INFECTION AND IMMUNITY, Dec. 1996, p. 5042–5046 Vol. 64, No. 12
0019-9567/96/$04.00+0

Roles of the Bacterial Cell Wall and Capsule in Induction of Tumor Necrosis Factor Alpha by Type III Group B Streptococci

JESUS G. VALLEJO,[1]* CAROL J. BAKER,[1,2] AND MORVEN S. EDWARDS[1]

Section of Infectious Diseases, Departments of Pediatrics,[1] and Microbiology and Immunology,[2] Baylor College of Medicine, Houston, Texas

Received 3 April 1996/Returned for modification 19 June 1996/Accepted 6 September 1996

Group B streptococci (GBS) are the major cause of sepsis and fatal shock in neonates in the United States. The precise role of tumor necrosis factor alpha (TNF-α) in the development of human GBS sepsis has not been defined; however, whole GBS have been shown to induce the production of this inflammatory cytokine. We sought to determine which bacterial cell wall components of GBS are responsible for triggering TNF-α production. Human cord blood monocytes were stimulated with encapsulated (COH1) or unencapsulated (COH1-13) whole type III GBS or with purified bacterial components, including type III capsular polysaccharide (III-PS), group B polysaccharide (GB-PS), lipoteichoic acid (LTA), or peptidoglycan (PG). Lipopolysaccharide from *Escherichia coli* served as a control. Supernatants were harvested at specific timed intervals, and TNF-α levels were measured by enzyme-linked immunosorbent assay. Monocytes exposed to COH1 and COH1-13 induced similar amounts of TNF-α. III-PS, GB-PS, LTA, and PG each induced TNF-α in a time- and concentration-dependent manner. However, TNF-α release was significantly greater after stimulation by the GB-PS or PG than after stimulation by III-PS or LTA ($P < 0.05$). Our findings indicate that GB-PS and PG are the bacterial cell wall components primarily evoking TNF-α release. These, alone or in concert with other factors, may be responsible for septic shock accompanying GBS sepsis.

Group B streptococci (GBS) are the leading cause of neonatal sepsis and meningitis in the United States. Despite advances in neonatal intensive care and early antibiotic treatment, neonatal GBS infection still carries a mortality of 5 to 10% (1). The clinical presentation of GBS infection may vary from asymptomatic bacteremia to fulminant systemic disease resembling the clinical syndrome of gram-negative sepsis (26). For gram-negative bacterial sepsis, it has been shown that the pathophysiology involves an excessive release of lipopolysaccharide (LPS)-induced cytokines, particularly tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) (8, 37). These two cytokines are believed to mediate reactions associated with clinical deterioration, multiorgan system failure, and death during endotoxic shock. Less is known regarding the cytokine-inducing effects of the cellular components of gram-positive organisms, including GBS. To date, no single mediator capable of initiating the entire cascade of inflammatory cytokines that underlie gram-positive sepsis has been identified. Nevertheless, cell walls and specific bacterial components from a variety of gram-positive organisms are known to trigger cytokine release by human monocytes (7, 13, 21, 32).

GBS infection has been shown to increase serum TNF-α levels in animal models of infection (29, 31) and in human neonates (10, 41). The precise role of TNF-α in the pathogenesis of GBS disease in human neonates has not been established. However, animal models have demonstrated its detrimental effects. In addition, the beneficial effect of antibodies directed against TNF-α in experimental GBS infection suggest that this mediator may be important in the pathogenesis of GBS sepsis (12, 31).

* Corresponding author. Mailing address: Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. Phone: (713) 798-4790. Fax: (713) 798-6407.

Although age susceptibility to infection with GBS has been attributed both to bacterial virulence factors and immaturity of the neonatal immune system (3, 4), the mechanisms by which tissue injury occurs during GBS infection are incompletely elucidated. In particular, the relative contribution of GBS bacterial components to the induction of the host inflammatory response has not been completely defined. Identifying the bacterial component(s) that induces the host immune response is important, as inflammatory mediators that are released during infection may contribute to tissue injury and deleteriously affect the clinical outcome of disease. In this study, we examined the capacity of whole type III GBS to induce TNF-α production by human neonatal monocytes and determined the relative contribution of purified type III capsular polysaccharide (III-PS), group B polysaccharide (GB-PS), lipoteichoic acid (LTA), and peptidoglycan (PG) of GBS to TNF-α production.

(This study was presented in part at the 33rd Infectious Diseases Society of America Annual Meeting, San Francisco, Calif., 17 September 1995 [35a].)

