Thalidomide protects mice against LPS-induced shock

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Abstract

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Thalidomide has been shown to selectively inhibit TNF-α production *in vitro* by lipopolysaccharide (LPS)-stimulated monocytes. TNF-α has been shown to play a pivotal role in the pathophysiology of endotoxic shock. Using a mouse model of LPS-induced shock, we investigated the effects of thalidomide on the production of TNF-α and other cytokines and on animal survival. After injection of 100-350 μg LPS into mice, cytokines including TNF-α, IL-6, IL-10, IL-1ß, GM-CSF and IFN-γ were measured in the serum. Administration of 200 mg/kg thalidomide to mice before LPS challenge modified the profile of LPS-induced cytokine secretion. Serum TNF-α levels were reduced by 93%, in a dose-dependent manner, and TNF-α mRNA expression in the spleens of mice was reduced by 70%. Serum IL-6 levels were also inhibited by 50%. Thalidomide induced a two-fold increase in serum IL-10 levels. Thalidomide treatment did not interfere with the production of GM-CSF, IL-1ß or IFN-γ. The LD$_{50}$ of LPS in this model was increased by thalidomide pre-treatment from 150 μg to 300 μg in 72 h. Thus, at otherwise lethal doses of LPS, thalidomide treatment was found to protect animals from death.

Introduction

Injection of lipopolysaccharide (LPS) into experimental animals induces a massive production of tumor necrosis factor-α (TNF-α) and other endogenous inflammatory mediators which are associated with pathological manifestations such as fever, anorexia, malaise and shock (1,2). The role of TNF-α in the pathogenesis of endotoxic shock has been extensively investigated. Injection of recombinant TNF-α into experimental animals mimics the pathological alterations found in endotoxic shock (3). Monoclonal antibodies directed against TNF-α prevent lethality in the mouse (4) and in the primate models (5), when given prior to LPS challenge. Further evidence for the role of TNF-α in the pathogenesis of endotoxic shock has emerged from studies with TNF-α receptor knock-out mice, which are unresponsive to TNF-α, and are thereby protected from endotoxic shock (6). Other LPS-induced cytokines, such as interleukin-1 (IL-1) and interferon gamma (IFN-γ), have also been implicated in the pathogenesis of endotoxic shock (2). Administra-

tion of IL-1 receptor antagonist has been shown to abolish lethality in experimental models (7,8). In addition, IFN-γ receptor knock-out mice are resistant to lethal doses of LPS (9). This suggests an interactive cytokine network or cascade of effector molecules contributing to the pathophysiology of endotoxic shock syndrome.

The development of therapies directed towards the inhibition of TNF-α production has been an important goal in improving the management of endotoxic shock and TNF-α-induced pathology. Drugs such as pentoxifylline (10,11), hydrazine sulfate (12), and some metalloprotease inhibitors (13) have been shown to inhibit TNF-α production *in vitro* and *in vivo*; this inhibition is associated with protection from lethal doses of LPS injected into experimental animals.

Thalidomide, α-N-phthalimidoglutarimide, has been shown to selectively inhibit TNF-α production *in vitro* in human peripheral blood-derived monocytes stimulated with LPS or mycobacterial agonists (14,15). In the present study we utilized the murine model of endotoxic shock to investigate the effects of thalidomide on LPS-induced cytokine production *in vivo* and on animal survival after endotoxic shock.

Material and Methods

Mice

Female B6D2/F1 (C57/b6 x DBA2) mice (Jackson Laboratories, Bar Harbor, ME), 7-9 weeks old, were used in the experiments. Mice were housed in group cages at the Animal Research Center, Rockefeller University.

Reagents

Thalidomide was supplied by Celgene Corporation (Warren, NJ). The drug was dissolved in sterile dimethylsulfoxide (DMSO) (Sigma Chemical Co., St. Louis,

MO) at 50 mg/ml (stock concentration), diluted in acidified pyrogen-free saline to the desired concentration and used immediately. The final concentration of DMSO was 2%. Lipopolysaccharide B from *E. coli* 026:B6 (Difco Laboratories, Detroit, MI) was reconstituted in saline to give a stock concentration of 1 mg/ml and stored at -20ºC until use.

