INFECTION AND IMMUNITY, Sept. 1993, p. 3873–3878
0019-9567/93/093873-06$02.00/0

Vol. 61, No. 9

Inhibition of Lipopolysaccharide-Associated Endotoxin Activities In Vitro and In Vivo by the Human Anti-Lipid A Monoclonal Antibody SdJ5-1.17.15

INGHWA S. FANG, MARK A. WISNIEWSKI, CAROLYN C. HUNTENBURG, LORI S. KNIGHT, J. ERIC BUBBERS, AND MARLOWE J. SCHNEIDKRAUT*

Hyland Division, Biotech Group, Baxter Healthcare Corporation, 1720 Flower Avenue, Duarte, California 91010

Received 25 January 1993/Returned for modification 12 April 1993/Accepted 18 June 1993

The present study evaluated the effect of a novel anti-lipid A monoclonal antibody, termed SdJ5, on the in vitro production of tumor necrosis factor alpha (TNF-α) and interleukin-1β by endotoxin- or lipopolysaccharide (LPS)-challenged human peripheral blood mononuclear cells (hPBMC). In addition, the present study determined whether SdJ5 could neutralize the in vivo toxicity of LPS. SdJ5, at a concentration equal to or greater than 3 μg/ml, specifically inhibited TNF-α and interleukin-1β production by hPBMC stimulated with every type of LPS and lipid A assessed. SdJ5 also showed a significantly greater inhibition of cytokine production than a nonrelevant human immunoglobulin M myeloma control. The SdJ5-mediated inhibition of TNF-α production was rapid, as the simultaneous addition of the SdJ5 and LPS still resulted in a marked decrease in hPBMC cytokine synthesis. The ability of SdJ5 to neutralize in vivo toxicity was also determined by using LPS from four different strains of gram-negative bacteria. LPS, when preincubated with SdJ5, resulted in a significant decrease in the 24-h mortality rate compared with that for the control. These studies show that the anti-lipid A monoclonal antibody SdJ5 can modulate LPS-induced cytokine production in vitro and increase the survival rate of rats challenged with lethal doses of LPS.

Septic shock is currently the most common cause of death in intensive care units in the United States (27). Current statistics estimate that there are 200,000 cases of septic shock per year in the United States and 100,000 of these cases result in death. There are several contributory causes for the high incidence of septic shock, including the increased use of cytotoxic and immunosuppressive drugs and the appearance of antibiotic-resistant bacteria. Endotoxin, or lipopolysaccharide (LPS), an integral component of the outer membrane of gram-negative bacteria, has been implicated as a primary initiator of the pathogenesis of this form of septic shock (23).

Recent studies showed that LPS, following its interaction with membrane components of monocytes (5, 15), induced the release of proinflammatory cytokines (3, 5, 9, 33) as well as other inflammatory mediators (20, 24, 28). Other studies have indicated that proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β), play significant roles in the pathogenesis of septic shock (3, 4, 7, 18, 21, 29).

Since LPS induces the release of proinflammatory cytokines, antibodies to LPS have been evaluated as a means to attenuate the endotoxin-associated pathology (2, 12–14, 30, 34, 35). Different investigators have assessed antibodies against the different regions of the LPS molecule, with variable results. For instance, antibodies to the type-specific O side chain of LPS, when administered to mice before endotoxin challenge, resulted in a significant increase in the survival rate (2). However, the protection afforded by these antibodies was not LPS cross-reactive. In order to provide protection against LPS derived from a broad range of gram-negative bacteria, monoclonal antibodies (MAb) against the structurally conserved and biologically active lipid A moiety of LPS have been developed. The effectiveness of these anti-lipid A MAb in both laboratory and clinical studies has been variable (2, 11, 13, 14, 30, 35). This variability may be attributed to different specificities of the MAb directed against unique lipid A epitopes, which, in turn, could be responsible for the different in vitro and in vivo activities (19).

In order to address these sources of variability, a novel human immunoglobulin M (IgM) MAb, SdJ5-1.17.15 (SdJ5), was developed against lipid A (8). Subsequent studies demonstrated that SdJ5 binds to an epitope on lipid A associated with its biologic activity (17). The present study evaluated the therapeutic potential of SdJ5 and characterized the in vitro inhibitory effects of this MAb on LPS-induced TNF-α and IL-1β production by human peripheral blood mononuclear cells (hPBMC). In addition, its effectiveness in neutralizing the in vivo toxicities of several strains of endotoxin was assessed.

MATERIALS AND METHODS

Human antibodies. Human IgM MAb SdJ5 (Baxter Healthcare Corporation Hyland Division, Duarte, Calif.) was utilized in the experiments. Antibody was formulated at 1 mg/ml in a sterile, pyrogen- and protein-free saline buffer solution and stored at 5°C. Vehicle controls were identical to the SdJ5 formulation buffer except that antibody was not present. Human myeloma IgM protein used as a negative control was purchased from Jackson Laboratory (Bar Harbor, Maine).

