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An Interleukin-6-Induced Acute-Phase Response Does Not Confer Protection against Lipopolysaccharide Lethality

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Lipopolysaccharide (LPS), a component of gram-negative bacterial outer cell walls, can stimulate lymphoreticular cells to produce cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6. One of these proinflammatory cytokines, IL-6, induces hepatic synthesis of a class of proteins termed acute-phase proteins. D-Galactosamine inhibits acute-phase protein synthesis and concurrently sensitizes mice to a lethal dose of LPS approximately 10,000-fold. From these observations, we hypothesized that the acute-phase response may serve as a defense mechanism for protection of the host against the deleterious effects of LPS. To test this hypothesis, murine recombinant IL-6 (mrIL-6) was used to induce an acute-phase response prior to a lethal LPS challenge in both D-galactosamine-treated and normal mice. Induction of the acute-phase response by mrIL-6 was quantitated by measuring the concentrations of fibrinogen and complement component C3, two well-characterized acute-phase proteins, in the circulation. The effect of acute-phase and normal serum on TNF-α release by peritoneal macrophages stimulated with LPS in vitro was also examined. The results of these studies confirmed the induction of the acute-phase response by mrIL-6, as reflected in an approximate doubling in circulating levels of fibrinogen and C3. However, when either D-galactosamine-sensitized or normal mice were challenged with a lethal dose of LPS at various times after mrIL-6 administration, the acute-phase response induced by mrIL-6 did not alter either cumulative lethality or the kinetics of lethality. Additionally, compared with normal serum, acute-phase serum did not affect TNF-α release by peritoneal macrophages following LPS-mediated stimulation in vitro. Collectively, these studies would not support a dominant role for an IL-6-mediated acute-phase response as contributing to the resistance of normal mice compared with D-galactosamine-sensitized mice in LPS-induced lethal toxicity.

Lipopolysaccharide (LPS) is an integral component of the outer membrane of gram-negative bacteria. This immunostimulatory molecule is a major contributing factor in the initiation of a generalized inflammatory response termed septic shock, which often accompanies gram-negative bacteremia. LPS manifests many biological properties, including, but not limited to, the stimulation of lymphoreticular cells to produce cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6 (14). It is thought that the induction of these cytokines is a pivotal event in the development of the septic syndrome, since administration of TNF-α alone can lead to a septic state and death (4), and TNF-α can induce IL-1 and IL-6 in vivo (19). Furthermore, pretreatment with both an antibody to TNF-α and an IL-1 receptor antagonist has been shown to protect experimental animals against the lethal effects of LPS (5, 22, 24). However, the exact roles of these mediators in sepsis remain to be fully defined.

One of the mediators produced by the host in response to LPS, IL-6, is a pluripotent cytokine which can stimulate both lymphoid and nonlymphoid cells. Although IL-6 has been shown to enhance B- and T-lymphocyte maturation (10, 11), it was originally described as a hepatocyte-stimulating factor (17). IL-6 has been documented to stimulate hepatocytes to increase the production of a unique class of proteins whose levels are, in general, elevated in the circulation during the acute phase of bacterial infections. These have been termed acute-phase proteins and include C-reactive protein, serum amyloid protein, mannose-binding protein, cysteine protease inhibitor, lipopolysaccharide-binding protein (21), several complement proteins, and fibrinogen (reviewed in reference 9). Although some of these proteins may be beneficial to the host during bacterial infections (23, 25), the exact role of acute-phase proteins during septic shock induced by LPS is not presently completely understood.

A potential role for the acute-phase response in endotoxemia might be inferred from the observation that the coadministration of LPS and D-galactosamine to experimental animals increases their sensitivity to a lethal dose of LPS by at least 10,000-fold compared with non-pretreated animals (8). While the D-galactosamine sensitization model has been used extensively to study LPS pathogenicity in mice, it presents complications with respect to normal mouse LPS lethality models, including a crisis period for lethality between 8 and 10 h and a potentially heightened dependence on TNF-α as the critical cytokine mediator.