Experimental design

Thalidomide was administered intraperitoneally to the mice at two doses of 200 mg/kg in saline with 2% DMSO (unless otherwise specified) 18 h and 2 h prior to LPS challenge. This regimen of thalidomide administration induced sedation of the mice 30 min after the drug had been injected into the peritoneal cavity. Control animals received injections of saline with 2% DMSO. LPS at doses ranging from 100 to 350 µg per mouse was injected intravenously into a lateral tail vein at the beginning of the experiment to characterize the dose-response effect of LPS. For blood collection by cardiac puncture mice were anesthetized with a solution of ketamine (44 mg/kg) (Aveco Co., Inc., Fort Dodge, IO) and xylazine (5 mg/kg) (Rompum, Mobay Corp., Shawnee, KS). Blood was collected at regular intervals from 15 min to 12 h after LPS injection. Serum was separated and stored at -80ºC until assay. Livers and spleens were obtained immediately after cardiac puncture and processed for RNA extraction (see below).

Serum TNF-α level determination

Serum TNF levels were measured by the cytotoxicity assay described by Fisch and Gifford (16). Briefly, the murine fibrosarcoma cell line L929B was used as the target for TNF-induced cytotoxicity. Cells were seeded at 5 x 10⁴ cells/well in a 96-well flat bottom plate (Costar, Inc., Cambridge, MA) in RPMI 1640 (Gibco, Grand Island, NY)

supplemented with 5% fetal calf serum (FCS) (Atlanta Biologicals, Norcross, GA) (culture medium) and incubated overnight at 37°C in a 5% CO_2 humidified incubator. Sequential two-fold dilutions of the sera were made in culture medium containing 2 μg/ml actinomycin-D (Calbiochem-Boehring Corp., La Jolla, CA). One hundred μl of each dilution was added to wells containing a confluent monolayer of L929B cells. After 18-20 h of incubation, plates were fixed and stained with 0.2% crystal violet in 2% ethanol solution. Plates were washed and analyzed with an ELISA plate reader (Dynatech Laboratories, Chantilly, VA) at 570 nm. TNF titer (U/ml) was defined as the inverse of the dilution that caused 50% destruction of the monolayer. Serum TNF-α levels were also evaluated by ELISA (see below), and the correlation coefficient between the TNF-α levels measured by ELISA or bioassay was r = 0.93 and P = 0.0001.

Cytokine determination

TNF-α, IFN-γ, GM-CSF, IL-6, and IL-10 levels were measured with commercial ELISA kits for these cytokines (Endogen, Inc., Boston, MA). Kits were used according to manufacturer specification.

Northern blots

Total cellular RNA was obtained from 0.1 g each of liver or spleen of LPS-injected mice treated with thalidomide or DMSO. Tissues were homogenized in 2 ml of RNAzol B (Cinna/Biotecx Lab., Inc., Houston, TX) and RNA was extracted according to manufacturer instructions. RNA was size fractionated by agarose/formaldehyde gel electrophoresis and transferred onto a nylon membrane (Biorad, Hercules, CA). Membranes were hybridized as described elsewhere (15) with ^{32}P-labeled random-primed probes specific for murine TNF-α (1.1-kb *Eco*RI fragment), IL-1ß (2.0-kb *Bam*HI-

*Hin*dIII fragment), IL-10 (1.34-kb *Bam*HI fragment), IFN-γ (0.7-kb *Pst*I fragment) (ATCC, Rockville, MD) and ß-actin (1.2-kb *Eco*RI-*Xho*I fragment) (17). Densitometry was performed with a Phosphorimager (Molecular Dynamics, Sunnyvale, CA) and results are reported as density units.

Statistical analysis

The paired *t*-test and the Fisher exact p-test were used when appropriate.

Results

Effect of thalidomide on TNF-α production *in vivo*

LPS was administered intravenously to mice and TNF-α production was induced in a dose-dependent manner. Serum TNF-α levels peaked at 1.5 h after LPS injection and were no longer detectable after 5 h (Figure 1A). There was no subsequent peak of TNF-α, in accordance with previous observations
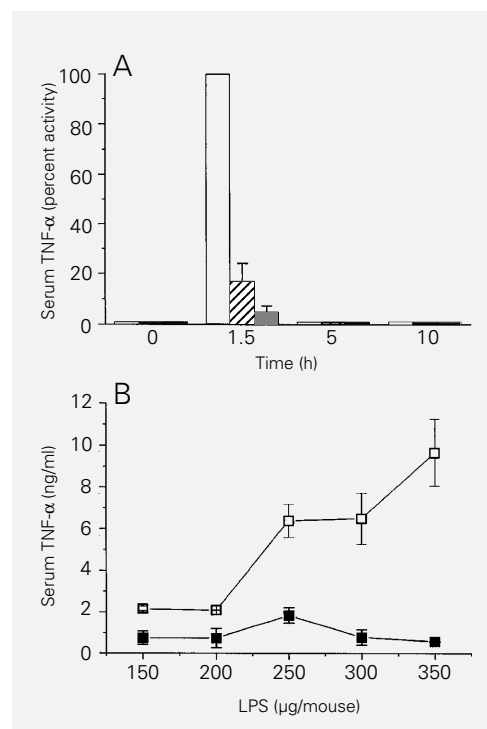

