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# Balance of Inflammatory Cytokines Related to Severity and Mortality of Murine Sepsis

KEITH R. WALLEY,[1] NICHOLAS W. LUKACS,[2] THEODORE J. STANDIFORD,[3] ROBERT M. STRIETER,[3] AND STEVEN L. KUNKEL[2]*

*Departments of Medicine[3] and Pathology,[2] Division of Pulmonary and Critical Care Medicine, The University of Michigan, Ann Arbor, Michigan 48109, and The Pulmonary Research Laboratory, University of British Columbia, Vancouver, British Columbia, Canada V6Z 1Y6[1]*

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**We tested the hypothesis that, during sepsis, the balance of pro- and anti-inflammatory cytokines is related to severity and survival. Cecal ligation and puncture (CLP) with a large (18-gauge)-, intermediate (21-gauge)-, or small (26-gauge)-diameter needle, or sham laparotomy, was performed on outbred CD-1 mice. Concentrations of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and the anti-inflammatory cytokine IL-10 were measured (by enzyme-linked immunosorbent assay) in serum, peritoneal lavage fluid, and liver and lung samples at 4, 8, 24, 48, and 96 h. As the diameter of the CLP needle decreased, the mortality rate decreased (at 48 h: large, 80%; intermediate, 40%; small, 20%; $P < 0.05$), the TNF-α and IL-6 concentrations decreased, and the time-to-peak TNF-α expression increased. In contrast, IL-10 concentration increased compared with baseline (serum at 24 h: large, 2.3-fold ± 1.6-fold; intermediate, 2.0-fold ± 0.5-fold; small, 49.9-fold ± 8.3-fold; $P < 0.05$). Administration of IL-10 (5 μg, intraperitoneal) prior to CLP decreased mortality ($P < 0.001$). Administration of polyclonal anti-IL-10 serum prior to CLP (0.5 ml intraperitoneal) had the opposite effect and increased mortality ($P < 0.001$) and TNF-α, IL-6, and TNF-α mRNA expression compared with controls. Thus, severe sepsis is associated with a largely unopposed inflammatory response, and a largely unopposed inflammatory response (with anti-IL-10) results in severe sepsis and death. Less severe sepsis is associated with greater anti-inflammatory mediator expression, and greater anti-inflammatory mediator expression (with IL-10) results in less severe sepsis. Thus, the balance of inflammatory mediators is related to the severity and mortality of murine sepsis.**

Overwhelming human septic shock is associated with dramatically elevated levels of inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) (8, 19, 32), interleukin-1 (IL-1) (8, 19), and IL-6 (8, 10). This systemic cytokine response appears to represent an uncontrolled and adverse inflammatory response. Infusion of these cytokines mimics many features of septic shock (7, 28), including the adverse cardiovascular response (15), while passive immunization with antibodies to specific cytokines, such as TNF-α, improves survival in animal models of severe septic shock (29) and in patients with severe septic shock (2). However, the expression of inflammatory cytokines during specific responses is not entirely deleterious, as they appear to play crucial roles in limiting and eliminating local infections and increasing host survival (3, 12, 13). Indeed, passive immunization with antibodies to TNF-α worsens mortality in murine CLP (13) and worsens outcome in patients with less severe sepsis (1, 2). Thus, the problem with the cytokine biology-sepsis axis is not the expression of inflammatory cytokines, but rather that they are not adequately modulated by anti-inflammatory mediators, including glucocorticoids, prostaglandin $E_2$, IL-1 receptor antagonist, and anti-inflammatory cytokines, etc. One cytokine which has been shown to possess modulating activity for the production of other cytokines is IL-10, as this cytokine can downregulate the expression of TNF-α and other inflammatory cytokines (24). Interestingly, IL-10 is expressed in elevated concentrations during sepsis (23). Conceivably, anti-inflammatory cytokine expression, including IL-10, is more effective in less severe sepsis in regulating inflammatory cytokines and, therefore, may be important in determining the outcome of sepsis.