D-Galactosamine has been demonstrated to function as a hepatocyte-specific mRNA synthesis inhibitor (12), which in turn reversibly inhibits hepatic protein synthesis, including the production of acute-phase proteins. The precise biochemical basis for the relationship between abrogation of hepatocyte protein synthesis and the profound increase in the sensitivity of mice to the lethal effects of LPS is, however, not known. We hypothesized, therefore, that the initiation of an acute-phase response by the host in response to LPS might provide protection against LPS challenge. If so, the increased sensitivity of mice after coadministration of LPS and D-galactosamine might reflect the fact that D-galac-

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tosamine prevented the production of acute-phase proteins. Treatment with IL-6 to induce an acute-phase response prior to LPS and D-galactosamine challenge might then be expected to increase the resistance of mice to the lethal effects of LPS.

In the studies reported here, we used murine recombinant IL-6 (mrIL-6) to induce an acute-phase response in mice. The kinetics of the acute-phase response to mrIL-6 were assessed by the quantitation of circulating levels of C3 and fibrinogen, two well-characterized acute-phase proteins. The mice were then challenged with LPS or LPS and D-galactosamine at various time points during this acute-phase response to mrIL-6, and cumulative lethality and the kinetics of lethality were then determined. In addition, serum obtained from mice following mrIL-6 administration was assessed for its capacity to modulate the secretion of TNF-α from peritoneal macrophages following LPS stimulation in vitro. The results of these studies do not support a protective role for IL-6-induced acute-phase protein synthesis in the host response to LPS.

MATERIALS AND METHODS

Mice. CF1 female mice (Charles River, Wilmington, Mass.), 8 to 14 weeks old, were used for the lethality studies. C3Heb/FeJ female mice (Jackson Laboratories, Bar Harbor, Maine), 8 to 14 weeks old, were used as a source of peritoneal macrophages for all in vitro studies. Both groups of mice were fed mouse chow ad libitum and were kept on a 12-h light, 12-h dark cycle.

Reagents. LPS from *Escherichia coli* O111:B4 (List Biological Companies, Campbell, Calif.) was used in all studies. D-Galactosamine was purchased from Sigma Chemical Co. (St. Louis, Mo.). mrIL-6 was obtained from Genzyme (Cambridge, Mass.). *Limulus* amoebocyte lysate was purchased from the Associates of Cape Cod (Woods Hole, Mass.). RPMI 1640 medium, fetal calf serum, L-glutamine, Hanks' balanced salt solution, and penicillin-streptomycin were all purchased from JRH Biosciences (Lenexa, Kans.). Polyclonal sheep anti-mouse C3 was purchased from the Binding Site (San Diego, Calif.).

Serum and plasma collection. Blood was collected from the tail vein and immediately anticoagulated by adding 225-μl samples to 25 μl of 3.8% citrate. The plasma was then isolated by centrifugation of the citrated blood. Serum was collected either by tail bleeding (for C3 determinations) or by cardiac puncture (for in vitro macrophage activation studies). Samples of blood were collected and then allowed to clot at 37°C for 1 h. Serum was then isolated by centrifugation.

Acute-phase response induction. mrIL-6 was purchased as a stock solution at a concentration of 10^5 U/ml and diluted in pyrogen-free saline just prior to use (1 U of mrIL-6 was defined by the manufacturer as the amount required to induce half-maximal proliferation of 7TD1 hybridoma cells). Fetal calf serum was then added to a final concentration of 1.0% (vol/vol). Mice were administered either mrIL-6 or saline vehicle (also containing 1.0% fetal calf serum) intraperitoneally in a final volume of 0.2 ml.

Lethality studies. For all lethality studies, mice were fasted for 12 h prior to lethal challenge (20). Mice were administered intraperitoneally either LPS alone (600 μg) or LPS (20 ng) plus D-galactosamine (20 mg) per 25 g of body weight. Deaths were monitored over the following 36 h for sensitized mice and 72 h for normal mice, and the results represent cumulative lethality at those times.