Figure 1 - TNF-α levels in serum of B6D2/F1 mice in response to LPS. *A*, 100 μg of LPS was injected into mice by the intravenous route (*iv*) (open bars). Thalidomide was administered 18 h and 2 h before LPS at 100 mg/kg (hatched bars) or at 200 mg/kg (filled bars). Blood was collected at different time intervals after LPS administration (20 mice per group). *B*, LPS dose response: mice were injected *iv* with different LPS concentrations (open squares), and thalidomide was administered to mice at 2 doses of 200 mg/kg as described above (filled squares). Blood for TNF-α evaluation was collected 1.5 h after injection (4 mice per data point). Results are reported as means ± SD.

(18). The administration of 100 mg/kg thalidomide 2 h before LPS injection resulted in 60-70% inhibition of TNF-α production (19). When the animals were pre-loaded with thalidomide using two doses of 100 mg/kg thalidomide, 18 h and 2 h before the injection of 100 μg of LPS, the reduction in TNF-α levels reached 83 ± 7%. This effect was improved further by administration of two doses of 200 mg/kg thalidomide, now giving an inhibition of 95 ± 2.3% in TNF-α levels, thus demonstrating a dose-dependent effect (Figure 1A). Thalidomide given together or after the LPS challenge did not affect the production of TNF-α *in vivo* (data not shown). Regardless of the dose of LPS used there was a marked reduction in the production of TNF-α in mice treated with two doses of 200 mg/kg thalidomide (Figure 1B). However, thalidomide treatment did not inhibit TNF-α production completely.

At 30 min and 1 h after LPS injection, TNF-α mRNA production was detectable in spleens by Northern blot (Figure 2). TNF-α mRNA was also expressed at low levels in

the livers (data not shown). No TNF-α mRNA expression was observed in organs of mice that had not been challenged with LPS (data not shown). Pre-treatment with two doses of 200 mg/kg thalidomide resulted in a 70% reduction in TNF-α mRNA levels in the spleens evaluated 30 min after LPS administration. A 65% reduction in TNF-α mRNA levels was observed 1 h after LPS challenge (Figure 2).

Effect of thalidomide on LPS-induced cytokines in the mouse

We also evaluated the effect of thalidomide on the production of other LPS-induced inflammatory cytokines. Serum GM-CSF levels peaked at 4 h and returned to baseline levels at 8 h after LPS injection; GM-CSF was not significantly affected by thalidomide treatment (Figure 3). Similarly, IFN-γ levels peaked in the serum at 8 h and were also not inhibited by thalidomide treatment. IL-10 peaked in the serum 1.5 h after the administration of LPS. In the presence of thalidomide, a significant increase in the level of this cytokine was observed (P = 0.02). Although thalidomide treatment did not modify the serum levels of IL-6 1.5 h after LPS injection, when TNF-α inhibition was maximal, a 50% reduction in serum IL-6 levels was observed 4 h after LPS injection in thalidomide-treated mice (Figure 3).

IL-1ß mRNA was detected in the spleens at 30 min after LPS injection, peaked at 1 h and was still detectable in the spleens 4 h after LPS injection. There was no significant inhibition in the expression of IL-1β mRNA in thalidomide-treated mice (Figure 4). We could not detect mRNA expression for IL-10 and IFN-γ in the spleens of mice injected with LPS, with or without thalidomide treatment.

LPS-mediated endotoxic shock
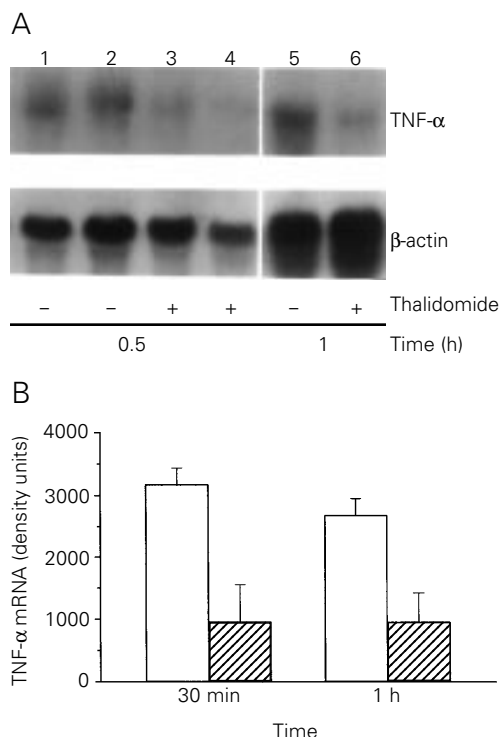
Having demonstrated a significant inhi-

