INFECTION AND IMMUNITY, July 1994, p. 2748–2753
0019-9567/94/$04.00+0

Vol. 62, No. 7

Induction of Tumor Necrosis Factor Alpha by the Group- and Type-Specific Polysaccharides from Type III Group B Streptococci

GIUSEPPE MANCUSO,[1] FRANCESCO TOMASELLO,[1] CHRISTINA VON HUNOLSTEIN,[2] GRAZIELLA OREFICI,[2] AND GIUSEPPE TETI[1]*

Istituto di Microbiologia, Facoltà di Medicina e Chirurgia, Università degli Studi di Messina, I-98122 Messina,[1] and Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, I-00161 Rome,[2] Italy

Received 28 January 1994/Returned for modification 3 March 1994/Accepted 8 April 1994

Previous studies suggested that circulating tumor necrosis factor alpha (TNF-α) may have a pathophysiologic role in experimental neonatal sepsis induced by group B streptococci (GBS). This study was undertaken to investigate the ability of the type III and group-specific polysaccharides of GBS to induce TNF-α production and TNF-α-dependent lethality in neonatal rats. The cytokine was detected in plasma samples by the L929 cytotoxicity assay. Intracardiac injections of either polysaccharide induced dose-dependent, transient elevations in plasma TNF-α levels that returned to baseline values after 5 h. The group-specific antigen induced significantly higher mean peak TNF-α levels than the type III antigen (125 ± 47 versus 44 ± 15 U/ml with 70 mg/kg of body weight). Glycogen (70 mg/kg), used as a negative control, did not induce TNF-α. The lipopolysaccharide-neutralizing agent polymyxin B did not decrease TNF-α levels induced by either polysaccharide, ruling out contamination with endotoxin as a possible cause of TNF-α induction. Fifty percent lethal doses of the type III and group-specific antigens given as intracardiac injections were 105 and 16 mg/kg, respectively. *Salmonella* endotoxin, used as a positive control, had a 50% lethal dose of 0.1 mg/kg. The lethal activities of GBS polysaccharides, as well as endotoxin, were completely prevented by pretreatment of neonatal rats with the respective specific antibodies or anti-murine TNF-α serum. To assess the relative importance of the type-specific substance in TNF-α induction by whole bacteria, two unrelated GBS transposon mutants devoid of only the type-specific capsular polysaccharide (COH1-13 and COH31-15) were employed. Each of the heat-killed unencapsulated mutants was able to produce plasma TNF-α level elevations or TNF-α-dependent lethality but was significantly less efficient in these activities than the corresponding encapsulated wild-type strain. These data suggest that the presence of type-specific material on GBS is not necessary for the stimulation of TNF-α production. Type III capsular polysaccharide, however, can significantly increase the ability of GBS to induce TNF-α. Further studies will be needed to assess the importance of TNF-α induction by the group- and type-specific antigens in the pathophysiology of GBS disease.

Tumor necrosis factor alpha (TNF-α) is a member of the cytokine family of low-molecular-weight proteins serving important functions in host-parasite interactions. TNF-α is produced by a variety of host cells, including mononuclear phagocytes, upon exposure to a range of unrelated microbial components (39).

The effects of this cytokine can be beneficial or detrimental to the host in different infections. TNF-α is a powerful activator of phagocyte antimicrobial properties and is believed to play an important role in controlling infections by intracellular pathogens (30, 35, 42) and localized infections (2, 9, 38). However, high levels of circulating TNF-α, alone or in combination with other proinflammatory cytokines, can mediate major hemodynamic changes and mortality. These effects are clearly observed in sepsis models involving intravascular injection of endotoxin (5) or whole gram-positive (10) and gram-negative (10, 29, 36) bacteria.

We have investigated the role of TFN-α and other cytokines in experimental sepsis induced by group B streptococci (GBS), a major cause of neonatal mortality and permanent disability throughout the world. In a neonatal rat model, circulating TNF-α levels were correlated with sepsis severity (33). Neutralization of TNF-α with specific antibodies resulted in a prolonged survival time, suggesting a possible role of this cytokine in the pathophysiology of GBS disease.

Although lipopolysaccharide (LPS) induces TNF-α, little is known about the identities of gram-positive components responsible for similar activities. Lipoteichoic acids from different gram-positive species can induce in vitro TNF-α production by monocytes (6) and macrophages (19). Staphylococcal peptidoglycan also has been shown to induce TNF-α production in vivo (38) and in vitro (34).