Our hypothesis is that severe fatal sepsis is better distinguished from less severe survivable sepsis by the balance of inflammatory mediators rather than by the concentration of a single mediator alone (6). That is, compared with overwhelming sepsis, a lesser septic insult would be associated with significant expression of the anti-inflammatory cytokine IL-10, with respect to other inflammatory mediators including TNF-α and IL-6. To test this hypothesis we first determined the extent and timing of TNF-α and IL-6 expression compared with that of IL-10 in severe and less severe CLP models of murine sepsis. Then we altered the balance of pro- and anti-inflammatory cytokines by administering IL-10 or polyclonal antibody to IL-10 (anti-IL-10). We found that severity of sepsis is associated with an altered balance of inflammatory cytokines, and conversely, altering the balance of inflammatory cytokines has a significant impact on severity of sepsis and overall mortality. In contrast, single mediator expression alone, such as early TNF-α expression at the primary site of inflammation, did not distinguish between severe and less severe sepsis. Thus, there is a close link between the severity of sepsis and the balance of inflammatory cytokines.

## MATERIALS AND METHODS

**Animal model.** Specific-pathogen-free CD-1 mice were used for CLP as previously described (4). Briefly, under sterile conditions, a 1- to 2-cm midline incision was first made on the anterior abdomen, and the cecum was exposed. With a 3-0 silk suture, the cecum was tightly ligated at its base without causing bowel obstruction. The cecum was then punctured through-and-through once with either a large (18-gauge)-, an intermediate (21-gauge)-, or a small (22- to 26-gauge)-diameter needle. Sham-operated animals underwent identical lapa-

* Corresponding author. Mailing address: Department of Pathology, The University of Michigan Medical School, M5214 Medical Science I, Box 0602, 1301 Catherine Rd., Ann Arbor, MI 48109-0602. Phone: (313) 936-1020. Fax: (313) 764-2397.

rotomy but did not undergo cecal ligation or puncture and served as controls. The abdominal incision was closed, and 1 ml of saline was administered subcutaneously for fluid resuscitation.

In the first set of experiments, survival curves were determined for 10 animals in each cecal puncture group. In the second set of experiments CLP animals were euthanized at 4, 8, 24, 48, 72, and 96 h, and serum, lung, and liver samples were collected. The peritoneal cavity was lavaged with 1 ml of sterile saline, and this peritoneal lavage fluid was collected. A minimum of 5 surviving animals at each time point was used. Because of the high mortality rate in the large-cecal-puncture-diameter group, at late time periods, the data are incomplete. In the third set of experiments the intermediate-diameter CLP model was used, and survival curves for 4 additional groups of animals were determined as follows. Mice were either treated with 5 μg of recombinant murine IL-10 (PeproTech, Rocky Hill, N.J.) ($n = 9$) or vehicle ($n = 9$) or treated with 0.5 ml of polyclonal rabbit antibody to murine IL-10 serum ($n = 12$) or control rabbit serum ($n = 12$) 2 h prior to CLP. In the fourth set of experiments TNF-α and IL-6 concentrations and TNF-α mRNA expression were measured 2 h after CLP in mice pretreated with IL-10 or control vehicle as well as in mice pretreated with anti-IL-10 or control pre-immune rabbit serum.

**Production of anti-cytokine antibodies.** Rabbit anti-murine TNF-α, IL-6, and IL-10 antibodies were prepared by multiple site immunization of New Zealand White rabbits with recombinant murine TNF-α, IL-6, or IL-10, respectively. Specificity of these polyclonal antibodies was verified by the failure to cross-react with the other two cytokines of this set and with murine IL-1, IL-3, IL-6, IL-8, macrophage inflammatory protein 1α (MIP-1α), MIP-1β, JE, and RANTES. The immunoglobulin G portion of the serum was purified by using a protein A column. These polyclonal antibodies were subsequently used either in sandwich enzyme-linked immunosorbent assays (ELISAs), for passive immunization, or for immunohistochemical staining.

**Cytokine measurement.** Measurements of antigenic TNF-α, IL-6, and IL-10 concentrations in serum, peritoneal lavage fluid, and homogenized aqueous lung and liver extracts were made by using a double-ligand ELISA. Briefly, flat-bottomed 96-well microtiter plates were coated with 50 μl per well of rabbit antibody specific to one of the above cytokines and incubated overnight at 4°C. After the plates were washed, nonspecific binding was blocked with 2% bovine serum. Diluted cell-free supernatants (1:2 and 1:10) were loaded in duplicate. Subsequently, streptavidin-peroxidase conjugate was added (Bio-Rad Laboratories, Richmond, Calif.) followed by chromogen substrate (OPD; Bio-Rad Laboratories). The plates were read at 490 nm in an ELISA reader. Sensitivities for the TNF-α, IL-6, and IL-10 ELISAs were 40 pg/ml, 250 pg/ml, and 60 pg/ml, respectively.