Figure 2 - Northern blot analysis of TNF-α mRNA in spleens of mice injected with 100 μg LPS. *A*, Total RNA was extracted 30 min and 1 h after LPS injection. Lanes 1, 2 and 5 contain RNA (40 μg/lane) extracted from spleens of DMSO-treated mice; lanes 3, 4, and 6 contain RNA from spleens of thalidomide- (2 doses of 200 mg/kg) treated mice. *B*, Densitometric analysis of the Northern blot. Densitometry was performed using a Phosphorimager. Results are reported as mean ± one SD of density units for TNF-α normalized to the amount of ß-actin mRNA in DMSO- (open bars) and thalidomide- (hatched bars) treated mice (5 mice per group).

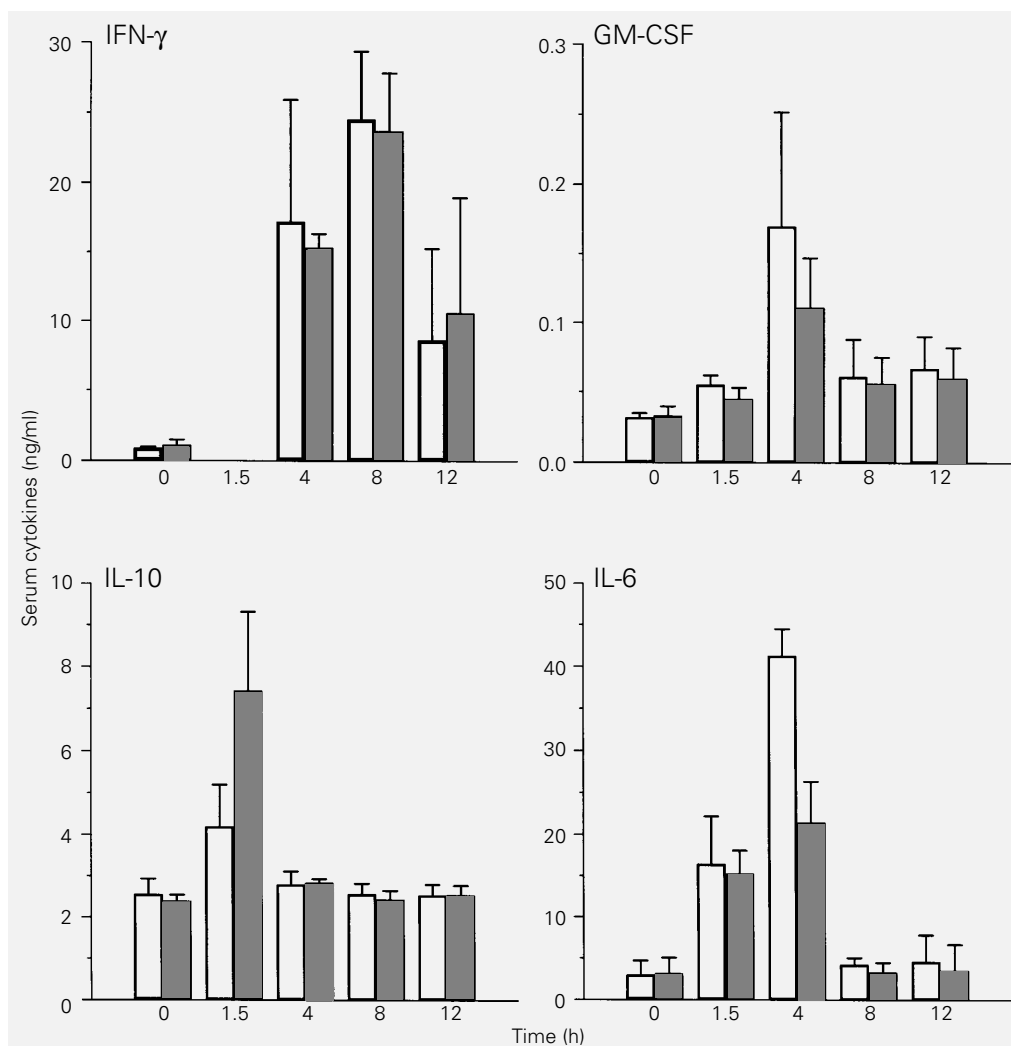Figure 3 - Serum cytokine levels of B6D2/F1 mice challenged with 100 µg LPS. Blood was collected at different times after LPS injection and cytokine levels were measured by ELISA for IFN-γ (6 mice per group), GM-CSF (10 mice per group), IL-10 (10 mice per group), and IL-6 (10 mice per group). Open bars represent DMSO-treated control mice and filled bars represent thalidomide- (2 doses of 200 mg/kg) treated mice.