Type-specific capsular polysaccharide is considered the major virulence factor of GBS (3). This notion is supported by the protective activity of type-specific antibodies (4, 20) and by the lack of virulence of isogenic GBS strains devoid of the type-specific capsule (26). One well-documented virulence mechanism of capsular polysaccharide is the inhibition of complement-mediated phagocytic killing (22). In addition, a GBS extract containing type III polysaccharide caused leukopenia and pulmonary hypertension in sheep (15) and serum-dependent in vitro aggregation of human neutrophils (24). A purified preparation of type III antigen enhanced the adherence of polymorphonuclear leukocytes to human endothelium (23). These effects may contribute to the leukopenia, vascular injury, and pneumonitis commonly observed in GBS disease.

Because TNF-α produces similar pathogenic responses (18, 31, 39), the toxic activities of GBS extracts enriched in

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

type-specific polysaccharide may be mediated at least in part by TNF-α. This study was undertaken to investigate the ability of type III antigen to induce plasma TNF-α level elevations and TNF-α-dependent lethality in neonatal rats. The activities of group-specific polysaccharide, a major cell-associated and extracellular product of GBS (1, 7), were also studied. Additionally, the ability of GBS mutants devoid of type-specific polysaccharide to induce TNF-α production was ascertained.

MATERIALS AND METHODS

Neonatal rats. Neonatal (24 to 48 h old) Sprague-Dawley rats were used in this study. Parental rats were obtained from Charles River Italia (Calco, Italy). Pups from each litter were randomly assigned to control or experimental groups, marked, and kept with the mother. To measure in vivo TNF-α production and for lethality tests, each animal received, under ether anesthesia, intracardiac (i.c.) injections of polysaccharides or bacteria in 25 μl of phosphate-buffered saline (PBS; 0.01 M phosphate, 0.15 M NaCl; pH 7.2).

GBS polysaccharides. The group- and type-specific polysaccharides were purified by anion-exchange and gel filtration chromatographies from culture supernatants of type III GBS (strain H738; a gift from Bascom Anthony, National Institutes of Health, Bethesda, Md.) as described previously (32, 40). These materials contained <0.5% protein and were free of carbohydrate contaminants, as determined by high-performance anion-exchange chromatography with a pulsed amperometric detector (40).

In some experiments, the LPS-neutralizing agent polymyxin B (25 μg/ml; Sigma Chimica, Milan, Italy) was mixed with the stimuli to rule out contamination with endotoxin. These mixtures were incubated for 30 min at room temperature before injection. LPS from *Salmonella enteritidis* (Difco, Diagnostic International Distribution, Milan, Italy) and rabbit glycogen (type X; Sigma) were used as positive and negative controls, respectively.

Heat-killed bacteria. The two pairs of isogenic strains used in the present study were generously provided by C. E. Rubens, University of Washington, Seattle. Strains COH1-13 and COH31-15 are unrelated, capsule-deficient transposon mutants of strains COH1 and COH31, respectively (22, 26). Levels of cell-associated type-specific antigen were 10.8 and 0.4 mg/g (dry weight) for strains COH1 and COH31, respectively, as quantitated by enzyme-linked immunosorbent assay inhibition (32, 33). As expected, type-specific antigen was undetectable in transposon mutants COH1-13 and COH31-15. All strains were grown in Todd-Hewitt broth (Difco) to the late logarithmic phase, washed with H_2O, killed by heating (80°C for 30 min), and lyophilized.

Measurement of TNF-α. To determine circulating TNF-α levels, groups of five animals were sacrificed by decapitation under ether anesthesia at different times after i.c. injection with polysaccharides or heat-killed bacteria. Mixed venous-arterial blood was collected in heparinized containers and centrifuged. Plasma (0.25 to 0.35 ml) was stored at −70°C until assayed for TNF-α activity by a cytotoxicity assay with L929 murine fibroblasts as previously described (33).

TNF-α activity was expressed in units per milliliter, 1 U being defined as the amount of TNF-α causing 50% lysis of L929 cells. Nine serial twofold dilutions (from 1:2 to 1:512) were tested in duplicate for each sample. The assay was calibrated with murine recombinant TNF-α (specific activity, 20 U/ng; Genzyme; distributed by Omnia Res., Cinisello Balsamo, Italy) 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 (Genzyme) but not by normal rabbit serum.

Lethality tests. Rat pups received i.c. injections of the materials being tested, and mortality was assessed every 12 h for 7 days. With this model, deaths rarely occurred after 72 h. To assess the protective effects of different antibodies, rat pups were injected intraperitoneally with 50 μl of purified monoclonal antibodies (MAbs) or rabbit sera 4 h before i.c. challenge. MAb P9D8, a mouse immunoglobulin M antibody (32), was used as a source of anti-type III antigen antibodies. The immunoglobulin M MAb P2E8, nonreactive against type III GBS (32), was used as a control. Both MAbs were dissolved in PBS and injected at 50 mg/kg of body weight.