In vitro macrophage cultures. Peritoneal exudate macrophages were harvested via lavage 4 days following a 1.5-ml intraperitoneal injection of 4.0% sterile thioglycolate to C3Heb/FeJ mice. The peritoneal cells were washed with RPMI 1640 medium supplemented with 10% fetal calf serum, 2% penicillin-streptomycin, and 2% L-glutamine and then plated at 10^5 cells per well in a 96-well microtiter plate (Costar, Cambridge, Mass.). After an adherence period of 5 h, nonadherent cells were removed by gently washing the cultures twice with Hanks' balanced salt solution.

Fibrinogen assay. Fibrinogen was quantitated by a clotting-time assay exactly as described by the manufacturer (Sigma procedure no. 880). The clotting time was measured after the addition of excess thrombin to citrated (0.38%) mouse plasma samples. According to this procedure, the time required for the first fibrin strands, detected visually, to form was defined as the clotting time.

C3 assay. C3 was quantitated by radial immunodiffusion. Polyclonal anti-mouse C3 was diluted 1:33 with 1.2% agarose prepared by adding 0.9% NaCl and 0.01 mM sodium borate (pH 8.4) to agarose and heating until it dissolved. Normal mouse serum, estimated to be approximately 1.2 mg/ml (16), was used as a standard to detect induction of C3 following mrIL-6 treatment. Serial dilutions of samples and standards were prepared, and 5-μl volumes were placed into preformed wells in anti-mouse C3 antibody-containing agarose. After 72 h of precipitation, the gels were extensively washed with saline for 48 h to remove nonprecipitated proteins. The gels were then dried, and the precipitin rings were stained with Coomassie blue. The stained rings were measured to within ±0.1 mm.

TNF-α assay. Macrophage culture supernatants were diluted 1:10 and then measured for TNF-α by the L929 cytotoxicity assay (1). After 14 to 18 h of incubation with the TNF-α-containing supernatants, the viability of the L929 cells was measured by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay (6). One unit of TNF was defined as that amount required for 50% lysis. Absorbance data were analyzed with a log-logit program.

Endotoxin quantitation. All reagents for lethality studies were assessed for endotoxin contamination by a *Limulus* amoebocyte lysate clotting assay. This assay was performed as described by the manufacturer (Associates of Cape Cod). All reagents were found to be negative within the sensitivity of the assay (0.5 endotoxin units [EU]/ml).

RESULTS

Quantitation of the murine acute-phase response. Circulating levels of both fibrinogen and C3 have been reported to increase following the induction of an acute-phase response. We therefore developed assays to quantitate the concentration of these proteins in plasma and serum, respectively, following the administration of mrIL-6. For these studies, mouse plasma (for fibrinogen) or serum (for C3) samples were obtained as described in Materials and Methods.

A standard curve for fibrinogen levels was established by using a sensitive fibrinogen-dependent clotting-time assay, and the results of studies with fibrinogen standards are shown in Fig. 1A. A standard curve was prepared from clotting-time values, and a linear graph was established for clotting time as a function of fibrinogen concentration. These data establish an approximately twofold change in clotting time as fibrinogen levels are varied twofold. Since clotting time can be reproducibly estimated to within 1 s, changes in