Lipid A and LPS preparation. LPSs from *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (List Biochemicals, Campbell, Calif.), *Salmonella minnesota* R595 (Re mutant), *Salmonella typhimurium* SL 1181 (Re mutant), and *Esche-*

* Corresponding author.

richia coli O55:B5 (Sigma Chemical Co., St. Louis, Mo.) and lipid A from *S. typhimurium* (Re mutant) (ICN Biochemicals, Irvine, Calif.) were used in this study. Unless otherwise stated, all lipid A and LPS samples were reconstituted in phosphate-buffered saline containing 20 mM EDTA and 0.1% (vol/vol) triethanolamine (Sigma), pulse sonicated (Branston Sonifier Cell Disrupter 200; duty cycle, 40%; output control, 3) for 10 min at 5°C, and stored at −70°C. Lipid A and LPS concentrations were adjusted from 4 to 40 ng/ml with complete RPMI medium consisting of RPMI-1640 (Whittaker M. A. Bioproducts, Walkersville, Md.) supplemented with 50 U of penicillin and 50 μg of streptomycin per ml, 2 mM L-glutamine (GIBCO, Grand Island, N.Y.), and 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (Sigma).

hPBMC isolation. hPBMC from healthy donors were isolated from heparinized (20 U/ml) (Elkins-Sinn, Cherry Hill, N.J.) blood. The hPBMC fraction was isolated on Ficoll-Hypaque (Sigma) by density centrifugation (400 × *g* for 30 min). The buffy coat was washed three times in RPMI (100 × *g* for 15 min) and then resuspended at 5×10^6 cells per ml in complete RPMI containing 2% heat-inactivated (30 min at 56°C) human AB serum.

In vitro cytokine induction procedure. SdJ5 and LPS were combined in a 1:1 (vol/vol) ratio and preincubated for 1 h (37°C, 5% CO_2) unless indicated otherwise. The SdJ5-LPS mixture was then added to an equal volume of hPBMC (5×10^6 cells per ml) and was cultured for 16 to 24 h (37°C, 5% CO_2) unless otherwise indicated. The cultures were then processed through three −70°C freeze-thaw cycles to disrupt the cell membranes. The cellular debris was pelleted by centrifugation at 500 × *g* for 2 min. The supernatant was removed, diluted 1:3 in complete RPMI medium containing 2% AB serum, and stored at −70°C until analyzed for TNF-α and IL-1β concentrations.

TNF-α and IL-1β ELISA. Both the total (freeze-thaw) lysates as well as hPBMC supernatants were evaluated for human TNF-α and IL-1β by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Quantikine, Minneapolis, Minn.). The cytokine levels were derived from standard curves generated for each experiment according to the manufacturer's suggested procedures (Molecular Devices Softmax Software).

LPS lethality model. Male Sprague-Dawley rats (175 to 200 g) (Charles River, Wilmington, Mass.) were sensitized to LPS by a modification of the methods of Freudenberg and Galanos (10). Rats were rendered unconscious by the inhalation of CO_2 and intravenously injected simultaneously with D-(+)-galactosamine (400 mg/kg of body weight) and one of the following strains of LPS: *S. minnesota* R595 (Re), *S. typhimurium* (Re), *E. coli* O55:B5, or *P. aeruginosa*. The LPS was administered intravenously at a dose range from 0.001 to 1,000 μg/kg, and the 24-h survival rate for each treatment group was determined. On the basis of these survival data, the dose of each LPS causing a 70 to 90% mortality rate (LD_{70-90}) was identified. Each LPS was preincubated with various amounts of SdJ5 (SdJ5/LPS ratio, 100:1, 400:1, 500:1, 600:1, or 10,000:1) or an equivalent volume of vehicle for 1 h at 37°C in a water bath (Durabath model 2975-22; Labline Instruments, Inc., Melrose Park, Ill.). Immediately following incubation, the previously determined LPS LD_{70-90} plus either SdJ5 or vehicle was transferred to a 5-ml syringe. To this was added D-(+)-galactosamine (400 mg/kg), and the injection volume was adjusted to 3.0 ml with sterile isotonic saline. The survival rate of the SdJ5-LPS-injected rats was compared with that for the appropriate vehicle-LPS-injected controls.