**TNF-α and IL-6 bioassays.** To confirm that antigenic TNF-α and IL-6 expression was associated with bioactivity, TNF-α concentrations were also measured by using the WEHI bioassay (14) and IL-6 concentrations were measured by using the B9 cell proliferation bioassay (25). In the TNF-α bioassay $5 \times 10^5$ WEHI 164 subclone 13 cells in 100 μl were added to 100-μl volumes of serial dilutions of test samples and were incubated overnight. In the IL-6 bioassay $5 \times 10^3$ IL-6-dependent murine hybridoma cell line B13.29 clone B9 cells in 100 μl of culture media were added to 100-μl volumes of serial dilutions of test samples and were incubated for 72 h in a humidified incubator at 37°C and 5% $CO_2$. Then, in both cases, cell viability was measured with a colorimetric assay by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma). During the final 6 h of the incubations, 20 μl of MTT (5 mg/ml in phosphate-buffered saline [PBS]) was added to each sample. Supernatant (150 μl) was aspirated from each well, and 100 μl of acidified isopropanol was added. $A_{550}$ was measured. A standard curve was generated by serial dilutions of either recombinant murine TNF-α or IL-6, respectively. These bioassays consistently detected TNF-α concentrations above 1 pg/ml and IL-6 concentrations above 0.1 pg/ml.

**Reverse transcriptase-PCR amplification of tissue mRNA.** The left upper lobe of the liver and of the left lung were harvested and immediately "snap frozen" in liquid nitrogen and stored at −80°C. Total cellular RNA was isolated by phenol-chloroform extraction. RNA was alcohol precipitated, and the pellet was dissolved in diethyl pyrocarbonate water. Five micrograms of total RNA was reverse transcribed into cDNA by using oligo(dT) 12 to -18 primers. The cDNA was amplified by using specific primers for TNF-α. β-actin cDNA was also amplified. The TNF-α sense primer sequence was 5′-CCTGTAGCCCACGTCGTAGC-3′, and the antisense primer sequence was 5′-TTGACCTCAGCGCTGAGTTG-3′. The β-actin sense primer sequence was 5′-ATGGATGACGATATCGCTC-3′, and the antisense primer sequence was 5′-GATTCCATACCCAGGAAGG-3′, giving an amplified product of 812 bp. Specific oligonucleotide primers were added (200 ng per sample) to the buffer, along with 5 μl of the reverse-transcribed cDNA samples. The mixture was first incubated for 5 min at 94°C and then 35 cycles of 93°C for 45 s, 52°C for 45 s, and elongation at 72°C for 90 s were performed. After amplification, the sample was separated on a 2% agarose gel containing 0.3 mg of ethidium bromide per ml, and bands were visualized and photographed with ultraviolet light.

**Immunohistochemistry.** Immunostaining of antigenic polypeptides from tissue was determined as previously described (9). Briefly, 7-μm-thick sections (10 total) were prepared and mounted on poly-L-lysine-coated glass slides. The mounted tissues were immediately fixed for 10 min in cold acetone. The slides were next rehydrated in PBS and treated with avidin and biotin to block binding sites for these molecules. Then, they were treated with a 1:50 dilution of blocking serum for 10 min at 37°C and then exposed to optimal dilutions of specific antibody and similar dilutions of control sera. After 20 min of incubation at 37°C, the slides were rinsed three times with PBS, overlaid with biotinylated secondary antibodies (1:100; Vector Laboratories) and incubated another 10 min; this was followed by three additional rinses with PBS. At this point, sections were treated with alkaline phosphatase-labeled streptavidin, rinsed three times, overlaid with substrate chromogen (Vector Laboratories), and incubated for 25 min at room temperature to allow for color development. Mayer's hematoxylin was used as a counterstain. Twenty random fields from each of the sections were evaluated for the presence of immunolocalization of the specific cytokines.