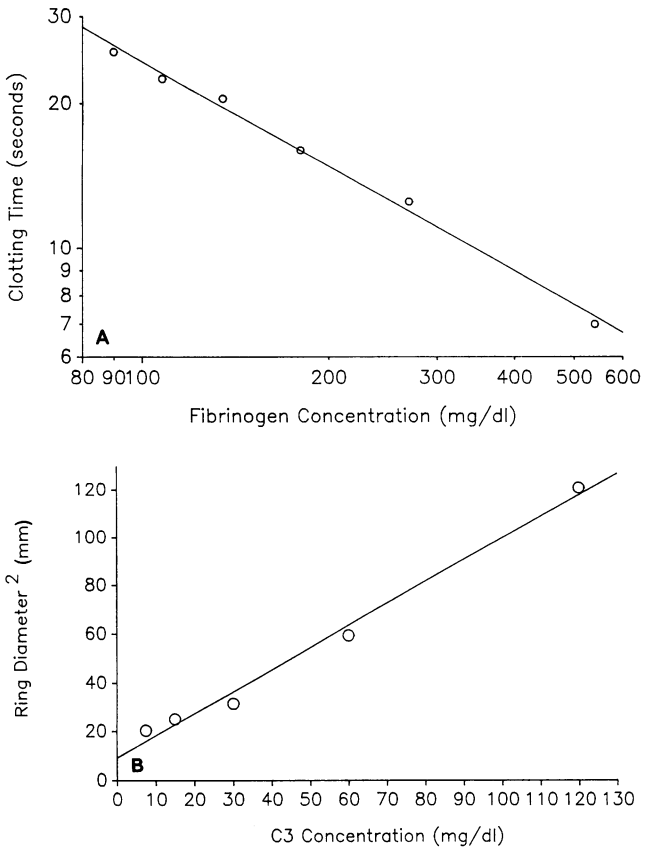

FIG. 1. (A) Clotting time of plasma as a function of fibrinogen concentration. A fibrinogen standard solution was diluted to various concentrations as described in Materials and Methods. The clotting time of these dilutions was assessed following the addition of excess thrombin. (B) Radial immunodiffusion assay for C3 determination. Normal mouse serum was diluted with 7.0% bovine serum albumin, and 5-μl samples were added to wells formed in agarose containing anti-mouse C3. After 72 h, the ring diameter was measured, and a standard curve was constructed based on the normal level of C3 (120 mg/dl) in mouse serum.

fibrinogen levels of approximately 10% can be determined by this assay.

Dilutions of normal mouse serum were used to quantitate C3 levels by a radial immunodiffusion assay system. The results of a standard curve are shown in Fig. 1B and establish that the zone of a detectable precipitin reaction at various dilutions of mouse serum was proportional to the diameter squared. Since increases in ring diameter of as little as 0.1 mm were measured, changes in C3 concentrations of approximately 5.0% can be detected by this assay.

mrIL-6-dependent induction of the acute-phase response. We initially investigated the time and dose dependence of the mouse acute-phase response induced by mrIL-6. For these experiments, groups of five mice were administered mrIL-6 or a saline control intraperitoneally in a final volume of 0.2 ml. At various times up to 25 h following injection, blood samples of approximately 50 μl per mouse were obtained by tail bleeding and pooled, and either serum or plasma was obtained. Aliquots were then assessed for serum C3 and plasma fibrinogen levels as described above. The results of this experiment are shown in Fig. 2. Increases in circulating levels of both of these acute-phase proteins were

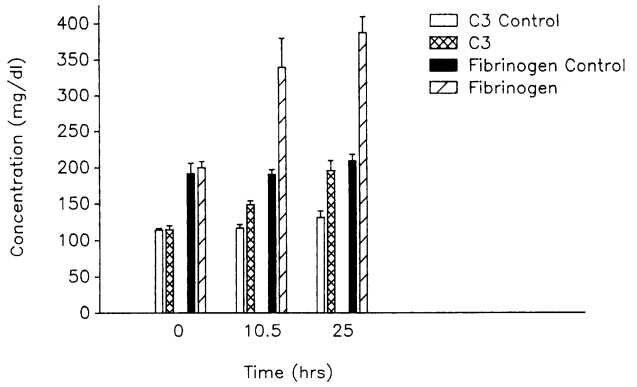

FIG. 2. Induction of the acute-phase response. Groups of five mice were administered 10,000 U of mrIL-6 intraperitoneally. Serum or plasma was then obtained at various times as described in Materials and Methods for C3 and fibrinogen quantitation, respectively. Circulating levels of these proteins were then measured by immunodiffusion or clotting time. Data are expressed as the means of three experiments ± standard deviations.