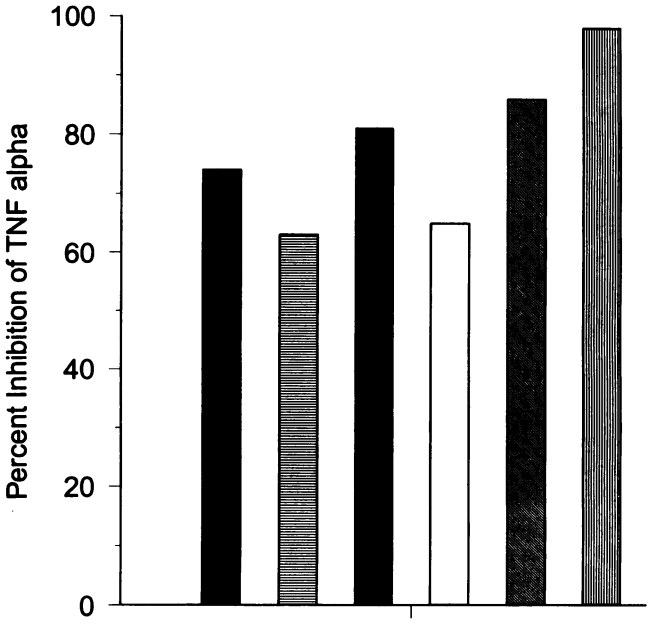

FIG. 1. SdJ5 inhibition of endotoxin-induced TNF-α production. One of five LPSs or lipid A (1 to 10 μg/ml) was preincubated with SdJ5 (3 μg/ml) or vehicle, added to hPBMC, and cultured for 16 to 24 h. SdJ5 markedly inhibited LPS- or lipid A-induced TNF-α synthesis by hPBMC assayed in triplicate. hPBMC of three donors were used with *E. coli* (■), *K. pneumoniae* (▤), *S. minnesota* R595 (□), and *S. typhimurium* (Re) (▧) LPSs. hPBMC of one donor were used with *P. aeruginosa* LPS (▨) as well as with *S. typhimurium* lipid A (▥).

Statistical analysis. The inhibitory effects of SdJ5 and myeloma IgM on LPS-induced TNF-α production by hPBMC were compared by an analysis of variance plus a Newman-Keul test. The effects of SdJ5 on LPS-induced cytokine levels in the hPBMC supernatants and cell lysates were evaluated by an unpaired Student's *t* test. Similarly, the effect of the duration of SdJ5 and LPS preincubation on the in vitro TNF-α production was assessed by an unpaired Student *t* test. The relationship between the dose of LPS and the survival rate of rats was evaluated by determining the Pearson product-moment correlation. Finally, the in vivo effect of SdJ5 was assessed by chi-square analysis. For each study and each statistical test, the confidence interval was set at 95%.

RESULTS

LPS-induced TNF-α production in vitro. In order to evaluate the ability of SdJ5 to inhibit cytokine production in vitro, it was first necessary to determine the concentrations of LPS required to induce cytokine production by hPBMC. Five LPSs and one lipid A were screened for TNF-α inductive potential. A marked increase in total TNF-α production occurred when the hPBMC were incubated with 1 to 10 ng of LPS or lipid A per ml, and this concentration range was chosen for all subsequent experiments evaluating SdJ5 function.

SdJ5 inhibition of LPS-induced TNF-α production by hPBMC. Once the concentration of LPS required to induce

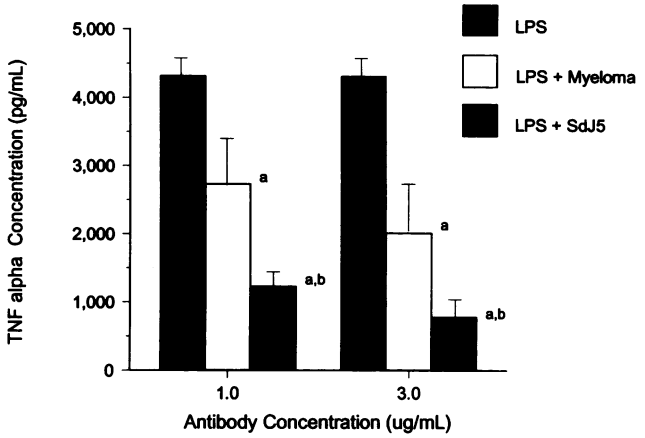

FIG. 2. Specificity of SdJ5-mediated inhibition of TNF-α production. *S. minnesota* R595 LPS (10 ng/ml) was preincubated with either vehicle, human IgM myeloma, or SdJ5, added to hPBMC, and cultured overnight. SdJ5-mediated inhibition of TNF-α production was compared with that in either LPS- or myeloma IgM-LPS-stimulated hPBMC by an analysis of variance plus a Newman-Keul test. The data are expressed as mean cytokine levels with standard errors (bars) for hPBMC isolated from four healthy donors and assayed in triplicate. a, $P < 0.05$ compared with the LPS-alone group; b, $P < 0.05$ compared with the myeloma-LPS group.