MATERIALS AND METHODS

Reagents. Ficoll was purchased from Sigma Chemical (St. Louis, Mo.), and Hypaque was purchased from Winthrop Pharmaceuticals (New York, N.Y.). RPMI 1640 (with L-glutamine and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES]), gentamicin, and sodium pyruvate were obtained from JRH Biosciences (Lexena, Kans.). Culture supplement (Nutridoma-HU) was purchased from Boehringer Mannheim (Indianapolis, Ind.), and phosphate-buffered saline (PBS) was obtained from Life Technologies GIBCO BRL (Grand Island, N.Y.).

Cell preparation. Cord blood was obtained immediately on delivery of the placenta from women delivering healthy term infants. Mononuclear cells were separated from citrated whole blood by gradient density centrifugation over Ficoll-Hypaque. The mononuclear cells obtained were washed with a solution of normal saline containing 1% (wt/vol) bovine serum albumin and 0.13% (wt/vol) EDTA. The cell concentration was adjusted to 10^7 cells per ml with PBS without calcium or magnesium. Subsequently, 0.3 ml of a cell suspension was added to medium (RPMI 1640 with L-glutamine, 25 mM HEPES, 50 μg of gentamicin per ml, 1 mM sodium pyruvate, and 1% culture supplement without serum) in

24-well tissue culture plates (Corning Glass Works, Corning, N.Y.) and allowed to adhere for 1 h at 37°C in 5% CO_2. Nonadherent cells were removed by two vigorous washes with RPMI 1640, and the final concentration in the well was adjusted to 10^6/ml, based on the calculated number of monocytes plated. The adherent cell population consisted of 85 to 88% monocytes as determined by differential counts using Neat Stain (Mid-Atlantic Biomedical, Paulsboro, N.J.). Viability, determined by trypan blue exclusion, was consistently >95%. All reagents and culture media used in monocyte isolation contained ≤0.03 endotoxin units/ml by *Limulus* amebocyte lysate assay (Associates of Cape Cod, Woods Hole, Mass.).

Bacterial strains. An encapsulated type III GBS strain (COH1) originally isolated from the blood of an infant with GBS sepsis was studied together with an unencapsulated mutant of this strain (COH1-13) (provided by Craig J. Rubens, University of Washington, Seattle). The isogenic mutant (COH1-13) was derived by transposon insertion mutagenesis as described previously (29). Bacteria were grown to log phase in Columbia broth and heat killed by incubating the organisms at 56°C for 1 h. Aliquots were stored at −70°C for later use in the experiments.