Group-specific serum was prepared with New Zealand White rabbits as described previously (40). Anti-murine TNF-α serum was obtained from Genzyme. Anti-group D *Salmonella* serum (Difco) was extensively dialyzed against PBS to remove preservatives and used at a final dilution of 1:2 in PBS.

Detection of endotoxin. GBS polysaccharides, bacterial suspensions, MAbs, and rabbit sera were tested for the presence of endotoxin with a *Limulus* amebocyte lysate assay kit (E-Toxate; Sigma). Levels of endotoxin were <0.05 ng/ml in rabbit sera and <5 pg/mg in all of the other materials.

Data expression and statistical analysis. TNF-α levels are expressed as means ± standard deviations of five independent observations. To calculate mean values, results below the detection level (2 U/ml) were assigned a theoretical value of 1 U/ml. Differences in plasma TNF-α levels were assessed by a 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.

RESULTS

Induction of TNF-α by GBS polysaccharides. Figure 1 shows plasma TNF-α levels in samples taken from rat pups at various times after i.c. injection of type- or group-specific polysaccharides. The cytokine was undetectable (<2 U/ml) in normal plasma samples obtained from five untreated animals (baseline or 0-h controls). Both polysaccharides induced transient, dose-dependent elevations in TNF-α activity (Fig. 1). This activity reached peak levels at 2 h and returned to the baseline at 5 h. TNF-α activity elevations were significant, compared with the baseline, at polysaccharide doses of 7, 28, or 70 mg/kg (Fig. 1). The group-specific antigen induced significantly higher mean peak TNF-α levels than the type III antigen (125 ± 47 versus 44 ± 15 U/ml with 70 mg/kg). TNF-α activity was undetectable in samples obtained at 1, 2, 3, or 5 h after i.c. injection of glycogen (70 mg/kg; data not shown).

Preincubation with polymyxin B (25 μg/ml) did not inhibit the TNF-α production induced by GBS polysaccharides (data not shown). However, polymyxin B produced an 82% reduction (23 ± 10 versus 128 ± 56 U/ml) in peak TNF-α levels induced by 0.35 mg of *S. enteritidis* LPS per kg. This result and the previous finding that endotoxin levels of polysaccharide preparations were determined to be <5 pg/mg by a *Limulus* assay ruled out contamination with endotoxin as a possible cause of TNF-α induction.

Induction of lethality by GBS polysaccharides. It was of interest to ascertain whether the observed elevations in TNF-α activity were associated with toxic effects. Mortality was assessed for groups of 10 animals receiving i.c. injections of increasing doses of group-specific and type III polysaccharides. Glycogen and LPS were used as negative and positive controls, respectively. The type III and group-specific polysaccharides