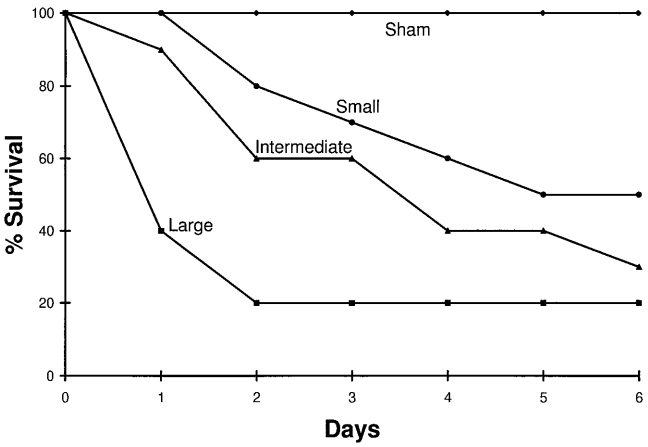


FIG. 1. Survival following large (18-gauge)-, intermediate (21-gauge)-, and small (26-gauge)-diameter CLP ($n = 10$ for each group) and following sham laparotomy ($n = 10$) is shown. Survival decreases significantly ($P < 0.001$) as cecal-puncture diameter increases.

**Data analysis.** A two-way analysis of variance was used to test for differences in cytokine levels between large-, intermediate-, and small-cecal-puncture groups. When a difference was found, specific differences were identified by using a sequentially rejective Bonferroni test procedure (21), choosing a $P$ value of $< 0.05$ as significant. A log-rank test was used to test for differences in survival curves. Cytokine measurements at the multiple time points were fit by using a beta distribution in order to estimate time-to-peak cytokine concentrations. All data are reported as means ± standard errors.

## RESULTS

**Mortality following CLP.** As shown in Fig. 1, survival following experimental CLP in mice varies dramatically, depending upon the size of the needle used to puncture the cecum ($P < 0.001$). At 48 h, 80% mortality was observed in the large-puncture-diameter group, 40% mortality was observed in the intermediate group, and 20% mortality was observed in the small group ($P < 0.05$). Time to 50% mortality increased from 0.9 days in the large-puncture-diameter group to 3.3 days in the intermediate group and to 5.5 days in the small group. Many animals in the small-puncture-diameter group survived long term.

**Cytokine expression following CLP.** Analogous to mortality, the extent and timing of expression of pro-inflammatory cytokines varied with cecal-puncture diameter. Serum TNF-α concentrations at 4 and 8 h after CLP decreased as cecal-puncture diameter decreased (Fig. 2). Eight hours after CLP in the large-cecal-puncture diameter group, serum TNF-α concentrations quickly decreased or the animals died. As cecal-puncture diameter decreased, the estimated peak in serum TNF-α concentration was delayed from 12.8 ± 1.4 to 17.0 ± 1.6 to 21.6 ± 2.1 h ($P < 0.05$) in the large-, intermediate-, and small-cecal-puncture diameter groups, respectively. Peak serum IL-6 concentrations occurred later than TNF-α peaks (Fig. 2). Serum IL-6 concentrations similarly demonstrated that small-cecal-puncture diameter was associated with decreased serum IL-6 concentrations compared with intermediate- and large-cecal-