Isolation and purification of specific components of GBS. III-PS and GB-PS, purified as previously described, were provided by Dennis L. Kasper, Harvard Medical School, Boston, Mass. (23, 40). Briefly, the III-PS was purified from the culture supernatant of type III GBS. Bacterial cells were removed by using a membrane filtration system, and the culture fluid was concentrated over a 10,000-molecular-weight-cutoff-pore-size membrane (Pellicon). Crude III-PS was precipitated from supernatant fluid by bringing the solution concentration to 80% (vol/vol) ethanol. The dried precipitate was dissolved with 10 mM Tris HCl (pH 7) and treated with RNase (250,000 U) and DNase (100,000 U) and then pronase (5,000 U). Contaminating GB-PS was depolymerized by treatment of the solution with 1 N NaOH overnight at 37°C. The preparation was dialyzed and then applied to a DEAE-Sephacel column equilibrated in 10 mM Tris-HCl (pH 7.2) and eluted with a gradient of 0 to 0.2 M NaCl in the same buffer. Column fractions were tested by enzyme-linked immunosorbent assay (ELISA) inhibition using specific rabbit antiserum for the presence of III-PS. Fractions showing inhibitory activity in the type III ELISA were pooled, dialyzed against water, and lyophilized. The GB-PS was isolated from a well-characterized unencapsulated mutant strain (COH31-15) of GBS (19, 29) as described by Michon et al. (23). The procedure for isolating the GB-PS is similar to that described for the III-PS. The purified material was tested by ELISA inhibition using specific rabbit antiserum for the presence of the group-specific antigen. GBS LTA, purified as previously described, was a gift from Stephen J. Mattingly, University of Texas Health Science Center—San Antonio (20). Briefly, LTA was obtained from GBS cell pellets by extraction with 90% phenol and chloroform-methanol (2:1, vol/vol). The extracted material was then purified by DEAE-Sephacel anion-exchange chromatography. This was identified as LTA by its reaction with a monoclonal antibody to poly(glycerolphosphate) and the 1:1 glycerol-to-phosphate ratio. PG was a gift from Michael R. Wessels, Harvard Medical School, Boston, Mass. Purification of GBS PG, not previously reported, was performed as described by Peterson et al. (28) for *Staphylococcus aureus*, with some modifications. COH1-13 was grown overnight in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) at 37°C. Bacteria were harvested by centrifugation (10,000 × *g*, 20 min, 4°C) and subsequently washed in sterile deionized water containing 0.01% thimerosal. Washed organisms were mixed with glass beads and disrupted by agitation in a Vibrogen Cell Mill for 2 h in an ice bath. The glass beads were allowed to settle, and the supernatant containing lysed cells was collected and centrifuged twice (1,500 × *g* for 20 min and 48,000 × *g* for 30 min, 4°C). The crude cell walls were washed six times with sterile deionized water (0.01% thimerosal) and resuspended in 100 ml of 40 mM sodium phosphate buffer (pH 7). To this suspension, 0.2 mg each of RNase and DNase per ml were added, and cells were incubated at 37°C for 2 h with gentle shaking. To remove proteinaceous material, trypsin (1 mg/ml) was added, and the mixture was incubated for 3 h at 37°C. Pellets were washed three times with sterile deionized water (0.01% thimerosal) and resuspended in 445 ml of 50 mM Tris-HCl containing 10 mM EDTA (pH 8.5). To this, 8.9 ml of Triton X-100 (Sigma) was added, and the mixture was incubated for 1 h at 37°C. Pellets were washed six times and resuspended in 445 ml of 0.5 N NaOH to destroy residual group antigen (50°C for 48 h). After extraction, pellets were washed three times in sterile deionized water (0.01% thimerosal) and lyophilized overnight. The purity of PG as determined by amino acid analysis showed the amino acids lysine, glutamate, alanine, serine, and methionine present in the following molar ratio (based on lysine + glutamate/2 = 1.00): 0.96:1.04:2.50:0.06:0.02. These molar ratios are in agreement with those reported by De Cueninck et al. (9) for GBS PG.

Endotoxin assay. Specific components of GBS, at a concentration of 10 μg/ml, had the following levels of endotoxin as determined by gel clot assay (test performed by Associates of Cape Cod): PG, 0.06 ng/ml; III-PS, 0.2 ng/ml; GB-PS, 1.0 ng/ml; and LTA, 1.25 ng/ml.

Endotoxin. *Escherichia coli* O55:B5 LPS (Sigma) was used as a positive control at a concentration of 100 ng/ml. Polymyxin B (10 μg/ml; Sigma) was incubated with LPS in selected experiments for 1 h at 37°C before cell exposure. Because of the amounts of endotoxin present in the purified components of GBS, each was exposed to polymyxin B (10 μg/ml) in a similar fashion prior to monocyte stimulation. Incubation of LPS (100 ng/ml) in polymyxin B (10 μg/ml) decreased TNF-α production by 53 ± 16% following 24 h of incubation (data not shown).

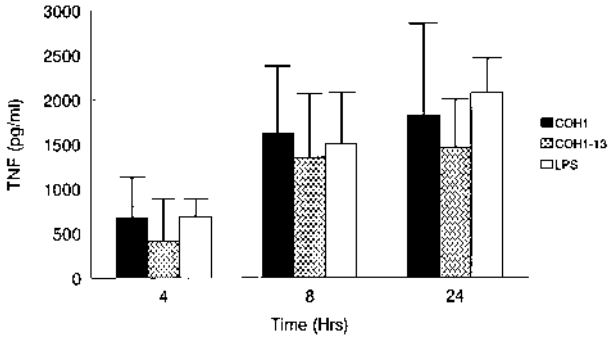

FIG. 1. TNF-α levels measured by ELISA in the supernatant of cord blood monocytes (10^6/ml) stimulated with heat killed (10^8 CFU/ml) COH1 and COH1-13. LPS served as a positive control. Means ± standard deviations of four experiments with four different donors are shown. The results shown are corrected for spontaneous TNF-α release by unstimulated cells.

Stimulation of monocytes. Whole GBS cells (10^8 CFU/ml), III-PS (10 μg/ml), GB-PS (10 μg/ml), LTA (10 μg/ml), PG (10 μg/ml), or LPS (100 ng/ml) was added to each well. Experiments comparing TNF-α production in response to the GBS parent and mutant strains were always run concurrently. Monocytes with medium alone served as controls. At 4, 8, and 24 h, culture supernatants were harvested from individual wells. All solutions and bacterial specimens used in these experiments were free of endotoxin contamination as determined by *Limulus* amebocyte lysate assay. The supernatants were aliquoted and frozen at −70°C until TNF-α determinations. The results shown are corrected for spontaneous TNF-α release by unstimulated cells.