bition of TNF-α production by thalidomide, and the additional changes in cytokine production described above, we next evaluated the effects of the drug on the survival of animals following LPS-induced shock. DMSO-treated mice injected with LPS became lethargic, and developed pilo-erection and diarrhea. The severity of symptoms and the extent of survival of mice was dependent on the LPS dose used (Figure 5). Control mice began to die 24-48 h after LPS injection. After 72 h there were no further changes in the lethality curve. At higher doses of LPS, death was recorded as early as 24 h (Figure 5B). The LD$_{50}$ for LPS in these experiments ranged from 150 to 250 µg LPS

per mouse. Mice treated with thalidomide had no diarrhea. Although these animals developed somnolence, they could be more easily aroused than the DMSO-treated control mice. Thalidomide (200 mg/kg) given 18 h and 2 h before the injection of 150 and 200 µg LPS protected mice from death (P = 0.03 for LPS injection of 150 µg/mouse and P = 0.04 for doses of 200 µg/mouse). The individual response of mice to LPS varied widely from experiment to experiment. At higher doses of LPS (250-300 µg/mouse) thalidomide treatment was not protective (P>0.05), but was associated with a delay in death. These results suggest that at the higher doses of LPS thalidomide may not be an

Figure 4 - Northern blot analysis of IL-1ß mRNA expression in spleens of mice injected with 100 µg LPS. *A*, Total RNA was extracted at 1 and 4 h after LPS injection. Each lane contains 40 µg of total RNA extracted from spleens of DMSO-treated mice (lanes 1 and 3) or RNA from spleens of thalidomide- (2 doses of 200 mg/kg) treated mice (lanes 2 and 4). *B*, Densitometric analysis of the Northern blot. Densitometry was performed using a Phosphorimager. Results are reported as mean ± SD of IL-1 density units normalized to the amount of ß-actin mRNA in DMSO-treated (open bars) and thalidomide-treated (hatched bars) mice (3 mice per group).
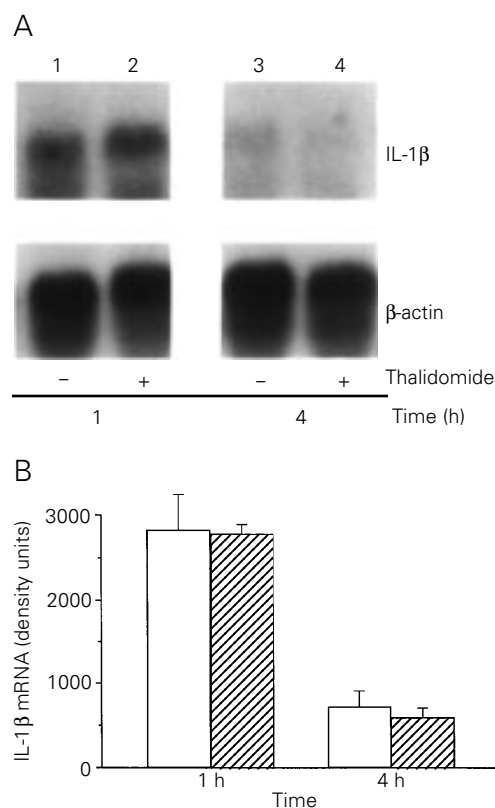
efficient enough drug for protection from the sequelae of LPS-induced cytokine production and shock.