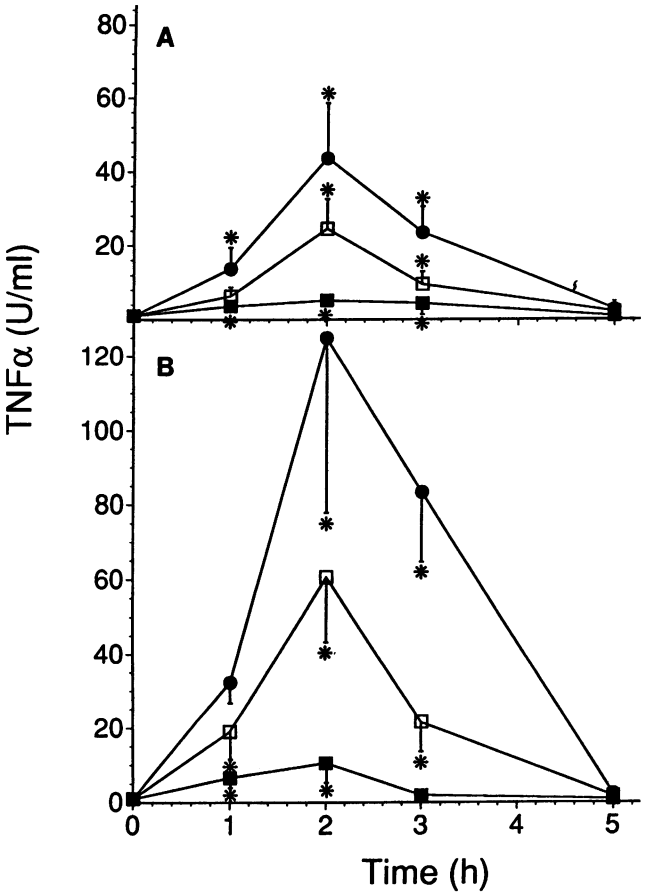

FIG. 1. Circulating TNF-α levels in neonatal rats injected i.c. with type III polysaccharide (A) or group-specific polysaccharide (B). Plasma was collected from pups (five per group) at various times after the injection of 7 (■), 28 (□), or 70 (●) mg of either polysaccharide per kg. Points and bars represent means ± standard deviations of five observations. *, significantly ($P < 0.05$) different from values for 0-h controls, as determined by a one-way analysis of variance and the Student-Newman-Keuls test.

had 50% lethal doses of 105 and 16 mg/kg, respectively. Glycogen was non toxic at doses as high as 200 mg/kg, while the 50% lethal dose of LPS was 0.1 mg/kg (data not shown).

As expected, pups were protected against *S. enteritidis* LPS toxicity by anti-group D *Salmonella* serum (Table 1). Similarly, the lethal effects of the type III and group-specific polysaccharides could be prevented by the corresponding antibodies (Table 1). These, however, were not effective against LPS-induced lethality. Thus, the lethal activities of GBS polysaccharides were specific and not caused by contamination with toxic materials, including endotoxin. Mortality induced by GBS polysaccharides, as well as LPS, was totally prevented by rabbit anti-TNF-α serum (Table 1).

Induction of TNF-α and lethality by isogenic strains. To assess the relative importance of the type-specific substance in TNF-α induction, two unrelated GBS mutants devoid of type-specific capsular polysaccharide were employed. The use of heat-killed bacteria allowed us to compare the in vivo responses induced by encapsulated and unencapsulated GBS without concern for the effects of the capsule in promoting the replication of live GBS within the host. Heat-killed GBS were recently shown to be as effective as live bacteria in causing dose-dependent hemodynamic changes in newborn lambs (28).

TABLE 1. Protection by specific antibodies against lethality induced by type III and group-specific antigens[a]

Pretreatment	Challenge antigen	Lethality[b]
Control MAb P2E8	Type III	8/11 (73)
Anti-type III antigen MAb P9D8	Type III	1/11 (9)[c]
Normal rabbit serum	Type III	7/8 (88)
Anti-TNF-α serum	Type III	0/8 (0)[d]
Normal rabbit serum	Group specific	9/12 (75)
Anti-group B serum	Group specific	0/12 (0)[d]
Anti-TNF-α serum	Group specific	0/12 (0)[d]
Normal rabbit serum	LPS	6/7 (86)
Anti-group D *Salmonella* serum	LPS	1/7 (14)[d]
Anti-TNF-α serum	LPS	0/7 (0)[d]
Anti-type III antigen MAb P9D8	LPS	6/7 (86)
Anti-group B serum	LPS	7/7 (100)

[a] Rat pups were injected intraperitoneally with 50 mg of MAbs per kg or with 50 μl of rabbit antisera 4 h before i.c. challenge with type III (120 mg/kg) or group-specific (20 mg/kg) polysaccharide or LPS (0.3 mg/kg).
[b] Number of dead rats/total number tested (percent).
[c] Significantly ($P < 0.05$) different from the value obtained with control MAb P2E8, as determined by a two-tailed Fisher's exact test.
[d] Significantly ($P < 0.05$) different from the values obtained with the respective normal rabbit serum controls, as determined by a two-tailed Fisher's exact test.

The efficacy of unencapsulated strains in inducing in vivo TNF-α production was compared with that of the encapsulated parental wild-type strains. Figure 2 shows circulating TNF-α kinetics in neonatal rats inoculated i.c. with 70 mg of GBS per kg. Each of the mutants induced significantly less TNF-α than the corresponding encapsulated parental strain.

Table 2 shows that all the strains, including unencapsulated mutants COH1-13 and COH31-15, caused mortality if given in sufficient amounts. However, encapsulated wild-type strains were able to induce mortality at lower doses. With all strains, mortality could be completely prevented by the administration of anti-TNF-α serum (data not shown).

DISCUSSION

Models of septic shock have classically used LPS or gram-negative bacteria to induce mediator release or hemodynamic and pathologic changes. It is becoming increasingly clear, however, that gram-positive bacteria can induce the same pathophysiologic phenomena as those observed in gram-negative shock and that endotoxin is not required to induce cytokine release (10, 33, 41). Little is known of bacterial components, other than LPS, that are responsible for TNF-α release. Some defined polysaccharides and LPS may stimulate human monocytes to produce TNF-α by similar mechanisms (25).