TNF-α assay. TNF-α levels in the culture supernatants were measured with an ELISA (Genzyme, Cambridge, Mass.) according to the manufacturer's recommendations. The lower limit of detection is 15 pg/ml. All determinations were performed in duplicate.

Statistical analysis. TNF-α values are expressed as mean ± standard deviation. Analysis of variance for repeated measures was used to simultaneously evaluate the effects of treatment (specific GBS components) and time and the interactions of these variables on TNF-α production. The detection of a significant interaction was followed by comparison of treatments at each time, using one-way analysis of variance followed by a multiple-comparison procedure (Fisher's least-significant-difference test) for pairwise treatment comparisons; a P value of <0.05 was considered to indicate statistical significance.

RESULTS

Induction of TNF-α by GBS. Figure 1 shows the time course and magnitude of TNF-α production by neonatal monocytes in response to stimulation with GBS (10^8 CFU/ml). There was no significant difference in TNF-α yield by monocytes exposed to the encapsulated strain (COH1) or the isogenic unencapsulated transposon mutant strain (COH1-13). These data suggest that bacterial components capable of causing TNF-α release are present in both encapsulated and unencapsulated strains of GBS.

Induction of TNF-α by purified GBS components. Figure 2 shows the corresponding TNF-α release induced by comparable concentrations of each GBS component following 8 h of incubation. Based on these studies, all subsequent experiments were performed with a concentration of 10 μg of each bacterial component per ml. The results shown are corrected for spontaneous TNF-α release by unstimulated cells (4 h = 10 ± 22 pg/ml; 8 h = 69 ± 67 pg/ml; 24 h = 66 ± 98 pg/ml). The kinetics of TNF-α release by neonatal monocytes in response to stimulation by specific components of GBS are summarized in Fig. 3. The cell-associated GB-PS was highly effective in inducing TNF-α release by cord blood monocytes. Supernatants were collected after 4, 8, and 24 h of incubation. LPS (100 ng/ml) from *E. coli* O55:B5 was used as a control. After 8 h of incubation, TNF-α release caused by GB-PS reached 59% of the maximum TNF-α value induced by this bacterial component. At 24 h, TNF-α levels induced by GB-PS (1,842 ± 336

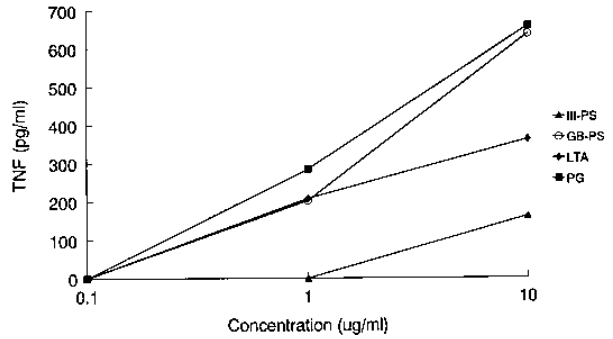

FIG. 2. Dose-response effects of specific GBS components on TNF-α production by cord blood monocytes. TNF-α was measured after 8 h of incubation at 37°C by ELISA.

pg/ml) were similar to those noted for the unencapsulated GBS mutant (1,816 ± 375 pg/ml). TNF-α release in response to GB-PS paralleled that induced by LPS (Fig. 3) at 8 and 24 h. However, the concentration of GB-PS was 100-fold greater than that of LPS.

Figure 3 also shows that purified PG (10 μg/ml) isolated from type III GBS was a potent inducer of TNF-α release by cord blood monocytes. As with the GB-PS, after 8 h of incubation, TNF-α release induced by PG reached 79% of the maximum value induced by this GBS component. While PG-induced TNF-α levels were similar to those triggered by GB-PS, the production of TNF-α was significantly different ($P < 0.05$) from that induced by III-PS or LTA.

The III-PS of GBS was a weak inducer of TNF-α release. The TNF-α levels produced in response to III-PS were significantly less ($P < 0.05$) than those induced by GB-PS (Fig. 3) at the same concentration. In addition, TNF-α release induced by III-PS was slower than that triggered by GB-PS or LPS. After 4 h of incubation, the TNF-α levels induced by GB-PS were equal to those induced by III-PS after 24 h.