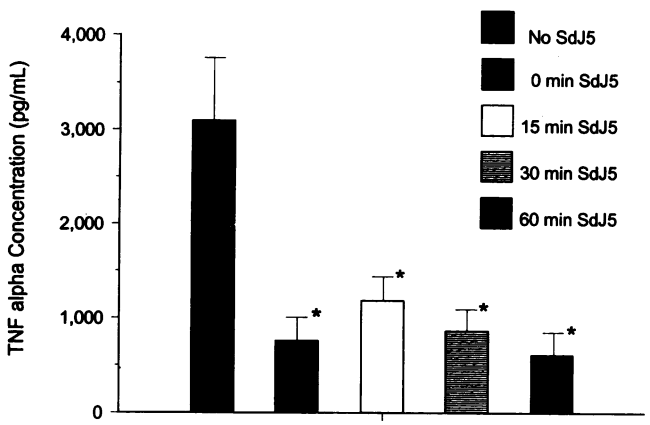

FIG. 3. Effect of preincubation time on SdJ5-mediated inhibition of TNF-α production. The production of TNF-α by hPBMC stimulated with *S. minnesota* R595 LPS (10 ng/ml) plus vehicle was compared with that induced by LPS preincubated with SdJ5 (3 μg/ml) for 0, 15, 30, or 60 min. The data are expressed as mean cytokine levels with standard errors (bars) for hPBMC isolated from five healthy donors and assayed in triplicate. *, $P < 0.05$ compared with the control by an analysis of variance plus a Dunnett test.

hPBMC production of TNF-α was established, the inhibitory activity of SdJ5 was evaluated. Preincubation of *S. minnesota* (Re) LPS with increasing concentrations of SdJ5 resulted in a MAb concentration-related inhibition of TNF-α production by hPBMC (data not shown). On the basis of these dose range studies, SdJ5 at 3 μg/ml was evaluated for its ability to inhibit TNF-α production induced by three wild-type LPSs, two mutant LPSs, and one lipid A (Fig. 1). SdJ5 inhibited TNF-α production by hPBMC stimulated with each LPS type tested or lipid A. The level of TNF-α inhibition ranged from 60 to 100%. There was no correlation between the extent of SdJ5-mediated inhibition of TNF-α synthesis and LPS strain type (rough versus smooth).

Specificity of the anti-lipid A MAb-mediated inhibition of TNF-α production. Since SdJ5 inhibited TNF-α production by hPBMC challenged with various types of LPS or lipid A, studies were then performed to determine whether the effect of SdJ5 was specific to this particular IgM. SdJ5 and a nonspecific myeloma IgM were compared for their abilities to inhibit hPBMC production of TNF-α induced by *S. minnesota* R595 LPS. Myeloma IgM nonspecifically decreased TNF-α production by LPS-stimulated hPBMC; however, the inhibition induced by SdJ5 was significantly greater than that induced by the myeloma IgM (Fig. 2). These data show that, although the myeloma protein mediated some inhibition, SdJ5 specifically inhibits LPS-induced TNF-α production by hPBMC.

The next series of studies determined whether preincubation of LPS with SdJ5 was necessary to inhibit hPBMC production of TNF-α. LPS and SdJ5 were either added to the cell suspension simultaneously or premixed for periods ranging from 15 min to 1 h before addition to hPBMC. SdJ5 decreased TNF-α synthesis by LPS-stimulated hPBMC independently of the duration of preincubation (Fig. 3). These data show that SdJ5-mediated inhibition of TNF-α production is rapid, specific, and quantitatively different from the inhibition seen with the myeloma IgM.

SdJ5-mediated inhibition of LPS-induced IL-1β production. In order to determine whether the inhibition of cytokine production by hPBMC was limited to TNF-α, the effect of SdJ5 on the in vitro production of IL-1β was assessed. Stimulation of hPBMC with one of six types of LPS resulted in IL-1β production which was inhibited by 20 to 90% with SdJ5 (Fig. 4). SdJ5 was able to inhibit IL-1β production

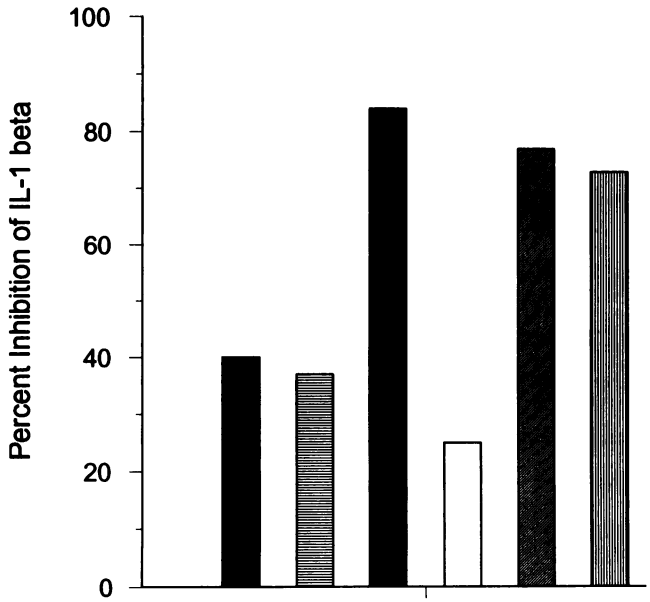

FIG. 4. SdJ5 inhibition of endotoxin-induced IL-1β production. One of five LPSs or lipid A (1 to 10 μg/ml) was incubated with SdJ5 (3 μg/ml) or vehicle, added to hPBMC, and cultured overnight. The data are expressed as the mean inhibition of IL-1β production by hPBMC assayed in triplicate. hPBMC of three donors were used with *S. minnesota* and *S. typhimurium* LPSs. hPBMC of two donors were used with *E. coli* and *K. pneumoniae* LPSs. hPBMC of one donor were used with *P. aeruginosa* LPS as well as with *S. typhimurium* lipid A. See the legend to Fig. 1 for symbols.