maximal at 10 h and were maintained for at least an additional 15 h. Increasing the dose of mrIL-6 above 10,000 U per mouse did not further enhance induction of the acute-phase response as assessed by fibrinogen levels, and a dose of less than 10,000 U per mouse did not maximally induce fibrinogen (data not shown). These results therefore confirm the ability of the mrIL-6 used in these studies to induce an acute-phase response in both a time- and dose-dependent manner and establish that 10,000 U per mouse was an optimal dose.

IL-6 versus LPS induction of the acute-phase response. LPS is recognized as an effective stimulus for induction of the acute-phase response. We therefore assessed the capacity of LPS relative to that of the mrIL-6 used in these studies to act as a stimulus for acute-phase protein synthesis. For these experiments, saline, LPS, or mrIL-6 was administered to mice intraperitoneally in a volume of 0.2 ml as described above. At various times, plasma was obtained from pooled blood samples from groups of five mice and used for fibrinogen quantitation. As shown by the data in Fig. 3, 2.0 μg of LPS induced a response equivalent to that of 10,000 U of mrIL-6, whereas 200 ng of LPS was somewhat less effective, as reflected in significantly lower fibrinogen levels at 25 h post-mrIL-6 treatment.

Effect of the acute-phase response on LPS-induced lethality. Having established that mrIL-6 can in fact induce an acute-phase response, that increases in circulating levels of C3 and fibrinogen were manifest by 6 h (data not shown), and that maximal responses required 10 h, we then carried out experiments to assess the consequences of induction of the acute-phase response on lethality induced by LPS. Groups of mice were treated with mrIL-6 or vehicle for various lengths of time and then challenged with a combination of LPS and D-galactosamine. Cumulative lethality was then monitored over the next 36 h. As shown by the data in Table 1, induction of an IL-6-dependent acute-phase response prior to lethal LPS challenge did not significantly change overall mortality in response to LPS. In this D-galactosamine model, lethality occurs 8 to 12 h post-LPS challenge. Therefore, those mice treated for 6 or 15 h with mrIL-6 prior to LPS challenge died at a time when circulating levels of IL-6-dependent acute-phase proteins were maximal. In con-

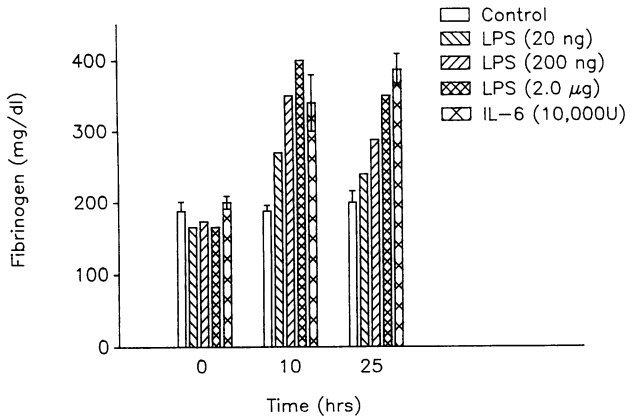

FIG. 3. Comparison of LPS and mrIL-6 in the induction of the acute-phase response. Groups of five mice were administered 10,000 U of mrIL-6 or LPS intraperitoneally. Fibrinogen levels were then assessed over a 25-h period. The absence of error bars indicates identical determinations.