Purified LTA of GBS also triggered TNF-α production by neonatal monocytes. The magnitude of the monocyte response to LTA was significantly less than that induced by GB-PS and PG at all time points ($P < 0.05$). In contrast, LTA and III-PS induced similar amounts of TNF-α at 4 and 8 h, but by 24 h LTA had induced significantly higher TNF-α levels.

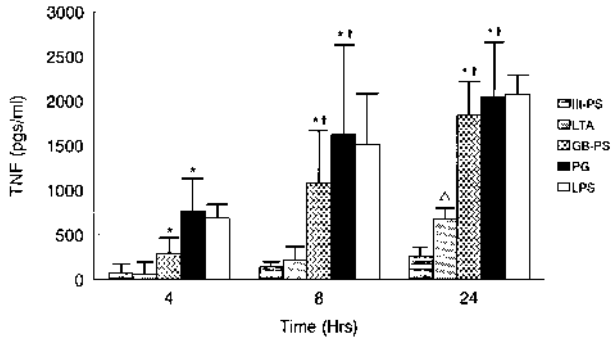

FIG. 3. Time curves of TNF-α responses by cord blood monocytes (10^6/ml) after stimulation with specific GBS components (10 μg/ml) and with LPS (100 ng/ml) from *E. coli* O55:B5. Means ± standard deviations of three to five experiments (III-PS, $n = 5$; LTA, $n = 3$; GB-PS, $n = 4$; PG, $n = 4$) with different donors are shown. The results shown are corrected for spontaneous TNF-α release by unstimulated cells. *, $P < 0.05$, GB-PS and PG versus III-PS; †, $P < 0.05$, GB-PS and PG versus LTA; △, $P < 0.05$, LTA versus III-PS.

DISCUSSION

Gram-negative bacterial infections are characterized by a marked inflammatory response that leads to significant tissue injury and multiorgan dysfunction. LPS is the predominant component of gram-negative organisms that triggers the release of host-derived proinflammatory cytokines, particularly TNF-α and IL-1β (37). Gram-positive bacterial infections also can be associated with vigorous inflammatory responses both clinically (8, 18) and experimentally (38). Infusion of live and heat-killed GBS induces the release of TNF-α, IL-1β, and IL-6 in experimental models of GBS infection (11, 30, 31). In these animal models, elevated levels of TNF-α have correlated with disease severity and mortality. In addition, no difference in TNF-α production was noted whether an encapsulated strain or an unencapsulated mutant strain of type III GBS was used. With respect to the release of TNF-α by human cord blood monocytes stimulated by GBS whole cells, our results confirm those reported by others (41) and emphasize the limited role of capsule in the induction of the host inflammatory response. Recently, Ling et al. (14) reported that heat-killed unencapsulated type III GBS were better than encapsulated GBS at inducing meningeal inflammation (characterized by increased TNF-α levels, leukocytosis, and increased protein levels) in a piglet model of GBS meningitis. Taken together, these data suggest that the cell wall is the active bacterial component causing inflammation and that the capsule plays a minor role in cytokine induction in vitro and in vivo.

The capsular polysaccharide of type III GBS is the major virulence factor of this organism (1). This conclusion is supported by the protective effect of type-specific antibody and by the ability of capsule to inhibit complement-mediated opsonization, phagocytosis, and intracellular killing of GBS (2, 3, 19). We found that the purified III-PS is not required to elicit TNF-α release by neonatal monocytes and that its contribution to TNF-α production is significantly less ($P < 0.05$; Fig. 3) than that of other cell wall-associated components of GBS. Similar findings were noted by Tuomanen et al. (33–35) for *Streptococcus pneumoniae* in an animal model of pneumonia and meningitis. Purified capsule of *S. pneumoniae* did not elicit an inflammatory response when directly instilled into rabbit lungs and induced 1,000-fold less meningeal inflammation than specific cell wall components. These findings do not imply that the GBS capsule is inert with respect to host defense but do suggest that eliciting proinflammatory cytokines is not its major function.