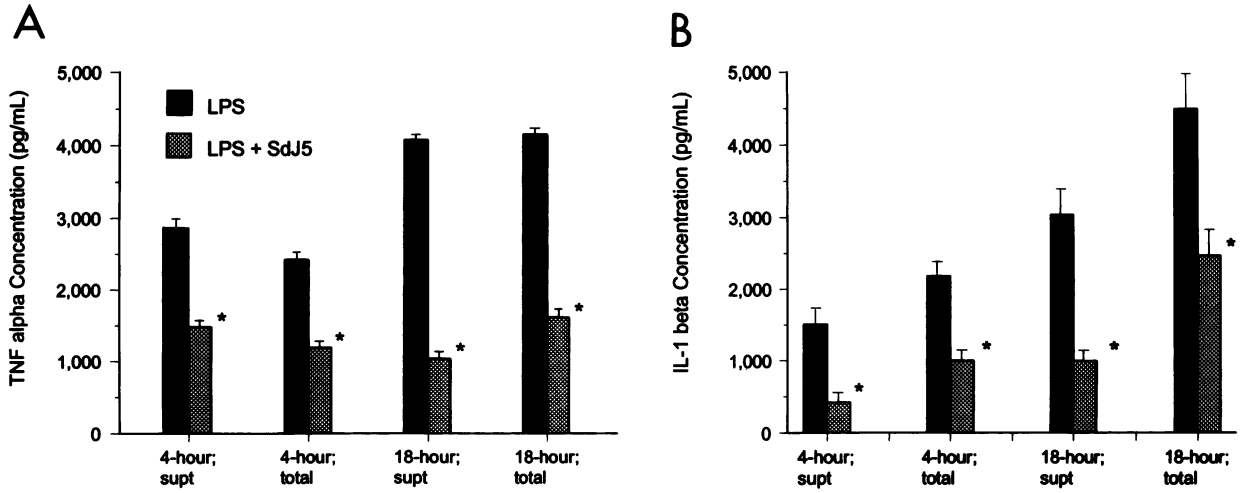

FIG. 5. SdJ5 inhibition of endotoxin-induced cytokine production: effects of cytokine source and culture time. *S. minnesota* LPS (10 ng/ml) preincubated with either SdJ5 (3 μg/ml) or vehicle was added to hPBMC cultures. After either 4 or 18 h in culture, cell lysate (total) or cell-free supernatant (supt) TNF-α (A) and IL-1β (B) levels were determined. The data are expressed as mean cytokine levels with standard errors (bars) for hPBMC isolated from three healthy donors and assayed in triplicate. *, $P < 0.05$ compared with vehicle control by an unpaired Student *t* test.

induced by both rough and smooth types of LPS as well as by lipid A. This indicated that the inhibition of in vitro cytokine production by SdJ5 was not limited to its actions against TNF-α synthesis.

Effects of cell culture time and cytokine source on SdJ5-mediated inhibition of cytokine production. In all of the experiments described above, total cytokine levels from both intracellular and extracellular pools following 16 to 24 h of incubation were measured. In order to determine whether the inhibitory activities of SdJ5 were limited to these conditions, experiments were performed to compare the levels of SdJ5-mediated inhibition of TNF-α cytokine production after 4 and 18 h of incubation. Total (cell lysate) as well as hPBMC supernatant TNF-α levels were measured at each of these time points. SdJ5 significantly inhibited TNF-α (Fig. 5A) and IL-1β (Fig. 5B) production irrespective of whether the cytokines were measured in cell lysates or culture supernatants. In addition, SdJ5 was effective at decreasing cytokine synthesis at both 4 and 18 h of cell culture.

SdJ5-mediated inhibition of LPS toxicity in vivo. Since cytokines have been implicated as mediators in the pathogenesis of gram-negative septic shock, studies were performed to determine whether SdJ5 would neutralize the in vivo toxicity of LPS. In order to evaluate the effect of SdJ5, it was first necessary to establish the toxic dose range of LPS. Intravenous administration of LPS plus D-(+)-galactosamine to rats resulted in a dose-dependent decrease in the 24-h survival rate. The toxicity of the LPS varied from type to type, with *E. coli* O55:B5 LPS being the most toxic (LD_{70-90} = 0.25 μg/kg) and *S. typhimurium* (Re) LPS being the least toxic (LD_{70-90} = 100 μg/kg).