Our results show that purified type III and group-specific polysaccharides of GBS can induce TNF-α and TNF-α-dependent lethality in rat pups. The group-specific antigen was significantly more potent in these activities than the type III antigen. To further assess the relative importance of the latter substance in TNF-α induction by whole bacteria, we used mutant strains differing in the expression of the type III antigen. We focused on the type-specific capsular polysaccharide because it is considered the major virulence factor of GBS and isogenic strains devoid of the type-specific antigen were available.

Our results show that the presence of the type-specific antigen on GBS is not essential to induce in vivo TNF-α

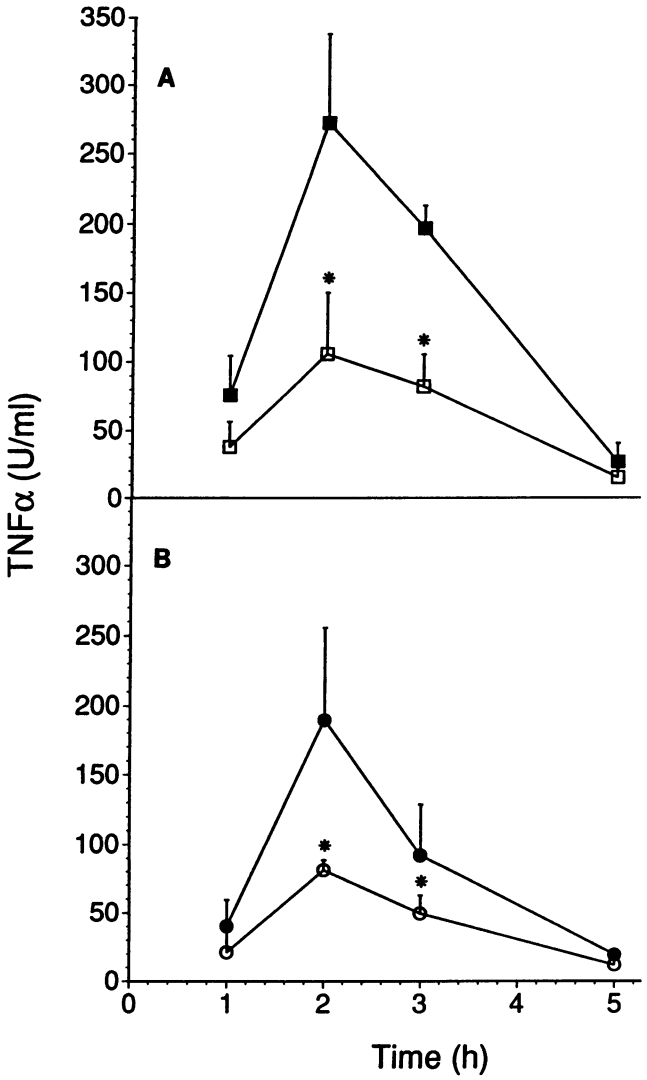

FIG. 2. Plasma TNF-α levels in neonatal rats injected with two unrelated pairs of isogenic strains. Strains COH1-13 (□) and COH31-15 (○) are unencapsulated mutants of strains COH1 (■) and COH31 (●), respectively. Plasma samples were obtained from five animals per group at various times after i.c. challenge with 70 mg of heat-killed bacteria per kg. Points and bars represent means ± standard deviations of five observations. *, significantly ($P < 0.05$) different from values for COH1 (A) or COH31 (B), as determined by a one-way analysis of variance and the Student-Newman-Keuls test.

production or TNF-α-mediated lethality. However, the presence of this polysaccharide significantly increased the effectiveness of whole bacteria in inducing TNF-α. This effect cannot be entirely accounted for by the TNF-α-inducing ability of purified type III antigen. In fact, the latter made up a small portion of the weight of the organisms (1.08 and 0.04% for strains COH1 and COH31, respectively). On the basis of the activity of the purified type-specific antigen, the amount injected with the encapsulated strains would be expected to produce only modest or no elevations in plasma TNF-α levels.