The contribution of the GB-PS, the species-specific cell wall antigen of GBS, in tissue injury during GBS infection has not been defined. In 1975, Lancefield et al. reported that GB-PS-specific antibodies, although broadly reactive among GBS serotypes, were not protective against infection (15). In the present study, the GB-PS was highly effective and significantly more efficient ($P < 0.05$; Fig. 3) than the type III capsule at inducing TNF-α release by human cord blood monocytes. Similar findings were reported by Mancuso et al. (17) in an experimental model of GBS infection where GB-PS induced significantly higher levels of TNF-α than the III-PS. In addition, antibody directed at the GB-PS was as effective as anti-TNF-α antibody in preventing the lethal effects of GB-PS. It is perhaps premature to speculate on the relevance of TNF-α induction by the GB-PS to the pathophysiology of GBS sepsis. However, easily detectable quantities of GB-PS are released in the serum, urine, and cerebrospinal fluid during human neonatal GBS infection (1, 5). The present findings have revealed a potentially important biologic effect of the GB-PS that merits attention.

PG is a major cell wall component of gram-positive bacteria and is considered to have an important role in inducing inflammation. Its effects on the host immune system include activation of the complement pathway (36) and inhibition of phagocytosis and killing of bacteria by neutrophils (24). Recent studies have documented that purified PG from staphylococci are capable of inducing TNF-α release by human monocytes and that the presence of serum may enhance the effects of PG (21, 32). It has been postulated that, analogous to LPS, PG may be the primary inducer of the inflammatory response associated with gram-positive sepsis. In our study, purified PG was a strong evoker of TNF-α release by cord blood monocytes and was significantly more potent than III-PS and LTA. The mechanism by which PG from GBS binds to monocytes and induces TNF-α release is unknown. Mattsson et al. (22) reported that PG-induced TNF-α production by human monocytes was independent of CD14 receptor binding. Anti-CD14 antibody did not inhibit TNF-α secretion by PG-stimulated cells but did inhibit that induced by LPS. In contrast, Weidemann et al. (39) noted that anti-CD14 antibodies inhibited IL-6 and IL-1β production induced by *S. aureus* PG, suggesting that PG and LPS shared a common biological pathway in cytokine production.

LTA has been implicated as an adhesin for GBS, but its role in the pathogenesis of GBS infection remains controversial (25). In this study, LTA from GBS was capable of triggering TNF-α release, but the magnitude was significantly less than that induced by the other two GBS cell wall components studied. Mancuso et al. (16) reported that anti-LTA antibodies enhanced TNF-α production by monocytes sensitized with LTA from group A streptococci. Whether antibodies directed against LTA from GBS would enhance cytokine release by monocytes sensitized with this cell wall component remains to be determined.

Our findings suggest that TNF-α production during GBS infection derives primarily from monocyte interactions with bacterial cell wall components rather than with the capsule. In particular, the cell wall-associated group B carbohydrate and PG were shown to be inducers of TNF-α. However, during GBS infection, any of the cell wall components may act together or with other extracellular molecules, perhaps synergistically, to induce TNF-α production. Recently, Peat et al. (27) reported that fibronectin enhanced TNF-α production by cultured-derived macrophages stimulated with type III GBS. Antibiotics may actually aggravate inflammation by generating inflammatory cell wall products, as a consequence of effective bacterial killing, that may induce further cytokine production. These factors working in concert may promote clinical deterioration and occasionally death despite bacteriologic cure of neonates with GBS infection.

ACKNOWLEDGMENTS

This work was supported by contract NO1-AI-25152 (Basic Science Component) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Jesus G. Vallejo is a recipient of a Pediatric Infectious Diseases Society Research Fellowship Award funded by Lederle Laboratories.

We thank Marisa Marques for performing the GBS PG amino acid analysis and E. O'Brian Smith for support with statistical analysis.

