, many of which may bind and inactivate proinflammatory mediators, thereby mitigating the course of septic shock (35, 37). IL-6 also induces adrenocorticotropin and, in turn, cortisol, a potent anti-inflammatory hormone (40).

The effects of experimental abrogation of endogenous IL-6 with specific antibodies varied in two gram-negative sepsis models (3, 29). Although in both of these models lethality could be totally prevented by anti-TNF-α antibodies, anti-IL-6 protected mice against lethal challenges with *E. coli* or rTNF-α (29) but had no effect in galactosamine-sensitized mice challenged with endotoxin (3). In the latter study, anti-IL-6 and IL-6 enhanced and decreased, respectively, lethality when TNF-α was partially neutralized by limited anti-TNF-α doses.

Little is known about the role of IL-6 in neonatal or gram-positive sepsis. Since anti-TNF-α antibodies or blocking compounds are beneficial in animal models of GBS disease (12, 31), we hypothesized that modulating TNF-α production by the administration of rIL-6 or anti-IL-6 would affect lethality. Results show that neutralization of endogenous IL-6 can result in a moderate decrease in survival time of neonatal mice rendered septic with GBS. This effect was associated with increased TNF-α levels at 48 h after challenge. Conversely, exogenous IL-6 markedly reduced TNF-α levels and prolonged survival. Since TNF-α is involved in IL-6 induction (31), our data support the hypothesis that IL-6 participates in a negative feedback loop regulating TNF-α levels during GBS sepsis. This is in agreement with observations in adult mice infected with *E. coli* (29). In that model, anti-IL-6 increased TNF-α levels, while anti-TNF-α decreased IL-6 levels.

Monocytes from very small premature (42) but not term (21, 27, 43) infants show defective production of IL-6 in response to GBS and other pathogens. The production of TNF-α was also reported to be lower in premature neonates (8, 38). However, mixed mononuclear cell from term neonates were recently shown to produce more TNF-α than those from adults in response to GBS (39). Thus, the possibility that altered regulation of TNF-α and other inflammatory mediators plays a role in the pathophysiology of neonatal sepsis should be considered in future studies.

Since IL-6 has pleiotropic effects on a number of different cell types, our data cannot exclude the possibility that mechanisms other than down regulation of TNF-α may contribute to or even be predominant in the effects of IL-6 in our model. The induction of an acute-phase response by low doses of rIL-6 did not alter the lethality of galactosamine-sensitized or normal mice challenged with endotoxin (5). Since, in the present study, the minimal effective dose of IL-6 was approximately 16-fold greater than that employed to induce the acute-phase response in adult (5) and neonatal (32) mice, it is unlikely that the latter effect of IL-6 contributed to increased survival. Further studies will be needed to assess whether IL-6-induced modulation of cortisol and acute-phase protein responses plays a role in GBS sepsis.

IL-6 pretreatment has been shown to prime host defenses against infection with the facultative intracellular pathogen *L. monocytogenes*, resulting in decreased bacterial numbers in the spleen and liver (18). As in the present study, timing of administration relative to challenge was crucial, as evidenced by lack of effects when the cytokine was given at the time of or after infection. In our study, no differences in bacterial numbers were noted between IL-6-treated and control mice. This may relate to differences in the defense mechanisms against facultative intracellular and encapsulated bacteria or in the time course of the infections. In the *Listeria* study, the more significant differences were observed at 4 days after infection, a time at which lethality approaches 100% in the GBS model. It should be noted, however, that the doses used in the *Listeria* study were at least 20-fold higher than the ones presently used. Thus, it cannot be excluded that IL-6 at high doses can increase host defenses against GBS as well.

Future studies will be needed to assess whether IL-6 can prove effective in the therapy of GBS diseases in conjunction with antibiotics, supportive measures, and, possibly, other anti-inflammatory agents.

## ACKNOWLEDGMENTS

This study was supported in part by the following grants: NIH GM27673, "40%" and "60%" of the Ministero dell' Università e della Ricerca Scientifica e Tecnologica, and Target Project "Biotechnology and Bioinstrumentation" of the Consiglio Nazionale delle Ricerche of Italy.