It is possible that the type-specific substance acts synergistically with some other GBS constituent in inducing TNF-α. It may be relevant that the majority of cell-associated type- and group-specific antigens are covalently linked to peptidoglycan

TABLE 2. Lethality of heat-killed mutant strains devoid of type-specific capsular polysaccharide

Dose (mg/kg)	Lethality[a] of:			
	COH1	COH1-13	COH31	COH31-15
3.5	5/13 (38)	0/14 (0)[b]	0/11 (0)	0/10 (0)
7.0	13/14 (93)	2/12 (17)[b]	10/15 (67)	1/10 (10)[c]
10.5	8/8 (100)	6/12 (50)[b]	12/14 (86)	4/12 (33)[c]
14.0	7/7 (100)	9/9 (100)	13/13 (100)	12/12 (100)

[a] Number of dead rats/total number tested (percent).
[b] Significantly ($P < 0.05$) different from the values for parental strain COH1, as determined by a two-tailed Fisher's exact test.
[c] Significantly ($P < 0.05$) different from the values for parental strain COH31, as determined by a two-tailed Fisher's exact test.

in the cell wall (8). In addition, the presence of a capsule may increase TNF-α induction by increasing cell size or via other physical factors. It was recently shown that the size, as well as the tertiary structure, of insoluble peptidoglycan fragments can dramatically affect their ability to induce TNF-α production by human monocytes (34). Collectively, our data indicate that the type-specific antigen may be more effective in inducing TNF-α when it is present on the surface of intact bacteria rather than when it is present as a soluble purified compound.

Gibson et al. did not detect differences in two of the isogenic strains used in the present study (COH31 and COH31-15) with regard to their potency in inducing early (≤1 h) pathophysiologic changes when infused into 16-day-old piglets (12). These early phenomena, consisting of pulmonary hypertension and hypoxia, are largely thromboxane A_2 mediated (27, 37) and occur before TNF-α production, which is detectable only at 2 to 6 h after GBS infusion in this model (11). Thus, the type III capsular polysaccharide may have a more evident role in inducing late, TNF-α-mediated abnormalities, as opposed to early, thromboxane A_2-associated events.

Possible differences in neonatal sepsis models should also be considered. Hoofed animals, but not other mammals, develop a large population of pulmonary intravascular macrophages within several days after birth (21). These cells are associated with increased pulmonary vascular reactivity and mediator release upon exposure to a wide range of particles (21).

In addition, differences between isogenic strains may be easily obscured by high GBS doses. In this study, no differences in the ability to produce TNF-α-dependent lethality were apparent between strains when high saturating doses of heat-killed bacteria were used.

While anti-TNF-α antibodies produced an increase in survival time but not permanent protection in pups injected intraperitoneally with viable GBS (33), in the present study anti-TNF-α completely prevented lethality in animals injected intravascularly with killed bacteria or polysaccharides. This result is in agreement with the notion that the site of inoculation and the compartmentalization of the inflammatory reaction can markedly influence the response to anticytokine therapy (2).

It is perhaps too early to speculate on the relevance of TNF-α induction by the type- and group-specific antigens to the pathophysiology of GBS sepsis. Unfortunately, detailed data on circulating levels of these polysaccharides in septic neonates are not available. However, it appears that considerable quantities of group-specific antigen are released during infection. On the basis of the sensitivity of latex agglutination reagents used for diagnostic purposes (14), it can be assumed that 62 to 83% of neonates with early- or late-onset GBS

disease have circulating group-specific antigen levels of >125 ng/ml (17).

Further studies are necessary to determine if, by increasing TNF-α levels, GBS polysaccharides contribute to the acceleration of the development of shock and mortality. Because whole bacteria induced larger amounts of TNF-α than purified polysaccharides, it is likely that components different from those examined also account for the ability of GBS to stimulate TNF-α release. Exotoxins from group A streptococci (13) and staphylococci (16) can stimulate human and murine leukocytes to produce cytokines, including TNF-α. Staphylococcal peptidoglycan has also been shown to induce TNF-α production in vivo (38) and in vitro (34). Therefore, it will be necessary in future studies to investigate the effects of other purified GBS products, including protein and peptidoglycan components, on the induction of various cytokines.

ACKNOWLEDGMENTS

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

We are very grateful to Craig Rubens for providing bacterial strains.