REFERENCES

1. **Baker, C. J., and M. S. Edwards.** 1995. Group B streptococcal infections, p. 980–1054. *In* J. S. Remington and J. O. Klein (ed.), Infectious diseases of the fetus and newborn infant, 4th ed. W. B. Saunders Co., Philadelphia.
2. **Baker, C. J., M. S. Edwards, and D. L. Kasper.** 1981. Role of antibody to native type III polysaccharide of group B *Streptococcus* in infant infection. Pediatrics **68:**544–549.
3. **Baker, C. J., and D. L. Kasper.** 1976. Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. N. Engl. J. Med. **294:**753–756.
4. **Baker, C. J., and D. L. Kasper.** 1977. Immunological investigation of infants with septicemia or meningitis due to group B *Streptococcus*. J. Infect. Dis. **136:**S98–S104.
5. **Baker, C. J., B. J. Webb, C. V. Jackson, and M. S. Edwards.** 1986. Countercurrent immunoelectrophoresis in the evaluation of infants with group B streptococcal disease. J. Pediatr. **154:**47–54.
6. **Bhakdi, S., T. Klonisch, P. Nuber, and W. Fischer.** 1991. Stimulation of monokine production by lipoteichoic acids. Infect. Immun. **59:**4614–4620.
7. **Bone, R. C.** 1994. Gram-positive organisms and sepsis. Arch. Intern. Med. **154:**26–34.
8. **Casey, R. B., R. A. Balk, and R. C. Bone.** 1993. Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome. Ann. Intern. Med. **119:**771–778.
9. **De Cueninck, B. J., G. D. Shockman, and R. M. Swenson.** 1982. Group B, type III streptococcal cell wall: composition and structural aspects revealed through endo-*N*-acetylmuramidase-catalyzed hydrolysis. Infect. Immun. **35:** 572–582.
10. **Dulkerian, S. J., L. Kilpatrick, A. T. Costarino, L. McCauley, J. Fein, L. Corcoran, S. Zirin, and M. C. Harris.** 1995. Cytokine elevations in infants with bacterial and aseptic meningitis. J. Pediatr. **126:**872–876.
11. **Gibson, R. L., G. J. Redding, W. R. Henderson, and W. E. Truog.** 1991. Group B *Streptococcus* induces tumor necrosis factor in neonatal piglets. Am. Rev. Respir. Dis. **143:**598–604.
12. **Givner, L. B., L. Gray, and T. M. O'Shea.** 1995. Antibodies to tumor necrosis factor-α: use as adjunctive therapy in established group B streptococcal disease in newborn rats. Pediatr. Res. **38:**551–554.
13. **Heumann, D., C. Barras, A. Severin, M. P. Glauser, and A. Tomasz.** 1994. Gram-positive cell walls stimulate synthesis of tumor necrosis alpha and interleukin-6 by human monocytes. Infect. Immun. **62:**2715–2721.
14. **Ling, E. W. Y., F. D. J. Noya, G. Ricard, K. Beharry, E. L. Mills, and J. V. Aranda.** 1995. Biochemical mediators of meningeal inflammatory response to group B *Streptococcus* in the newborn piglet model. Pediatr. Res. **38:**981–987.
15. **Lancefield, R. C., M. McCarty, and W. Everly.** 1975. Multiple mouse-protective antibodies directed against group B streptococci. J. Exp. Med. **142:** 165–179.
16. **Mancuso, G., F. Tomasello, T. Ofek, and G. Teti.** 1994. Antilipoteichoic acid antibodies enhance release of cytokines by monocytes sensitized with lipoteichoic acid. Infect. Immun. **62:**1470–1473.
17. **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.
18. **Marks, J. D., C. B. Marks, J. M. Luce, A. B. Montgomery, J. Turner, C. A. Metz, and J. F. Murray.** 1990. Plasma tumor necrosis factor in patients with septic shock. Am. Rev. Respir. Dis. **141:**94–97.
19. **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.
20. **Mattingly, S. J., and B. P. Johnston.** 1987. Comparative analysis and localization of lipoteichoic acid in *Streptococcus agalactiae* and *Streptococcus pyogenes*. Infect. Immun. **55:**2383–2386.
21. **Mattsson, E., J. Rollof, J. Verhoef, V. H. Dijk, and A. Fleer.** 1994. Serum-induced potentiation of tumor necrosis factor alpha production by human monocytes in response to staphylococcal peptidoglycan: involvement of different serum factors. Infect. Immun. **62:**3837–3843.
22. **Mattsson, E., H. Van Dijk, K. V. Kessel, J. Verhoef, A. Fleer, and J. Rollof.** 1996. Intracellular pathways of tumor necrosis factor-α release by human monocytes on stimulation with lipopolysaccharide or staphylococcal peptidoglycan are partly similar. J. Infect. Dis. **173:**212–218.
23. **Michon, F., E. Katzenellenbogen, D. L. Kasper, and H. L. Jennings.** 1987. Structure of the complex group-specific polysaccharide of group B *Streptococcus*. Biochemistry **26:**476–486.
24. **Musher, D. M., H. Verbrugh, and J. Verhoef.** 1981. Suppression of phagocytosis and chemotaxis by cell wall components of *Staphylococcus aureus*. J. Immunol. **127:**84–88.
25. **Nealon, T. J., and S. J. Mattingly.** 1985. Kinetic and chemical analysis of the biologic significance of lipoteichoic acids mediating adherence of serotype III group B streptococci. Infect. Immun. **50:**107–115.
26. **Payne, N. R., B. A. Burke, D. L. Day, P. D. Christenson, T. R. Thompson, and P. Ferrieri.** 1988. Correlation of clinical and pathologic findings in early onset neonatal group B streptococcal infection with disease severity and prediction of outcome. Pediatr. Infect. Dis. J. **7:**836–847.
27. **Peat, E. B., N. H. Augustine, W. K. Drummond, J. F. Bohnsack, and H. R. Hill.** 1995. Effects of fibronectin and group B streptococci on tumor necrosis factor-α production by human cultured-derived macrophages. Immunology **84:**440–445.
28. **Peterson, P. K., B. J. Wilkinson, Y. Kim, D. Schmeling, S. D. Douglas, P. G. Quie, and J. Verhoef.** 1978. The key role of peptidoglycan in the opsonization