Administration of LPS preincubated with SdJ5 to rats resulted in a significant increase in the 24-h survival rate compared with that for animals injected with LPS plus

TABLE 1. Survival rates (24 h) of rats injected intravenously with various strains of LPS preincubated with either MAb SdJ5 or a vehicle control[a]

Therapeutic agent	MAb/LPS (wt/wt)	*S. typhimurium* (Re) (10 μg/kg)			*S. minnesota* (Re) (10 μg/kg)			*E. coli* O55:B5 (0.25 μg/kg)			*P. aeruginosa* (3 μg/kg)		
		No. of animals	mg of MAb per kg	Survival (%)	No. of animals	mg of MAb per kg	Survival (%)	No. of animals	mg of MAb per kg	Survival (%)	No. of animals	mg of MAb per kg	Survival (%)
SdJ5	100:1	13	1	54	11	1	45	13	0.25	39*	16	0.3	56*
Vehicle		12		17	11		27	15		7	16		31
SdJ5	400:1	13	4	54*	19	4	74*	13	0.1	39	20	1.2	85*
Vehicle		12		8	19		26	12		50	20		45
SdJ5	500:1	13	5	46*	19	5	84*	13	0.125	46	20	1.5	85*
Vehicle		12		8	19		32	12		42	20		45
SdJ5	600:1	14	6	64*	20	6	80*	13	0.15	62*	20	1.8	68*
Vehicle		12		8	20		20	16		25	20		48
SdJ5	10,000:1			ND			ND	16	2.5	63*	ND		ND
Vehicle				ND			ND	16		32	ND		ND

[a] *, $P < 0.05$. ND, not done.

vehicle (Table 1). The ability of SdJ5 to neutralize the in vivo toxicity of LPS appeared to vary with the LPS type; however, SdJ5 was capable of increasing the 24-h survival rate for each of the four types of LPS evaluated (Table 1). In addition, the ability of SdJ5 to neutralize the in vivo toxicity of LPS was not limited to either rough or smooth strains of endotoxin (Table 1). These data show that SdJ5 can neutralize the in vivo toxicity of LPS.

DISCUSSION

TNF-α and IL-1β have been implicated as major mediators of endotoxic shock (13, 21). These cytokines, produced by LPS-stimulated mononuclear leukocytes, act synergistically to produce the clinical symptoms of endotoxic shock. Consequently, agents such as antibodies or receptor antagonists which block the effects of TNF-α or IL-1β have been found to be effective in ameliorating the lethal effects of LPS (1, 16, 26, 32). It was of interest, then, to determine whether an anti-lipid A MAb could, by virtue of its capacity to inhibit LPS-induced cytokine production, produce the same effects as reagents which block cytokine activity.

The human IgM MAb SdJ5 was generated against a heat-killed *S. minnesota* R595 whole-organism vaccine and has previously been reported to recognize an epitope expressed on the lipid A moiety of LPS (17). The present study was designed to determine whether the anti-lipid A specificity of SdJ5 conferred upon this antibody the ability to neutralize LPS-induced cytokine production in vitro and whether this activity correlated with the ability of SdJ5 to protect rats from lethal endotoxin challenge.

SdJ5 specifically inhibited LPS-induced cytokine production in a concentration-dependent manner. This specific inhibition was seen at an equimolar or greater SdJ5/LPS ratio and was observed to be independent of the LPS or lipid A type tested. These data agree with those of Kazemi et al. (17), who showed that SdJ5 bound to various smooth and rough LPSs in the liquid phase. It is this interaction between the LPS and SdJ5 that apparently prevented the production of cytokines by hPBMC challenged with various types of LPS. While most of the data presented here involved TNF-α and IL-1β measurements taken after 16 to 24 h of culture, SdJ5 was also found to block cytokine production after only 4 h. This is especially relevant since in many in vivo models of endotoxic shock serum TNF-α levels are observed to peak acutely after LPS challenge (16, 22, 25).

Other investigators have studied the capacity of anti-lipid A MAb to inhibit LPS-induced cytokine production, with variable results. Chia et al. (6) tested an extensive panel of murine MAb directed against either core LPS or lipid A epitopes and did not detect any MAb able to prevent LPS-induced TNF-α production in vitro by RAW 264.7 macrophages. Similarly, the human IgM anti-lipid A MAb, HA-1A, was found not to inhibit circulating TNF-α production in mice challenged with LPS (2). However, Vacheron et al. (31) studied three murine anti-core LPS MAb and found that two of the MAb effectively lowered murine serum TNF-α levels when administered 3 h prior to LPS challenge. Thus, different MAb directed against either the lipid A or the core region of LPS apparently differ in their capacities to block LPS-induced cytokine production. Whether this variability can be ascribed to differences in the epitopes recognized by different MAb, to differences in the experimental systems employed, or to other factors cannot be determined.