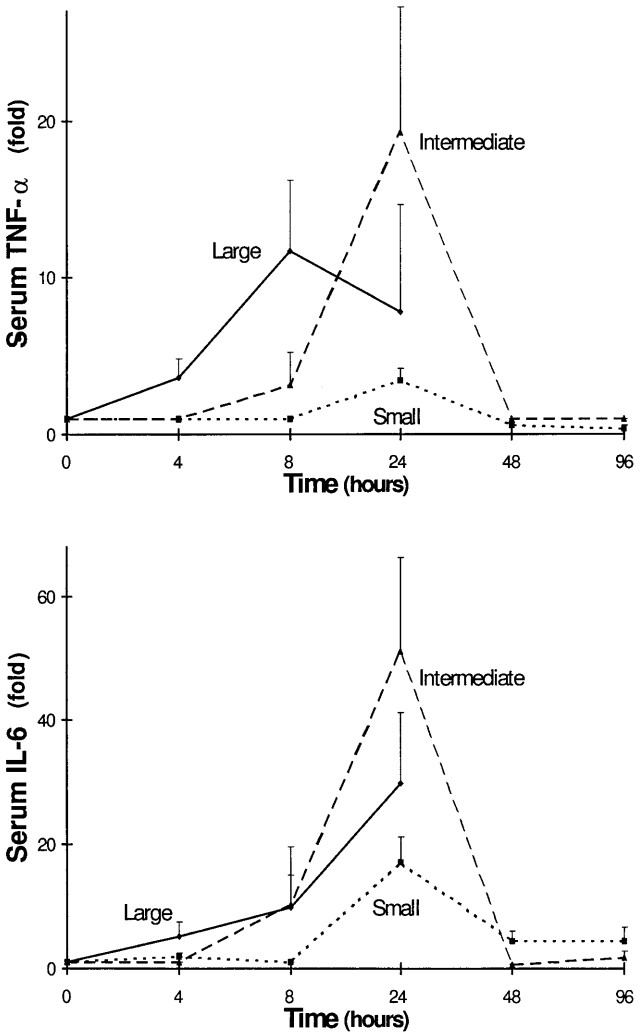


FIG. 2. Serum TNF-α (top) and IL-6 (bottom) concentrations (means ± standard errors), expressed as fold increases relative to time-matched sham laparotomy controls, are shown for large-, intermediate-, and small-diameter cecal puncture. As cecal-puncture diameter decreases, the time-to-peak TNF-α concentration is delayed. Peak concentrations of both TNF-α and IL-6 in serum are lowest at the smallest puncture diameter ($P < 0.05$).

puncture diameters (Fig. 2). TNF-α concentrations in the lung were increased to 1.5 times ± 0.2 times that of control ($P < 0.05$) at 4 h post-CLP but were not different between groups and were not significantly elevated at later time points. Liver TNF-α concentrations were 30-fold greater than serum concentrations at baseline (liver, 2,810 ± 460 pg/ml; serum, 95 ± 25 pg/ml). Liver TNF-α concentrations did not change significantly after CLP, possibly because any change was small compared with the high baseline values. IL-6 concentrations in the lung and liver did not change significantly from baseline, but similar to TNF-α, liver IL-6 concentrations were high at baseline (liver, 19,790 ± 3,750 pg/ml; serum, 210 ± 50 pg/ml).

In contrast to the pro-inflammatory mediators, serum IL-10 concentration was greater in the small-cecal-puncture-diameter group compared with the intermediate and large groups (Fig. 3). Similar to TNF-α, constitutive concentrations of IL-10 were elevated in the lung (1,570 ± 130 pg/ml) and liver (6,830 ± 130 pg/ml) at baseline compared with serum (83 ± 14 pg/ml). However, no significant increase in IL-10 concentration was detectable in the lung and liver after CLP.

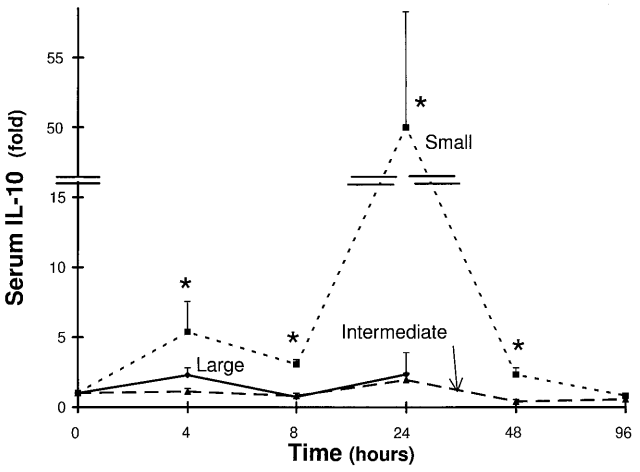


FIG. 3. Serum IL-10 concentrations (means ± standard errors), expressed as fold increases relative to time-matched sham laparotomy controls, are shown. In contrast to TNF-α and IL-6, serum IL-10 concentrations were increased at all time points in the small-cecal-puncture-diameter group. *, significantly different from baseline ($P < 0.05$).