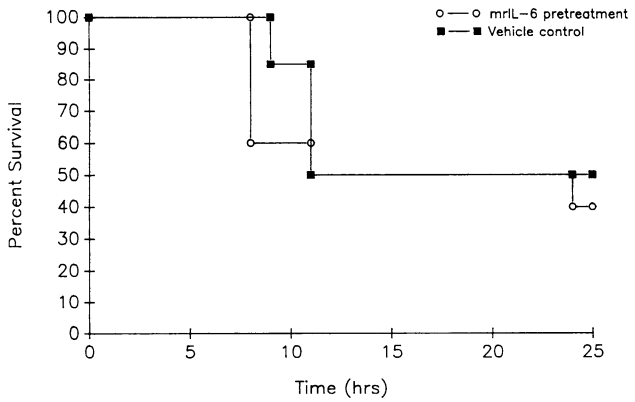

FIG. 4. Effect of the acute-phase response on the kinetics of lethality. Groups of six mice were treated with mrIL-6 or vehicle control for 9 h and then challenged with LPS (20 ng per mouse) and D-galactosamine. The mice were then monitored each hour for 36 h to assess the kinetics of lethality. The data represent the results of two independent experiments.

trast to the results obtained after treatment with mrIL-6, treatment with LPS (100 ng per mouse) protected mice from a subsequent lethal challenge with LPS and D-galactosamine. Furthermore, treatment with 100 ng of LPS resulted in only minor increases in circulating levels of fibrinogen (185 mg/dl in control mice versus 260 mg/dl in mice administered 100 ng of LPS versus 320 mg/dl in mice given 10,000 U of mrIL-6).

Since D-galactosamine-sensitized mice were not protected by the mrIL-6-induced acute-phase response, additional experiments were performed to address a potential role for the IL-6-dependent acute-phase response in non-D-galactosamine-sensitized mice. In two independent experiments, groups of mice were treated with 10,000 U of mrIL-6 for 12 h and then challenged with 600 μg of LPS. Cumulative lethality was subsequently monitored for 72 h. As can be seen from the data in Table 1, prior induction of an IL-6-dependent acute-phase response also did not protect these mice against LPS lethality.

TABLE 1. Effect of IL-6 on LPS-induced lethality[a]

Mouse group	Pretreatment time (h)	mrIL-6 (10^3 U)	LPS (ng)	Lethality (no. dead/ no. in group)
D-Galactosamine treated	3	0		5/6
		2.0		5/6
		5.0		6/6
		10.0		6/6
			0	4/5
			100	0/5
	6	0		5/6
		2.0		6/6
		5.0		4/6
		10.0		5/6
			100	0/5
	15	0		5/5
		10.0		5/6
			100	0/5
Normal	12	0		13/20[b]
		10.0		16/20

[a] Mice were pretreated with mrIL-6, LPS, or vehicle control in a volume of 200 μl given intraperitoneally. After pretreatment, mice were given 20 mg of D-galactosamine and 20 ng of LPS also intraperitoneally in a final volume of 400 μl. Normal mice were pretreated with either vehicle or mrIL-6 and subsequently challenged with 600 μg of LPS in a final volume of 400 μl. Lethality was monitored at 12-h intervals for 36 h (sensitized mice) or 72 h (normal mice) post-LPS challenge, and cumulative lethality is reported.

[b] Not statistically significant by the chi square test ($P > 0.25$).

In the experiments described above, the dose of LPS administered was approximately an 85% lethal dose (LD_{85}) for D-galactosamine-treated mice and an LD_{75} for normal mice. If the induction of the IL-6-dependent acute-phase response had only a marginal effect on LPS lethality, such an effect might not be detected at the relatively high doses of LPS used. Additionally, the acute-phase response may not alter cumulative lethality, but it might still have some effect on the kinetics of lethality. Therefore, if the acute-phase response were only partially protective, pretreated mice might survive for a longer period than saline-pretreated control animals when challenged with LPS. To investigate this question, LPS was administered at a lower dose (approximately an LD_{50}), and the kinetics of lethality in the animals were subsequently studied. As shown by the data in Fig. 4, the kinetics of lethality in this D-galactosamine model was not altered by mrIL-6 pretreatment, nor was cumulative lethality. Similarly, mrIL-6-pretreated mice did not survive for a longer period of time in the normal mouse model (data not shown). Therefore, we conclude that induction of an IL-6-dependent acute-phase response is not protective at lower doses of LPS, nor does it increase survival times.