To address the question of whether the in vitro activity of SdJ5 correlated with the in vivo activity, SdJ5 was tested for its capacity to protect rats from lethal LPS challenge. Incubation of SdJ5 with LPS prior to intravenous administration in rats resulted in a significant decrease in the 24-h mortality rate compared with the vehicle-preincubated control group. This decreased mortality rate was seen with four types of LPS. To our knowledge, this study is the first report that a cross-reactive anti-lipid A MAb which inhibits LPS-induced cytokine release in vitro can also protect against the lethal effects of endotoxin. These data do not preclude the possibility that MAb such as SdJ5 exert their in vivo effects by other mechanisms in addition to their effects on cytokine production. Nor do these data speak to the possibility that other anti-lipid A MAb, which do not inhibit cytokine production, are capable of affording in vivo protection by mechanisms such as antigen clearance. Given that multiple epitopes are expressed on lipid A (19), it is possible that different anti-lipid A MAb recognizing different epitopes may manifest their biological functions via different mechanisms.

In summary, preincubation of various strains of LPS with the anti-lipid A MAb SdJ5 resulted in a significant decrease in the in vivo toxicity of endotoxin. Studies of the in vitro interaction between SdJ5 and LPS indicated that the MAb may produce its in vivo effects by virtue of its ability to inhibit LPS-induced cytokine production. These data support the notion that a further preclinical evaluation of this anti-lipid A MAb is warranted.

ACKNOWLEDGMENTS

We thank Elise Greenlee and Leslie Atkinson for their assistance in the preparation of the manuscript.

REFERENCES

1. **Alexander, H. R., G. M. Doherty, C. M. Buresh, D. J. Venzon, and J. A. Norton.** 1991. A recombinant human receptor antagonist to interleukin 1 improves survival after lethal endotoxemia in mice. J. Exp. Med. **173**:1029–1032.
2. **Baumgartner, J. D., D. Heumann, J. Gerain, P. Weinbeck, G. E. Grau, and M. P. Glauser.** 1990. Association between protective efficacy of anti-lipopolysaccharide (LPS) antibodies and suppression of LPS-induced tumor necrosis factor alpha and interleukin 6. Comparison of O side chain-specific antibodies with core LPS antibodies. J. Exp. Med. **171**:889–896.
3. **Beutler, B., and A. Cerami.** 1986. Cachectin and tumour necrosis factor as two sides of the same biological coin. Nature (London) **320**:584–588.
4. **Beutler, B., and A. Cerami.** 1987. Cachectin: more than a tumor necrosis factor. N. Engl. J. Med. **316**:379–385.
5. **Cavaillon, J. M., and N. Haeffner-Cavaillon.** 1990. Signals involved in interleukin 1 synthesis and release by lipopolysaccharide-stimulated monocytes/macrophages. Cytokine **2**:319–329.
6. **Chia, J. K. S., M. Pollack, G. Guelde, N. L. Koles, M. Miller, and M. E. Evans.** 1989. Lipopolysaccharide (LPS)-reactive monoclonal antibodies fail to inhibit LPS-induced tumor necrosis factor secretion by mouse-derived macrophages. J. Infect. Dis. **159**:872–880.
7. **Dinarello, C. A.** 1989. Interleukin-1 and its biologically related cytokines. Adv. Immunol. **44**:153–205.
8. **Dorai, H., J. E. Bubbers, and S. D. Gillies.** 1992. Cloning and re-expression of a functional human IgM anti-lipid A antibody. Hybridoma **11**:667–675.
9. **Fong, Y., L. L. Moldawer, M. Marano, H. Wei, S. Tatter, R. Clarick, U. Santhanam, D. Sherris, L. May, P. Sehgal, and S. Lowry.** 1989. Endotoxemia elicits increased circulating B2-IFN/IL-6 in man. J. Immunol. **142**:2321–2324.
10. **Freudenberg, M. A., and C. Galanos.** 1987. Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine-treated mice. J. Exp. Med. **165**:657–663.