Discussion

Thalidomide was initially developed and used as a sedative without the knowledge of its mechanism of action or teratogenic effects. Recently, the drug has been shown to inhibit the production of TNF-α by reducing the half-life of the TNF-α mRNA in LPS-stimulated human monocytes (15) without directly affecting the production of other cytokines produced by monocytes. Thalidomide is insoluble in water and alcohol, and is very unstable in aqueous solutions. Studies on the pharmacokinetics of thalidomide are not available in the mouse; in a rabbit model, however, treatment of the animals with two or three doses of 150 mg/kg thalidomide improved absorption and accumulation of

the drug in the serum (20). Peak serum levels obtained after three injections of 150 mg/kg thalidomide (20) are similar to those obtained in humans treated with thalidomide for graft-versus-host disease (21). In our experiments, the need to use two doses of 200 mg/kg thalidomide to achieve cytokine modulation may reflect the poor solubility and bioavailability of the drug (22). In fact, in our experiments thalidomide crystals were seen in the peritoneal cavity of the animals after injections of the drug. Based on these considerations, the animals were pre-loaded with thalidomide at doses adequate for sedation, thereby indicating effective pharmacological levels.

The present study demonstrates that administration of thalidomide to mice modified the cytokine response to the intravenous injection of LPS. The drug suppressed TNF-α and IL-6 production and enhanced the production of IL-10. However, thalidomide does not affect IL-6 production in LPS-stimulated monocytes *in vitro* (15,19). Indeed, following treatment with thalidomide, no inhibition of serum IL-6 was observed at 1.5 h, when TNF-α inhibition was maximal. We did observe a 50% reduction in serum IL-6 levels at 4 h after LPS injection. This temporal relationship suggests that the effect of thalidomide on IL-6 may be secondary to the inhibition of TNF-α production. Since it has been shown that TNF-α up-regulates IL-6 production (23), a reduction in the level of TNF-α may account for the lower levels of IL-6 observed at the later time point in our studies. Indeed, in animals treated with antibodies against TNF-α, a reduction in IL-6 and IL-1 production in response to bacteremia has also been observed (23).

Thalidomide-treated mice had a consistent elevation in serum IL-10 levels, although we could not detect IL-10 mRNA in the organs of LPS-injected mice whether or not mice were treated with thalidomide. The inhibition of TNF-α and IL-6 production

observed in thalidomide-treated mice could be secondary to the enhancement of IL-10 production, since IL-10 has been shown to down-modulate the production of TNF-α and other inflammatory cytokines (24,25). However, in an *in vitro* system, thalidomide inhibited TNF-α production in the presence of neutralizing antibodies to IL-10, thus confirming that thalidomide has a direct effect on the inhibition of TNF-α production (19). This inverse relationship of TNF-α and IL-10 production in LPS-stimulated macrophages has been previously observed in cells treated with drugs that increase intracellular cAMP levels (26), as well as in mice injected with LPS and treated with cyclosporine *in vivo* (27). Therefore, the increase in IL-10 levels observed in our experiments may reflect an interference by thalidomide in a regulatory pathway between TNF-α and IL-10. The enhancement of IL-10 production by thalidomide may provide additional benefit in the treatment of inflammatory diseases (28,29).

The inhibition of TNF-α by thalidomide in LPS-challenged mice was also associated with protection from LPS-induced shock and death. This result confirms the importance of TNF-α in the pathophysiology of endotoxic shock. However, the protective effect of thalidomide in preventing death was dependent on the dose of LPS used. Mice could not be protected against very high doses of LPS, despite a measurable inhibition of TNF-α production (Figure 1). Endotoxic shock is multifactorial and the reduction in the levels of TNF-α achieved by the drug may not be sufficient for the prevention of death in every case. Cytokines other than TNF-α such as IL-1, IL-6 and IFN-γ (1,7,9,30,31), and inflammatory mediators such as nitric oxide (31-34) have been shown to contribute to the development of endotoxic shock and death. Higher doses of LPS might induce other pathways independent of TNF-α in the development of shock in this model.
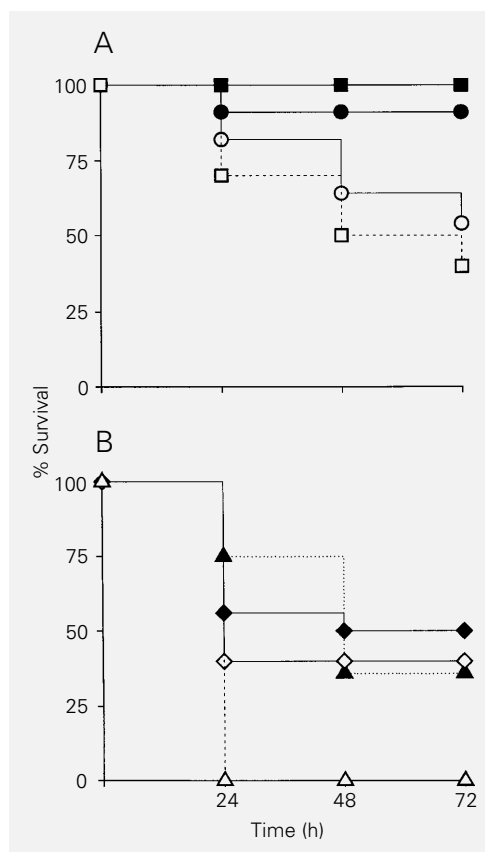