**Peritoneal lavage cytokine expression.** Peritoneal lavage cytokine concentrations reflect the primary inflammatory focus and were extraordinarily high after CLP. Figure 4 illustrates that during severe sepsis, IL-10 levels do not rise to the same extent that they do in less severe sepsis at all time points. Lower IL-10 concentrations were associated with sustained high TNF-α concentrations and progressively increasing IL-6 concentrations in peritoneal fluid during severe sepsis. In contrast, during less severe sepsis (Fig. 5), the early and sustained rise in IL-10 concentration is associated with a rapid decrease in TNF-α concentration in peritoneal fluid after the first 24 h and a marked attenuation of the rise in IL-6 concentration. The fold increase in TNF-α at 4 h in less severe sepsis (32-fold ± 12-fold) is no less than, and not significantly different from, the fold increase in severe sepsis (21-fold ± 10-fold). Cytokine concentrations increased most markedly in the peritoneal lavage fluid and did not increase significantly in the lung

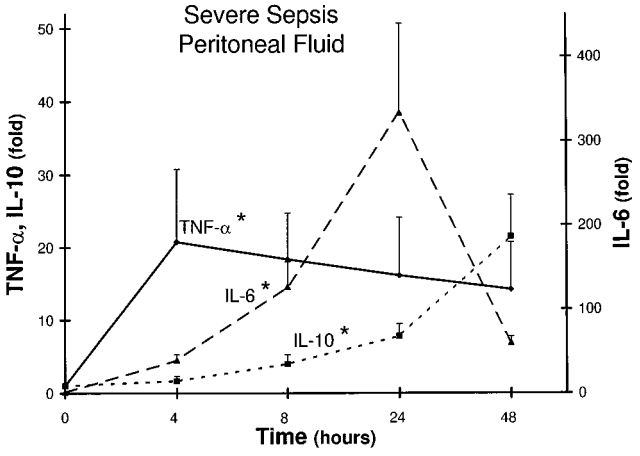


FIG. 4. Peritoneal lavage fluid TNF-α, IL-10 (left axis), and IL-6 (right axis) concentrations (means ± standard errors), expressed as fold increases relative to time-matched sham laparotomy controls, are shown for severe sepsis (large- and intermediate-cecal-puncture-diameter groups). TNF-α concentration increases early and is sustained while IL-6 concentration is extraordinarily elevated at 24 h. *, significantly different from baseline by analysis of variance ($P < 0.05$).

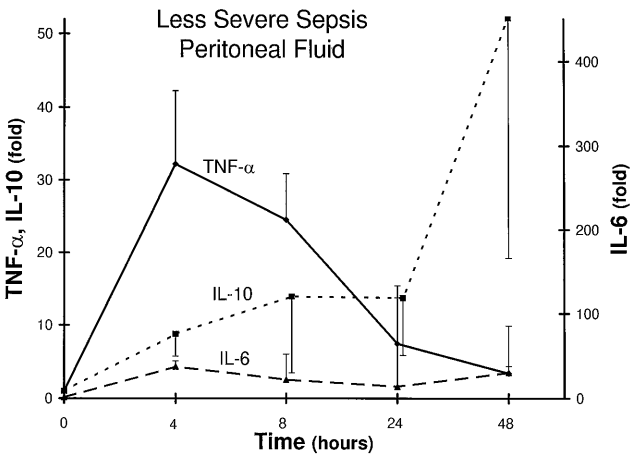


FIG. 5. Peritoneal lavage fluid TNF-α, IL-10 (left axis), and IL-6 (right axis) concentrations, expressed as fold increases relative to time-matched sham laparotomy controls, are shown for less severe sepsis (small-cecal-puncture-diameter group). The early increase in TNF-α concentration is equal to that in the severe sepsis groups (no significant difference). However, IL-10 concentration increases more in less severe sepsis compared with the severe sepsis group ($P < 0.05$). This may contribute to the subsequent decrease in TNF-α and the failure of IL-6 to increase dramatically.

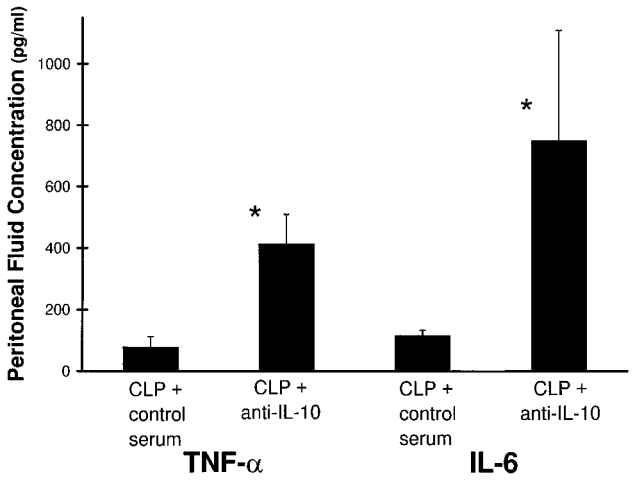


FIG. 7. Peritoneal lavage fluid TNF-α and IL-6 concentrations (means ± standard errors) at 2 h after CLP are greater ($P < 0.01$) when anti-IL-10 is given prior to CLP compared with control serum given prior to CLP. *, statistically significant compared with control serum.