11. **Gazmuri, R. J., C. Mecher, and M. H. Weil.** 1991. The HA-1A monoclonal antibody for gram-negative sepsis (correspondence). N. Engl. J. Med. **325**:279–280.
12. **Goto, M., P. Zeller, R. M. Hurley, J. S. Jong, and C.-H. Lee.** 1991. Prophylaxis and treatment of newborn endotoxic shock with anti-lipid A monoclonal antibodies. Circ. Shock **35**:60–64.
13. **Greenberg, R. N., K. M. Wilson, A. Y. Kunz, N. I. Wedel, and K. J. Gorelick.** 1992. Observations using antiendotoxin antibody (E5) as adjuvant therapy in humans with suspected, serious, gram-negative sepsis. Crit. Care Med. **20**:730–735.
14. **Greenman, R. L., R. M. H. Schein, M. A. Martins, R. P. Wenzel, N. R. MacIntyre, G. Emmanuel, H. Chanel, R. B. Kohler, M. MacCarthy, J. Plouffe, J. A. Russell, et al.** 1991. A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of Gram negative sepsis. JAMA **266**:1091–1102.
15. **Haeffner-Cavaillon, N., J.-M. Cavaillon, and L. Szabó.** 1985. Cellular receptors for endotoxin, vol. 3, p. 1–24. Elsevier Science Publishers B.V., New York.
16. **Hinshaw, L. B., P. Tekamp-Olson, A. C. K. Change, P. A. Lee, F. B. J. Taylor, C. K. Murray, G. T. Peer, T. E. J. Emerson, R. B. Passey, and G. C. Kuo.** 1990. Survival of primates in LD100 septic shock following therapy with antibody to tumor necrosis factor. Circ. Shock **30**:279–292.
17. **Kazemi, M., C. C. Huntenburg, and J. E. Bubbers.** Lipopolysaccharide epitope specificity and binding cross reactivity of the human IgM anti-lipid A monoclonal antibody SdJ5-1.17.15. Mol. Immunol., in press.
18. **Kiener, P. A., F. Marek, G. Rodgers, P. F. Lin, G. Warr, and J. Desiderio.** 1988. Induction of tumor necrosis factor, IFN-, and acute lethality in mice by toxic and non-toxic forms of lipid A. J. Immunol. **141**:870–874.
19. **Kuhn, H., L. Brade, B. J. Appelmelk, S. Kusumoto, E. T. Rietschel, and H. Brade.** 1992. Characterization of the epitope specificity of murine monoclonal antibodies directed against lipid A. Infect. Immun. **60**:2201–2210.
20. **Kunkel, S. L., S. W. Chensue, and S. H. Phan.** 1986. Prostaglandins as endogenous mediators of interleukin 1 production. J. Immunol. **136**:186–192.
21. **Okusawa, S., J. A. Gelfand, T. Ikejima, R. J. Connolly, and C. A. Dinarello.** 1988. Interleukin 1 induces a shock-like state in rabbits: synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition. J. Clin. Invest. **81**:1162–1172.
22. **Peel, J. E., M. J. Voirol, C. Kolly, D. Gobet, and S. Martinod.** 1990. Induction of circulating tumor necrosis factor cannot be demonstrated during septicemic salmonellosis in calves. Infect. Immun. **58**:439–442.
23. **Rietschel, E. T.** 1984. Chemistry of endotoxin. Handb. Endotoxin **1**:187–214.
24. **Semeraro, N., A. Biondi, R. Lorenzet, D. Locati, A. Mantovani, and M. B. Donati.** 1983. Direct induction of tissue factor synthesis by endotoxin in human macrophages from diverse anatomical sites. Immunology **50**:529–535.
25. **Silva, A. T., B. J. Appelmelk, W. A. Buurman, K. F. Bayston, and J. Cohen.** 1990. Monoclonal antibody to endotoxin core protects mice from Escherichia coli sepsis by a mechanism independent of tumor necrosis factor and interleukin-6. J. Infect. Dis. **162**:454–459.
26. **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.
27. **Snell, R. J., and J. E. Parrillo.** 1991. Cardiovascular dysfunction in septic shock. Chest **99**:1000–1009.
28. **Strunk, R. C., A. S. Whitehead, and F. S. Cole.** 1985. Pretranslational regulation of the synthesis of the third component of complement in human mononuclear phagocytes by the lipid A portion of lipopolysaccharide. J. Clin. Invest. **76**:985–990.
29. **Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Melsaik, R. J. Hariri, T. J. Fahey III, A. Zentella, J. D. Albert, G. T. Shires, and A. Cerami.** 1986. Shock and tissue injury induced by recombinant human cachectin. Science **234**:470–474.
30. **Tune, B. M., C. Y. Hsu, M. M. Bieber, and N. N. H. Teng.** 1989. Effects of anti-lipid A human monoclonal antibody on lipopolysaccharide-induced toxicity to the kidney. J. Urol. **141**:1463–1466.
31. **Vacheron, F., E. Mandine, R. Lennour, P. Smets, R. Zalisz, and M. Guenounou.** 1992. Inhibition of production of tumor necrosis factor by monoclonal antibodies to lipopolysaccharides. J. Infect. Dis. **165**:873.
32. **Wakabayashi, G., J. A. Gelfand, J. F. Burke, R. C. Thompson, and C. A. Dinarello.** 1991. A specific receptor antagonist for interleukin 1 prevents Escherichia coli-induced shock in rabbits. FASEB J. **5**:338–343.
33. **Yoshimura, T., K. Matsushima, and J. J. Oppenheimer.** 1987. Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL-1). J. Immunol. **139**:788–793.
34. **Young, L., R. Gascon, S. Alam, and L. Bermudez.** 1989. Monoclonal antibodies for treatment of gram-negative infections. Rev. Infect. Dis. **II**:S1564–S1571.
35. **Ziegler, E. J., C. J. Fisher, C. L. Sprung, R. C. Straube, J. C. Sadoff, G. E. Foulke, C. H. Wortel, M. P. Fink, R. P. Dellinger, N. N. H. Teng, I. E. Allen, H. J. Berger, G. L. Knatterud, A. F. LoBuglio, C. R. Smith, et al.** 1991. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. N. Engl. J. Med. **324**:429.