Acute-phase serum factor effects on macrophages in vitro. It has been documented that the increased sensitivity of mice to the lethal effects of LPS in the D-galactosamine model is also manifested by an increased sensitivity to the lethal effects of TNF-α (13). It has also been reported that an acute-phase protein termed lipopolysaccharide-binding protein will markedly potentiate the capacity of LPS to stimulate rabbit peritoneal exudate cells to release TNF-α (18). Experiments were therefore carried out to assess the relative capacity of normal and mrIL-6-induced acute-phase sera to modulate LPS-dependent stimulation of murine peritoneal macrophages to produce TNF-α in vitro. Serum was obtained from mice by cardiac puncture 14 h following admin-

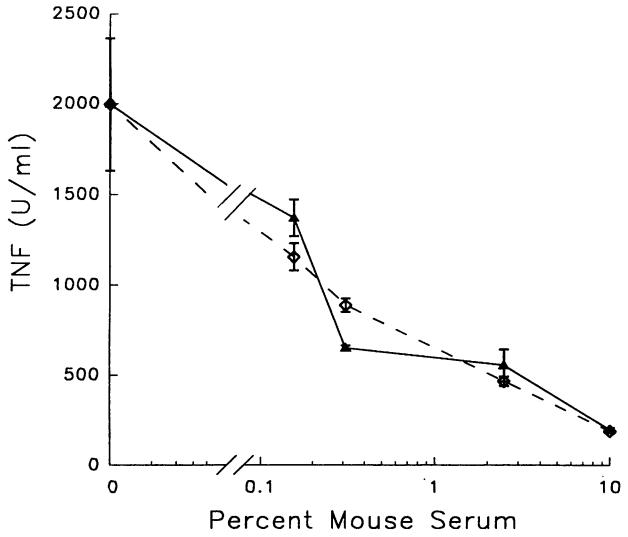

FIG. 5. In vitro comparison of the effect of acute-phase and normal sera on TNF-α production by elicited peritoneal macrophages. Peritoneal macrophages were cultured as described in Materials and Methods. They were then stimulated with LPS (1.0 ng/ml) for 5 h in the presence of either acute-phase (▲) or normal (◇) serum. Culture supernatants were then obtained for TNF-α measurements. Data are expressed as the means of three experiments ± standard deviation. Unstimulated macrophages in the presence of serum did not produce any detectable TNF-α.

istration of either 10,000 U of mrIL-6 or saline. Thioglycolate-elicited peritoneal macrophages from C3Heb/FeJ mice were then stimulated in vitro with LPS in the presence of various dilutions of either acute-phase or normal serum (macrophages from C3Heb/FeJ mice rather than from CF-1 mice were used to avoid potential allogeneic effects during culture), and the amount of TNF-α in culture supernatants was quantitated after 5 h. To prevent the possibility that low serum concentrations might affect the assay, all culture medium was supplemented with 10% fetal calf serum. Of interest, incubation of murine peritoneal macrophages with increasing concentrations of mouse serum resulted in a significant decrease in TNF-α release following LPS stimulation (Fig. 5). However, we detected no significant differences between acute-phase serum and normal serum in relative ability to modulate macrophage responses in vitro.

DISCUSSION

Our studies have confirmed the induction of an acute-phase response in mice by administration of either mrIL-6 or LPS, as assessed by increases in the circulating levels of fibrinogen and C3 of approximately twofold. A dose of LPS of between 200 ng and 2.0 μg was equivalent to 10,000 U of mrIL-6 in inducing an acute-phase response. Our studies have also demonstrated that induction of the acute-phase response by mrIL-6 neither protects nor sensitizes mice to a subsequent lethal challenge with LPS and D-galactosamine or LPS alone. Since pretreatment with 100 ng of LPS prior to challenge with LPS and D-galactosamine can protect mice without inducing maximal circulating levels of fibrinogen, it is unlikely that the failure of mrIL-6 to induce a protective acute-phase response in the D-galactosamine model is the result of inadequate doses of mrIL-6. Therefore, the exact role of IL-6 as a mediator of the pathogenesis of endotoxemia is still not certain.