REFERENCES

1. **Anthony, B. F., N. F. Conception, S. A. McGeary, J. I. Ward, D. C. Heiner, P. Shapshak, and R. A. Insel.** 1982. Immunospecificity and quantitation of an enzyme-linked immunosorbent assay for group B streptococcal antibody. J. Clin. Microbiol. **16:**350–354.
2. **Bagby, G. J., K. J. Plessala, L. A. Wilson, J. J. Thompson, and S. Nelson.** 1991. Divergent efficacy of antibody to tumor necrosis factor-α in intravascular and peritonitis models of sepsis. J. Infect. Dis. **163:**83–88.
3. **Baker, C. J.** 1986. Group B streptococcal infections in newborns. N. Engl. J. Med. **314:**1702–1708.
4. **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:**752–756.
5. **Beutler, B., I. W. Milsark, and A. Cerami.** 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science **229:**869–871.
6. **Bhakdi, S., T. Klonish, P. Nuber, and W. Fisher.** 1991. Stimulation of monokine production by lipoteichoic acids. Infect. Immun. **59:**4614–4620.
7. **Carey, R. B., T. K. Eisenstein, G. D. Shockman, T. F. Greber, and R. M. Swenson.** 1980. Soluble group- and type-specific antigens from type III group B streptococcus. Infect. Immun. **28:**195–203.
8. **Doran, T. I., and S. J. Mattingly.** 1982. Association of type- and group-specific antigens with the cell wall of serotype III group B streptococcus. Infect. Immun. **36:**1115–1122.
9. **Echtenacher, B., W. Falk, D. M. Mannel, and P. H. Krammer.** 1990. Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. J. Immunol. **145:**3762–3766.
10. **Freudenberg, M. A., and C. Galanos.** 1991. Tumor necrosis factor mediates lethal activity of killed gram-positive and gram-negative bacteria in D-galactosamine-treated animals. Infect. Immun. **59:**2110–2115.
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. **Gibson, R. L., G. J. Redding, W. E. Truog, W. R. Henderson, and C. E. Rubens.** 1989. Isogenic group B streptococci devoid of capsular polysaccharide or β-hemolysin: pulmonary hemodynamic and gas exchange effects during bacteremia in piglets. Pediatr. Res. **26:**241–245.
13. **Hackett, S. P., and D. L. Stevens.** 1992. Streptococcal toxic shock syndrome: synthesis of tumor necrosis factor and interleukin 1 by monocytes stimulated with pyrogenic exotoxin A and streptolysin O. J. Infect. Dis. **165:**879–885.
14. **Hemming, V. G., W. T. London, L. P. Smith, B. L. Curfman, G. W. Fisher, and J. L. Sever.** 1983. Detection of group B streptococcal antigens in amniotic fluid of rhesus monkeys. J. Clin. Microbiol. **17:**1127–1131.
15. **Hemming, V. G., W. F. O'Brien, G. W. Fisher, S. M. Golden, and S. F. Noble.** 1984. Studies on short-term pulmonary and peripheral vascular responses induced in oophorectomized sheep by the infusion of a group B streptococcal extract. Pediatr. Res. **18:**266–269.
16. **Ikejiima, T., S. Okusawa, J. M. van der Meer, and C. A. Dinarello.** 1988. Induction by toxic-shock-syndrome toxin 1 of a circulating tumor necrosis-like substance in rabbits and of immunoreactive tumor necrosis factor and interleukin 1 from human mononuclear cells. J. Infect. Dis. **158:**1017–1025.
17. **Ingram, D. L., D. M. Suggs, and A. W. Pearson.** 1982. Detection of group B streptococcal antigen in early-onset and late-onset group B streptococcal disease with the Wellcogen Strep B latex agglutination test. J. Clin. Microbiol. **16:**656–658.
18. **Johnson, J., B. Meyrick, G. Jesmok, and K. L. Brigham.** 1989. Human recombinant tumor necrosis factor alpha infusion mimics endotoxemia in awake sheep. J. Appl. Physiol. **66:**1448–1454.
19. **Keller, R., W. Fisher, R. Keist, and S. Bassetti.** 1992. Macrophage response to bacteria: induction of marked secretory and cellular activities by lipoteichoic acids. Infect. Immun. **60:**3664–3672.
20. **Lancefield, R. C., M. McCarthy, and W. N. Everly.** 1975. Multiple mouse-protective antibodies directed against group B streptococci: special references to antibodies effective against protein antigens. J. Exp. Med. **142:**165–179.
21. **Longworth, K. E., A. M. Westgate, M. K. Grady, J. Y. Westcott, and N. C. Staub.** 1992. Development of pulmonary intravascular macrophage function in newborn lambs. J. Appl. Physiol. **73:**2608–2615.
22. **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.
23. **Mcfall, T. L., G. A. Zimmerman, N. H. Augustine, and H. R. Hill.** 1987. Effect of group B streptococcal type-specific antigen on polymorphonuclear leukocyte function and polymorphonuclear leukocyte-endothelial cell interaction. Pediatr. Res. **21:**517–523.
24. **Olson, T. A., G. W. Fisher, V. G. Hemming, W. F. O'Brien, S. M. Golden, and D. A. Maybee.** 1987. A group B streptococcal extract reduces neutrophil counts and induces neutrophil aggregation. Pediatr. Res. **21:**326–330.
25. **Otterlei, M., A. Sundan, G. Skjåk-Bræk, L. Ryan, O. Smidsrød, and T. Espevik.** 1993. Similar mechanisms of action of defined polysaccharides and lipopolysaccharides: characterization of binding and tumor necrosis factor alpha induction. Infect. Immun. **61:**1917–1925.
26. **Rubens, C. E., M. R. Wessels, L. M. Heggen, and D. L. Kasper.** 1987. Transposon mutagenesis of group B streptococcal type III capsular polysaccharide: correlation of capsule expression with virulence. Proc. Natl. Acad. Sci. USA **84:**7208–7212.
27. **Runkle, B., R. N. Goldberg, M. M. Streitfeld, M. R. Clark, E. Buron, E. S. Setzer, and E. Bancalari.** 1984. Cardiovascular changes in group B streptococcal sepsis in the piglet: response to indomethacin and relationship to prostacyclin and thromboxane A_2. Pediatr. Res. **18:**874–878.
28. **Schreiber, M. D., R. F. Covert, and L. J. Togerson.** 1992. Hemodynamic effects of heat-killed group B β-hemolytic streptococcus in newborn lambs. Pediatr. Res. **31:**121–126.
29. **Silva, A. T., K. F. Bayston, and J. Cohen.** 1990. Prophylactic and therapeutic effects of a monoclonal antibody to tumor necrosis factor-α in experimental gram negative shock. J. Infect. Dis. **162:**421–427.
30. **Smith, G. M., D. M. Magee, D. M. Williams, and J. R. Graybill.** 1990. Tumor necrosis factor-α plays a role in host defence against *Histoplasma capsulatum*. J. Infect. Dis. **162:**1349–1353.
31. **Stephens, K. E., A. Ishizaka, J. W. Larrik, and T. A. Raffin.** 1988. Tumor necrosis factor causes increased pulmonary permeability and edema: comparison to septic acute lung injury. Am. Rev. Respir. Dis. **137:**1364–1370.
32. **Teti, G., M. Calapai, G. Calogero, F. Tomasello, G. Mancuso, A.**