or liver, suggesting that local inflammation at the site of CLP may have contributed to increased serum cytokine levels. Immunostaining of the cecum and surrounding tissues demonstrated that by 8 h after CLP, mononuclear cells surrounding the abscess cavity stained strongly for TNF-α and IL-6.

**Altered mortality with IL-10 and anti-IL-10.** Having observed a difference in inflammatory cytokine balance between severe and less severe sepsis, we then sought to primarily alter the inflammatory cytokine balance. We administered recombinant IL-10 or polyclonal antibody to IL-10 prior to intermediate-diameter CLP. The group that was treated with anti-IL-10 had greatly decreased survival compared with intermediate-diameter CLP alone (Fig. 6) ($P < 0.001$), with most of the mice dying within hours postprocedure. This rapid time course of lethality correlated with the early peak of TNF-α normally observed in the sera of 21-gauge CLP mice. In contrast, the group of mice that were treated with recombinant IL-10 had increased survival rates ($P < 0.001$). In the IL-10-treated group, 7 of 9 mice were alive at 96 h, appeared completely normal, and lived well after the 1-week experiment (Fig. 6).

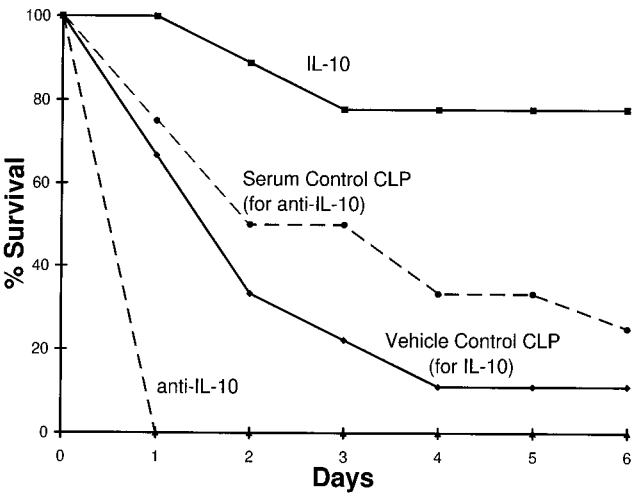


FIG. 6. Survival following intermediate-diameter (21-gauge) CLP is shown for two experiments. First, when anti-IL-10 is given prior to CLP ($n = 12$), survival decreases compared with CLP alone ($P < 0.001$). Second, when IL-10 is given prior to CLP ($n = 9$), survival is greatly increased compared with CLP alone ($P < 0.001$).

**Altered cytokine protein and mRNA expression with anti-IL-10.** In associated experiments, anti-IL-10 pretreatment increased TNF-α mRNA expression by the liver at 2 h after CLP 3.2-fold ± 1.5-fold compared with time-matched controls that received control rabbit serum ($P < 0.05$). Furthermore, anti-IL-10 pretreatment resulted in increased concentrations of TNF-α and IL-6 in peritoneal lavage fluid (Fig. 7). The increase in IL-6 concentration was similar by the ELISA (650% ± 310%) and B9 bioassay methods (810% ± 410%). At this early time point intraperitoneal IL-10 administration did not alter expression of TNF-α or IL-6 in the serum and peritoneal lavage fluid and did not alter expression of TNF-α mRNA in the liver or lung. These findings fit with the observation in Fig. 4 and 5 that greater early IL-10 expression in peritoneal lavage fluid is not associated with a decrease in TNF-α or IL-6.