of *Staphylococcus aureus*. J. Clin. Invest. **61:**597–609.
29. **Rubens, C. E., J. M. Kupers, L. M. Heggen, D. L. Kasper, and M. R. Wessels.** 1991. Molecular analysis of the group B streptococcal capsule genes, p. 179–183. *In* G. M. Dunny, P. P. Cleary, and L. L. McKay (ed.), Genetics and molecular biology of streptococci, lactococci, and enterococci. American Society for Microbiology, Washington, D.C.
30. **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.
31. **Teti, G., G. Mancuso, F. Tomasello, and M. S. Chiofalo.** 1992. Production of tumor necrosis factor-α and interleukin-6 in mice infected with group B streptococci. Circ. Shock **38:**138–144.
32. **Timmerman, C. P., E. Mattsson, L. Martinez-Martinez, L. De Graaf, J. A. E. VanStrijp, H. A. Verbrugh, J. Verhoef, and A. Fleer.** 1995. Induction of release of tumor necrosis factor from human monocytes by staphylococci and staphylococcal peptidoglycan. Infect. Immun. **61:**4167–4172.
33. **Tuomanen, E., H. Liu, B. Hengstler, O. Zak, and A. Tomasz.** 1985. The induction of meningeal inflammation by components of the pneumococcal cell wall. J. Infect. Dis. **151:**859–868.
34. **Tuomanen, E., R. Rich, and O. Zak.** 1986. Induction of pulmonary inflammation by components of the pneumococcal cell surface. Am. Rev. Respir. Dis. **135:**869–874.
35. **Tuomanen, E., A. Tomasz, B. Hengstler, and O. Zak.** 1985. The relative role of bacterial cell wall and capsule in the induction of inflammation in pneumococcal meningitis. J. Infect. Dis. **151:**535–540.
35a. **Vallejo, J. G., C. J. Baker, M. S. Edwards.** 1995. The group B cell wall antigen of group B *Streptococcus* induces tumor necrosis factor alpha production by human neonatal monocytes. Clin. Infect. Dis. **21:**806. (Abstract.)
36. **Verbrugh, H., W. Van Dijk, R. Peters, M. Van der Tol, and J. Verhoef.** 1979. The role of *Staphylococcus aureus* cell-wall peptidoglycan, teichoic acid, and protein A in the processes of complement activation and opsonization. Immunology **37:**615–621.
37. **Waage, A., P. Brandtzaeg, T. Espevik, and A. Halstensen.** 1991. Current understanding of the pathogenesis of gram-negative shock. Infect. Dis. Clin. North Am. **5:**781–791.
38. **Wakabayashi, G., J. A. Gelfand, W. K. Jung, R. J. Conolly, J. F. Burke, and C. A. Dinarello.** 1991. *Staphylococcus epidermidis* induces complement activation, tumor necrosis factor and interleukin-1, a shock-like state and tissue injury in rabbits without endotoxemia. J. Clin. Invest. **87:**1925–1935.
39. **Weidemann, B., H. Brade, E. T. Rietschel, R. Dziarski, V. Bazil, S. Kusumoto, H.-D. Flad, and J. Ulmer.** 1994. Soluble peptidoglycan-induced monokine production can be blocked by anti-CD14 monoclonal antibodies and by lipid A partial structures. Infect. Immun. **62:**4709–4715.
40. **Wessels, M. R., L. C. Paoletti, D. L. Kasper, J. L. DiFabio, F. Michon, and H. J. Jennings.** 1990. Immunogenicity in animals of a polysaccharide-protein conjugate vaccine against type III group B *Streptococcus*. J. Clin. Invest. **86:**1428–1433.
41. **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 cell in vitro and in vivo, induced by group B streptococci. J. Pediatr. **123:** 292–300.

Editor: R. E. McCallum