The induction of a protective acute-phase response may require multiple stimulatory signals, only one of which is provided by IL-6. There is some in vitro evidence to suggest that acute-phase protein synthesis induced in hepatoma cells by IL-6 may be affected by the addition of other cytokines, such as IL-1 and TNF-α (3). However, only a select few acute-phase proteins induced in response to IL-6 appeared to be influenced by additions of IL-1 or TNF-α. In this respect, LPS itself may, in addition to inducing the production of IL-6, provide unique activation signals to hepatocytes, which then result in a novel set of potentially protective proteins not induced by IL-6, either alone or in combination with IL-1 or TNF-α. Receptors for LPS on hepatocytes have been described and may participate in hepatocyte stimulation (15). It is noteworthy that treatment of mice with LPS, unlike treatment with IL-6, prior to LPS and D-galactosamine challenge will protect these animals against LPS lethality in this model (7). Furthermore, it has been shown that an acute-phase response induced by turpentine can afford some protection against a subsequent lethal challenge with both LPS and TNF-α (2). However, a nonspecific inflammatory stimulus such as turpentine may well induce multiple classes of mediators, which may then lead to a desensitization phenomenon similar to that which occurs following pretreatment with LPS.

Since the increased sensitivity to LPS in the D-galactosamine model has been shown to be due, at least in part, to an increased sensitivity to TNF-α (13), we constructed an in vitro system which would detect serum factors that might have an effect on TNF-α release by peritoneal macrophages stimulated with LPS. Although addition of serum had an apparent inhibitory effect on the capacity of LPS to induce macrophage TNF-α secretion, acute-phase serum did not differ from normal mouse serum in this respect. Differential results between normal and acute-phase sera might have been anticipated if acute-phase proteins affected macrophage release of TNF-α. The results of these in vitro studies with acute-phase sera are in accord with our in vivo data showing no effect of IL-6-dependent acute-phase serum on LPS lethality.

Although IL-6 has been shown to be a key mediator of the acute-phase response, the results of our studies do not provide any evidence for protection against LPS challenge, particularly in the D-galactosamine model of LPS lethality. These results might be considered with the framework of LPS-binding proteins recently reported to be produced by the liver as acute-phase proteins (21). If these LPS-binding proteins were present in acute-phase serum, significant effects on the ability of LPS to stimulate macrophages and consequent alterations in lethality or in TNF-α release from macrophage culture supernatants might have been anticipated. One interpretation of the results obtained in these studies would be that mrIL-6, although clearly capable of inducing increased levels of fibrinogen or C3, does not induce the production of LPS-binding proteins. Alternatively, an acute-phase protein such as LPS-binding protein produced by the liver may have only a marginal effect in the outcome of sepsis. However, both the in vivo and in vitro experimental protocols used in these studies are inherently complex because of the multiple interactions of LPS, serum, and macrophages. As a consequence, an effect which can be ascribed to a purified acute-phase protein in vitro might conceivably be opposed when the protein is present in whole serum by the activity of other constituents.

In conclusion, we have shown that mrIL-6 can induce an acute-phase response in mice. However, these studies do not support a protective role for an acute-phase response induced by IL-6. Therefore, these results do not shed additional light on the exact significance of IL-6 in the induction of an acute-phase response during sepsis. IL-6, a pluripotent cytokine, does have other activities during sepsis that are not fully defined. It may very well be that the complex interaction between IL-6 and other factors produced during sepsis has importance for the host by mechanisms which have yet to be defined.

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