## REFERENCES

1. **Aderka, D., J. M. Le, and J. Vilcek.** 1989. IL-6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. J. Immunol. **143:**3517–3523.
2. **Aiura, K., J. A. Gelfand, J. F. Burke, R. C. Thompson, and C. A. Dinarello.** 1993. Interleukin-1 (IL-1) receptor antagonist prevents *Staphylococcus epidermidis*-induced hypotension and reduces circulating levels of tumor necrosis factor and IL-1 beta in rabbits. Infect. Immun. **61:**3342–3350.
3. **Barton, B. E., and J. V. Jackson.** 1993. Protective role of interleukin-6 in the lipopolysaccharide-galactosamine septic shock model. Infect. Immun. **61:**1496–1499.
4. **Bermudez, L. E., M. Wu, M. Petrofsky, and L. S. Young.** 1992. Interleukin-6 antagonizes tumor necrosis factor-mediated mycobacteriostatic and mycobactericidal activities in macrophages. Infect. Immun. **60:**4245–4252.
5. **Bucklin, S. E., R. Silverstein, and D. C. Morrison.** 1993. An interleukin-6-induced acute-phase response does not confer protection against lipopolysaccharide lethality. Infect. Immun. **61:** 3184–3189.
6. **Caracciolo, D., S. C. Clark, and G. Rovera.** 1989. Human interleukin-6 supports granulocytic differentiation of hematopoietic progenitor cells and acts synergistically with GM-CSF. Blood **73:**666–670.
7. **de Bont, E., A. Martens, J. van Raan, G. Samson, W. P. Fetter, A. Okken, and L. H. F. M. de Leij.** 1993. Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 plasma levels in neonatal sepsis. Pediatr. Res. **33:**380–383.
8. **English, B. K., S. K., Burchett, J. D. English, A. J. Ammann, D. W. Wara, and C. B. Wilson.** 1988. Production of lymphotoxin and tumor necrosis factor by human neonatal mononuclear cells. Pediatr. Res. **24:**717–722.
9. **Fong, Y., L. L. Moldawer, M. Marano, H. Wei, S. B. Tatter, R. H. Clarick, U. Santhanam, D. Sherris, L. T. May, P. B. Sehgal, and S. F. Lowry.** 1989. Endotoxemia elicits increased circulating beta 2-IFN/IL-6 in man. J. Immunol. **142:**2321–2324.
10. **Freudenberg, M. A., and C. Galanos.** 1991. Tumor necrosis factor alpha mediates lethal activity of killed gram-negative and gram-positive bacteria in D-galactosamine-treated mice. Infect. Immun. **59:**2110–2115.
11. **Gauldie, J., C. Richards, D. Harnish, P. Lansdorp, and H. Baumann.** 1987. Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc. Natl. Acad. Sci. USA **84:**7251–7255.
12. **Gibson, R. L., G. J. Redding, W. R. Henderson, and W. E. Truog.** 1991. Group B streptococcus induces tumor necrosis factor in neonatal piglets. Effect of the tumor necrosis factor inhibitor pentoxifylline on hemodynamics and gas exchange. Am. Rev. Respir. Dis. **143:**598–604.
13. **Girardin, E., G. E. Grau, J. M. Dayer, L. P. Roux, and P. H. Lambert.** 1988. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. N. Engl. J. Med. **319:**397–400.
14. **Hack, C. E., G. E. De, B. R. Felt, J. H. Nuijens, V. S. R. Strack, B. A. Eerenberg, L. G. Thijs, and L. A. Aarden.** 1989. Increased plasma levels of interleukin-6 in sepsis. Blood **74:**1704–1710.
15. **Havell, E. A., and P. B. Sehgal.** 1991. Tumor necrosis factor-independent IL-6 production during murine listeriosis. J. Immunol. **146:**756–761.
16. **Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, et al.** 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature (London) **324:**73–76.
17. **Houssiau, F. A., P. G. Coulie, D. Olive, and S. J. Van.** 1988. Synergistic activation of human T cells by interleukin 1 and interleukin 6. Eur. J. Immunol. **18:**653–656.
18. **Liu, Z., R. J. Simpson, and C. Cheers.** 1992. Recombinant interleukin-6 protects mice against experimental bacterial infection. Infect. Immun. **60:**4402–4406.
19. **Mancuso, G., F. Tomasello, C. von Hunolstein, G. Orefici, and G. Teti.** 1994. Induction of tumor necrosis factor alpha by the group- and type-specific polysaccharides from type III group B streptococci. Infect. Immun. **62:**2748–2753.
20. **Marques, M. B., D. L. Kasper, M. K. Pangburn, and M. R. Wessels.** 1992. Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci. Infect. Immun. **60:**3986–3993.
21. **Matsuzaki, N., F. Saji, T. Kameda, K. Yoshizaki, T. Okada, K. Sawai, and O. Tanizawa.** 1990. In vitro and in vivo production of interleukin-6 by fetal mononuclear cells. Clin. Immunol. Immunopathol. **55:**305–314.
22. **Miller, L. C., S. Isa, P. G. Lo, J. G. Schaller, and C. A. Dinarello.** 1990. Neonatal interleukin-1 beta, interleukin-6, and tumor necrosis factor: cord blood levels and cellular production. J. Pediatr. **117:**961–965.
23. **Morrison, D. C., and J. L. Ryan.** 1987. Endotoxins and disease mechanisms. Annu. Rev. Med. **38:**417–432.
24. **Morrone, G., G. Ciliberto, S. Oliviero, R. Arcone, L. Dente, J. Content, and R. Cortese.** 1988. Recombinant interleukin 6 regulates the transcriptional activation of a set of human acute phase genes. J. Biol. Chem. **263:**12554–12558.
25. **Ohlsson, K., P. Bjork, M. Bergenfeldt, R. Hageman, and R. C. Thompson.** 1990. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature (London) **348:**550–552.
26. **Rodewald, A. K., A. B. Onderdonk, H. B. Warren, and D. L. Kasper.** 1992. Neonatal mouse model of group B streptococcal infection. J. Infect. Dis. **166:**635–639.
27. **Saito, S., M. Saito, Y. Kato, M. Maruyama, I. Moriyama, and M. Ichijo.** 1990. Production of IL-6 (BSF-2/IFN beta 2) by mononuclear cells in premature and term infants. J. Reprod. Immunol. **17:**17–26.
28. **Saunders, B. M., Z. Liu, Y. Zhan, and C. Cheers.** 1993. Interleukin-6 production during chronic experimental infection. Immunol. Cell Biol. **71:**275–280.
29. **Starnes, H. J., M. K. Pearce, A. Tewari, J. H. Yim, J. C. Zou, and J. S. Abrams.** 1990. Anti-IL-6 monoclonal antibodies protect against lethal Escherichia coli infection and lethal tumor necrosis factor-alpha challenge in mice. J. Immunol. **145:**4185–4191.
30. **Takai, Y., G. G. Wong, S. C. Clark, S. J. Burakoff, and S. H.**