## DISCUSSION

Decreasing cecal-puncture diameter resulted in decreased mortality and was associated with delayed and decreased expression of the inflammatory mediators TNF-α and IL-6. In contrast, concentrations of the anti-inflammatory mediator, IL-10, increased. Administration of IL-10 or anti-IL-10 altered the balance of inflammatory mediators and resulted in decreased or increased mortality, respectively. We conclude that a largely unopposed pro-inflammatory mediator response occurs in severe sepsis and leads to death. In less severe sepsis, relatively greater IL-10 expression delays and decreases the pro-inflammatory response, resulting in improved survival. It is interesting to note that at the site of initial inflammation (reflected by peritoneal fluid) early TNF-α concentrations in less severe sepsis (32-fold ± 12-fold) are equal to or greater than those in severe sepsis (21-fold ± 10-fold). However, IL-10 concentrations are also greater in less severe sepsis, which is associated with a subsequent decrease in TNF-α concentra-

tions and prevention of the later increase in IL-6 in peritoneal fluid. Thus, the balance and time course of pro- and anti-inflammatory mediators are related to the severity of sepsis and survival and are likely more important than single inflammatory cytokine concentrations taken alone.

Of several sepsis-related mediator pathways with anti-inflammatory actions, we chose to focus on IL-10 as a representative cytokine in this class. IL-10 is a 178-amino-acid cytokine with anti-inflammatory properties and is expressed by $CD4^+$ T cells, activated B cells, monocytes-macrophages, and keratinocytes (24). IL-10 inhibits macrophage synthesis and expression of cytokines in vitro, including IL-1, IL-6, IL-8, and TNF-α (11, 16). A role for IL-10 in modulating the septic inflammatory response is suggested by the observation that IL-10 is expressed in animal models of sepsis (17) and in human sepsis (23). In animal models of sepsis, IL-10 results in decreased production of pro-inflammatory mediators including TNF-α and gamma interferon (18, 22). Previous observations have demonstrated a protective role for IL-10 in animal models of the septic inflammatory response (24, 27, 28). It is interesting to note that IL-10 release is regulated in part by TNF-α (29), resulting in an autoregulatory feedback loop.

The new observation that the severity of the pro-inflammatory cytokine response and mortality vary markedly with cecal-puncture diameter may account for some of the variation in published CLP studies (10, 11) and may be explained by the altered balance between pro- and anti-inflammatory cytokines. In the large-cecal-puncture-diameter model, an overwhelming systemic inflammatory response occurs with elevated levels of TNF-α and IL-6. In this model the expression of the regulatory mediator, IL-10, was also significantly elevated during the first 6 h, but this modulating polypeptide was not elevated to the extent seen during less severe sepsis. In the small-cecal-puncture-diameter model of systemic inflammation, IL-10 is elevated to a greater extent and remains elevated, reaching a zenith in serum at approximately 24 h. In peritoneal fluid, which reflects the primary site of inflammation in these models, IL-10 expression continues to increase, even at 48 h. The sustained production of regulatory mediators is likely an important aspect relating to in vivo cytokine balance which dictates resolution of inflammation and may be a positive prognostic indicator for recovery and survival.

In the present study, we have performed experiments using a previously described model of CLP (17). This is quite different from endotoxin models of the septic inflammatory response in several respects. Compared with endotoxin administration, the CLP model of sepsis produces delayed and prolonged pro-inflammatory cytokine expression. An additional important difference is that a specific focus of inflammation does not exist in endotoxin models. Although the use of endotoxin models has helped to determine numerous pathways of activation which are operative in multiple diseases, the model itself may not be completely representative of a typical episode of clinical human sepsis. The subsequent response to the cecal products, which leak into the abdomen, resembles that described for septic patients. We found that, as a result, the cytokine profile is different from endotoxin models, with peak expression being delayed and prolonged. It is conceivable that rapid endotoxin administration may result in death due to the effects of the intense inflammatory response on cardiovascular and pulmonary physiology (30, 31). In contrast, death at later time points following CLP may be due to multiorgan failure, as in most septic humans (32). The accelerated expression pattern of inflammatory cytokines in severe sepsis supports the notion that early and aggressive therapy is important in treating multiorgan injury syndromes.

In summary, these results show that varying the severity of sepsis induced by CLP results in substantial differences in the balance of inflammatory mediators. On one hand, largely unopposed inflammatory mediator expression leads to death, while on the other, less severe sepsis is associated with relatively increased anti-inflammatory mediator expression. Directly altering the balance of inflammatory mediators in either direction, by exogenous administration of IL-10 or anti-IL-10, leads to decreased or increased mortality, respectively. Thus, the balance of inflammatory mediators is closely related to severity and outcome of sepsis.

## ACKNOWLEDGMENTS

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