Galli, and G. Riggio. 1992. Specificity and protective activity of murine monoclonal antibodies directed against the capsular polysaccharide of type III group B streptococci. Hybridoma **11:**13–22.

33. **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.
34. **Timmerman, C. P., E. Mattson, L. Martinez-Martinez, L. De Graaf, J. A. G. van Stijp, H. A. Verbrugh, J. Verhoef, and A. Fleer.** 1993. Induction of release of tumor necrosis factor from human monocytes by staphylococci and staphylococcal peptidoglycans. Infect. Immun. **61:**4167–4172.
35. **Titus, R., B. Sherry, and A. Cerami.** 1989. Tumor necrosis factor plays a protective role in experimental murine cutaneous leishmaniasis. J. Exp. Med. **170:**2097–2104.
36. **Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry, and A. C. Cerami.** 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature (London) **330:**662–665.
37. **Truog, W. E., G. K. Soresen, T. A. Standaert, and G. J. Redding.** 1986. Effects of the thromboxane synthetase inhibitor, Dazmegrel (UK 38,485), on pulmonary gas exchange and hemodynamics in neonatal sepsis. Pediatr. Res. **20:**481–486.
38. **Vandaux, P., G. E. Grau, E. Huggler, F. Shumacher-Perdreau, F. Fiedler, F. A. Waldvogel, and D. P. Lew.** 1992. Contribution of tumor necrosis factor to host defences against staphylococci in a guinea pig model of foreign body infections. J. Infect. Dis. **166:**58–64.
39. **Vassalli, P.** 1992. The pathophysiology of tumor necrosis factors. Annu. Rev. Immunol. **10:**411–452.
40. **von Hunolstein, C., S. D'Ascenzi, B. Wagner, J. Jelinkova, G. Alfarone, S. Recchia, M. Wagner, and G. Orefici.** 1993. Immunochemistry of the type capsular polysaccharide and virulence properties of type VI *Streptococcus agalactiae* (group B streptococcus). Infect. Immun. **61:**1272–1280.
41. **Wakabayashi, G., J. A. Gelfand, W. K. Jung, R. J. Connolly, 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. Comparison to *Escherichia coli*. J. Clin. Invest. **87:**1925–1935.
42. **Williams, D. M., D. M. Magee, L. F. Bonewald, J. G. Smith, C. A. Bleicker, G. I. Byrneand, and J. Schachter.** 1990. A role for tumor necrosis factor alpha in host defense against *Chlamydia trachomatis*. Infect. Immun. **58:**1572–1576.