**Herrmann.** 1988. B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. J. Immunol. **140**:508–512.

31. **Teti, G., G. Mancuso, and F. Tomasello.** 1993. Cytokine appearance and effects of anti-tumor necrosis factor alpha antibodies in a neonatal rat model of group B streptococcal infection. Infect. Immun. **61**:227–235.
32. **Teti, G., G. Mancuso, and F. Tomasello.** Unpublished observations.
33. **Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and A. Cerami.** 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature (London) **330**:662–664.
34. **Van Snick, J.** 1990. Interleukin-6: an overview. Annu. Rev. Immunol. **8**:253–278.
35. **van Vugt, H., J. van Gool, and L. de Ridder.** 1986. Alpha 2 macroglobulin of the rat, and acute phase protein, mitigates the early course of endotoxin shock. Br. J. Exp. Pathol. **67**:313–319.
36. **Vassalli, P.** 1992. The pathophysiology of tumor necrosis factors. Annu. Rev. Immunol. **10**:411–452.
37. **Warren, H. S., and L. A. Chedid.** 1987. Strategies for the treatment of endotoxemia: significance of the acute-phase response. Rev. Infect. Dis. **9**(Suppl. 5):S630.
38. **Weatherstone, K. B., and E. A. Rich.** 1989. Tumor necrosis factor/cachectin and interleukin-1 secretion by cord blood monocytes from premature and term neonates. Pediatr. Res. **25**:342–346.
39. **Williams, P. A., J. F. Bohnsack, N. H. Augustine, W. K. Drummond, C. E. Rubens, and H. R. Hill.** 1993. Production of tumor necrosis factor by human cells in vitro and in vivo, induced by group B streptococci. J. Pediatr. **123**:292–300.
40. **Woloski, B. M., E. M. Smith, W. J. Meyer, G. M. Fuller, and J. E. Blalock.** 1985. Corticotropin-releasing activity of monokines. Science **230**:1035–1037.
41. **Wong, G. G., and S. C. Clark.** 1988. Multiple actions of interleukin 6 within a cytokine network. Immunol. Today **9**:137–139.
42. **Yachie, A., N. Takano, K. Ohta, T. Uehara, S. Fujita, T. Miyawaki, and N. Taniguchi.** 1992. Defective production of interleukin-6 in very small premature infants in response to bacterial pathogens. Infect. Immun. **60**:749–753.
43. **Yachie, A., N. Takano, T. Yokoi, K. Kato, Y. Kasahara, T. Miyawaki, and N. Taniguchi.** 1990. The capability of neonatal leukocytes to produce IL-6 on stimulation assessed by whole blood culture. Pediatr. Res. **27**:227–233.