Figure 5 - Survival curve of B6D2/F1 mice injected with different doses of LPS. *A*, Circles indicate that mice were injected with 150 µg of LPS (11 mice per group) and squares indicate LPS at 200 µg/mouse (14 mice per group). *B*, Doses of LPS were used at 250 µg/mouse (diamonds) (16 mice per group) and 300 µg/mouse (triangles) (8 mice per group). Mice were treated with 2 doses of 200 mg/kg thalidomide (filled symbols) or DMSO (open symbols) before LPS injection. Occurrence of death was recorded during the 72-h study period.

The use of thalidomide as a TNF-α inhibitor is currently being tested in diseases that are associated with TNF-α toxicity such as wasting observed in AIDS (35). Whether thalidomide can be added to the current arsenal of drugs used for the therapy of endotoxic shock requires further investigation.

Acknowledgments

References

1. Waage A (1992). Presence and involvement of TNF in septic shock. In: Beutler B (Editor), *Tumor Necrosis Factor: The Molecule and the Emerging Role in Medicine.* Raven Press, New York, 275-284.

2. Doherty GM, Lange JR, Langstein HN, Alexander HR & Buresh CM (1992). Evidence for interferon-gamma as a mediator of the lethality of endotoxin and tumor necrosis factor. *Journal of Immunology,* 149: 1666-1670.

3. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey 3rd TJ, Zentella A & Albert JD (1986). Shock and tissue injury induced by recombinant cachectin. *Science,* 234: 470-474.

4. Beutler B, Milsark IW & Cerami AC (1985). Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. *Science,* 229: 869-871.

5. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF & Cerami A (1987). Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. *Nature,* 330: 662-664.

6. Pfeffer K, Matsuyama T, Kundig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Kronke M & Mak TW (1993). Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. *Cell,* 73: 457-467.

7. Ohisson K, Björk P, Bergenfeld M, Hageman R & Thompson RC (1990). Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature,* 348: 550-552.

8. Alexander HR, Doherty GM, Buresh CM, Venzon DJ & Norton JA (1991). A recombinant human receptor antagonist to interleukin-1 improves survival after lethal endotoxemia in mice. *Journal of Experimental Medicine,* 173: 1029-1032.

9. Kamijo R, Le J, Shapiro D, Havell EA, Huang S, Aguet M, Bosland M & Vilcek J (1993). Mice that lack the interferon-γ receptor have profoundly altered responses to infection with bacillus Calmette-Guérin and subsequent challenge with lipopolysaccharide. *Journal of Experimental Medicine,* 178: 1435-1440.

10. Schade FV (1990). Pentoxifylline increases survival in murine endotoxin shock and decreases formation of tumor necrosis factor. *Circulatory Shock,* 31: 171-181.

11. Zabel P, Schade FV & Schlaak M (1993). Inhibition of endogenous TNF formation by pentoxifylline. *Immunobiology,* 187: 447-463.

12. Silverstein R, Turley BR, Christoffersen CA, Johnson DC & Morrison DC (1991). Hydrazine sulfate protects D-galactosamine-sensitized mice against endotoxin and tumor necrosis factor/cachectin lethality: Evidence of a role for the pituitary. *Journal of Experimental Medicine,* 173: 357-365.

13. Mohler KM, Sleath PR, Fitzner JN, Cerretti DP, Aldreson M, Kerwar SS, Torrance DS, Otten-Evans C, Greenstreet T, Weerawarna K, Kronheim SR, Petersen M, Gehart M, Kozlosky CJ, March CJ & Black RA (1994). Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. *Nature,* 370: 218-220.

14. Sampaio EP, Sarno EN, Galilly R, Cohn ZA & Kaplan G (1991). Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. *Journal of Experimental Medicine,* 173: 699-703.

15. Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA & Kaplan G (1993). Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. *Journal of Experimental Medicine,* 177: 1675-1680.

16. Fisch H & Gifford GE (1983). *In vitro* production of rabbit macrophage tumor cell cytotoxin. *International Journal of Cancer,* 32: 105-112.

17. Gunning P, Okayama H, Engles J, Blau H & Kedes L (1983). Isolation and characterization of full-length cDNA clones for human α-ß- and γ-actin mRNA: Skeletal but not cytoplasmic actins have an amino terminal cysteine that is subsequently removed. *Molecular and Cellular Biology,* 3: 787-795.

18. Remick DG, Strieter RM, Eskaudari MK, Nguyen DT, Genord MA, Raiford CL & Kunkel SL (1990). Role of tumor necrosis factor-α in lipopolysaccharide-induced pathologic alterations. *American Journal of Pathology,* 136: 49-60.

19. Corral LG, Muller GW, Moreira AL, Chen Y, Wu M, Stirling D & Kaplan G (1996). Selection of novel analogs of thalidomide with enhanced tumor necrosis factor α inhibitory activity. *Molecular Medicine,* 2: 506-515.

20. Burroughs MH, Tsenova-Berkova L, Sokol K, Ossig J, Tuomanem E & Kaplan G (1995). Effect of thalidomide on the inflammatory response in cerebrospinal fluid in experimental bacterial meningitis. *Microbial Pathogenesis,* 19: 245-255.

21. Heney D, Norfolk DR, Wheeldon J, Bailey CC, Lewis IJ & Barnard DL (1991). Thalidomide treatment for chronic graft-versus-host disease. *British Journal of Haematology,* 78: 23-27.

22. Zwingenberger K & Wnendt S (1996). Immunomodulation by thalidomide: Systemic review of the literature and of unpublished observations. *Journal of Inflammation,* 46: 177-211.

23. Fong Y, Tracey KJ, Moldawer LL, Hesse DG, Manogue KB, Kenney JS, Lee AT, Kuo GC, Allison AC, Lowry SF & Cerami A (1989). Antibody to cachectin/TNF reduces interleukin-1ß and interleukin-6 appearance during lethal bacteremia. *Journal of Experimental Medicine,* 170: 1627-1633.

24. Ishida H, Hastings R, Thompson-Snipes L & Howard M (1993). Modified immunological status of anti-IL-10 treated mice. *Cellular Immunology,* 148: 371-384.

25. Marchant A, Bruyns C, Vandenabeele P, Ducarne M, Gerard C, Delvaux A, de Grootte D, Abramowicz D, Velu T & Goldman M (1994). IL-10 controls IFN-γ and TNF production during experimental endotoxemia. *European Journal of Immunology,* 24: 1167-1171.

26. Platzer C, Meisel C, Vogt K & Volk HD (1994). Up-regulation of monocytic IL-10 by tumor necrosis factor-α and cAMP elevating drugs. *International Immunology,* 7: 517-523.

27. Durez P, Abramowicz D, Gerard C, Van Mechelen M, Amraoui Z, Dubois C, Leo O, Velu T & Goldman M (1993). *In vivo* induction of interleukin-10 by anti-CD3 monoclonal antibody or bacterial lipopolysaccharide: Differential modulation by cyclosporin A. *Journal of Experimental Medicine,* 177: 551-555.

28. Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, Fiers W, Goldman M & Velu T (1993). Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *Journal of Experimental Medicine,* 177: 547-550.

29. Howard M, Muchamuel T, Andrade S & Menon S (1993). Interleukin 10 protects mice from lethal endotoxemia. *Journal of Experimental Medicine,* 177: 1205-1208.

30. Calandra T, Baumgartner JD, Grau GE, Wu MM, Lambert PH, Schellekens J, Verhoef J & Glauser MP (1990). Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-alpha and interferon-gamma in the serum of patients with septic shock. *Journal of Infectious Diseases*, 161: 982-987.

31. Bucala R (1994). MIF, a previously unrecognized pituitary hormone and macrophage cytokine, is a pivotal mediator in endotoxic shock. *Circulatory Shock,* 44: 35-39.

32. Petros K, Bennett D & Vallance P (1991). Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet*, 338: 1557-1558.

33. Kilbourn RG, Jubran A, Gross AA, Griffith OW, Levi R, Adams J & Lodato RF (1990). Reversal of endotoxin-mediated shock by N^G-methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochemical and Biophysical Research Communications*, 172: 1132-1139.

34. Nava E, Palmer RMJ & Moncada S (1991). Inhibition of nitric oxide synthesis in septic shock: How much is beneficial? *Lancet*, 338: 1555-1556.

35. Klausner DJ, Freedman VH & Kaplan G (1996). Thalidomide as an anti-TNF-α inhibitor: Implications for clinical use. *Clinical Immunology and Immunopathology*, 81: 1-5.