Image Cover Sheet

CLASSIFICATION	SYSTEM NUMBER
UNCLASSIFIED	513557

TITLE

Peritoneal Cytokine Concentrations and Survival Outcome in an Experimental Bacterial Infusion Model of Peritonitis

System Number:

Patron Number:

Requester:

Notes:

DSIS Use only:

Deliver to:

This page is left blank

This page is left blank

Peritoneal cytokine concentrations and survival outcome in an experimental bacterial infusion model of peritonitis

Lucie Martineau, PhD; Pang N. Shek, PhD

Objective: To correlate the dynamics of peritoneal cytokines with systemic concentrations and survival outcome.

Design: Randomized, controlled study using a recently developed rat model of peritonitis.

Setting: Government research facility.

Subjects: Male Sprague-Dawley rats.

Interventions: Infected animals (INF) received an intraperitoneal infusion of 6.5×10^8 colony-forming units of *Escherichia coli* over 12 hrs, whereas control rats (CON) received a sterile inoculum. Peritoneal fluid and plasma samples were obtained from all rats at the end of the 12-hr infusion period as well as from all animals that survived the 7-day study (SURV).

Measurements and Main Results: Interleukin (IL)-1β concentration in the peritoneal fluid at 12 hrs tended to be higher in nonsurvivors (NONSURV) than in SURV. Tumor necrosis factor-α and IL-6 peritoneal concentrations at 12 hrs were significantly greater in NONSURV than in SURV. There were no significant differences in IL-2 and IL-4 peritoneal concentrations at 12 hrs between SURV and NONSURV. Although the concentrations of IL-1β and tumor necrosis factor-α in the peritoneal fluid of INF decreased gradually during the study, these concentrations remained significantly higher than those of CON at 7 days. In contrast, peritoneal IL-2 concentrations remained lower in INF than in CON for most of the experiment. Peritoneal IL-6 concentrations in INF were transiently elevated above those of CON for 12 hrs. Cytokine concentrations in the peritoneal fluid of INF were always higher than those in plasma, which remained relatively unchanged throughout the study. For most of the variables assessed, CON showed no significant changes compared with INF.

Conclusions: This model of peritonitis is associated with a significant and prolonged peritoneal inflammatory response that is adversely correlated with survival outcome. Our data would suggest that to be effective, novel immunotherapies should target mainly the peritoneal compartment. (Crit Care Med 2000; 28: 788–794)

KEY WORDS: peritonitis; intra-abdominal infection; compartmentalization; cytokines; survival; rats; tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6 (IL-6)

Despite the availability of potent antimicrobial agents and recent advances in critical care technology, sepsis remains a major cause of morbidity and mortality after intra-abdominal surgery. Although a large variety of endogenous mediators have been implicated in the development and pathogenesis of sepsis, the excessive early release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β plays a pivotal role in the uncontrolled and adverse host immune response associated with this syndrome (1). Although these cytokines contribute to the demise of the host, others involved in the late phase of inflammation (e.g., IL-4, IL-10, and IL-13) appear to protect it as a result of their anti-inflammatory activities (1). Sepsis also increases the production of IL-6, a multifunctional cytokine inhibiting the appearance of both agonist and antagonist cytokines (2), as well as that of IL-2, an important immunomodulator implicated in sepsis.

Many attempts have been made to design rodent models of peritonitis that simulate the human disease. Although these experimental models do have some shortcomings (for a review, see Deitch [3]), it is generally acknowledged that the relative magnitude of the systemic cytokine response of animals subjected to peritonitis is similar to that observed in patients with intra-abdominal infection (4, 5). Although the contribution of systemic cytokines to the inflammatory cascade in sepsis has been well characterized, the magnitude of the peritoneal concentrations of cytokines and their role in supporting local host defenses in peritonitis have received considerably less attention. An early and short-lived increase in peritoneal concentrations of TNF-α, IL-1β, and IL-6 has been observed in experimental models of rapidly lethal peritonitis involving bolus injection of various pathogens (6, 7). However, Cross et al. (4) have suggested that the cytokine patterns in such models of fulminant infection may not be typical of those observed in clinical sepsis, because the animals are overwhelmed with the bacterial challenge and cannot fully express the appropriate local host defense mechanisms. The murine model of cecal ligation and puncture is generally believed to mimic more closely the clinical condition of a patient with a septic focus that continually seeds the body with bacteria over time (5). Although the expression of inflammatory peritoneal cytokines after cecal ligation and puncture was reported recently (8), data were presented only for the acute phase after the infection (i.e., <48 hrs). Furthermore, these data reflected the "natural" course of untreated peritonitis, not necessarily the pattern resulting from the combined effects of in-

From the Operational Medicine Sector, Defence and Civil Institute of Environmental Medicine, West Toronto, ON, Canada

Supported, in part, by the Department of National Defence of Canada

Address requests for reprints to L. Martineau, PhD, DCIEM/OM, 1133 Sheppard Avenue, West Toronto, ON, Canada M3M 3B9. E-mail: lucie@dciem.dnd.ca

tra-abdominal infection and supportive therapy.

Our laboratory has recently developed a rat model of chronic intra-abdominal infection that simulates the clinical condition of a trauma-induced, slow, sustained bacterial release from the gut (9). In this model, a standardized *Escherichia coli* inoculum is slowly and continuously infused into the peritoneal cavity of conscious, unrestrained rats over 12 hrs. This time period was selected based on the observation that in a large number of clinical cases, patients with slow-onset peritonitis may not be treated for at least 12 hrs (10). Unlike in most animal models of peritonitis, the bacterial burden within the peritoneal cavity was mechanically reduced at the end of the 12-hr infusion period, as recommended in clinical practice (10, 11). Furthermore, the infected animals received repeated bolus fluid replacement. The objective of the present study was to measure the concentrations of inflammatory cytokines in peritoneal fluid at various times after the induction of infection in our model of experimental peritonitis. More specifically, data are presented that correlate the dynamics of peritoneal cytokines with systemic concentrations and survival outcome.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (n = 85) with a mean body weight of 369 ± 4 g (mean ± SEM) were obtained from Charles River (St Constant, PQ, Canada). The animals were housed individually, allowed to adapt to the environmental conditions (22°C [71.6°F], 12-hr light/dark cycle), and handled daily before undergoing surgery 7 days later. All animals had free access to standard rodent chow and water at all times during the experimental period. All procedures described in this study were performed in adherence with the Canadian Council on Animal Care regulations for the use of experimental animals (12) and were reviewed and approved by the institutional animal ethics committee.

Preparation of Bacterial Inoculum. E. coli (ATCC 25922) was obtained from a commercial source (PML Microbiologics, Mississauga, ON, Canada). This particular strain of *E. coli* was used in previous studies in our laboratory (9, 13, 14). These bacteria, harvested in the log phase of growth, were encapsulated, as assessed by Anthony's staining method (15). Preliminary experiments to determine the relative bactericidal action of various active human (n = 4) and rat (n = 4) sera *in vitro* revealed that this strain of *E. coli* is serum resistant. Because most clinical isolates of *E. coli* are also encapsulated and serum resistant (4), the choice of this particular strain of *E. coli* for the study of intra-abdominal infection seems appropriate. We acknowledge that human clinical intra-abdominal infections after perforation of the gut are usually characterized by a polymicrobial contamination. However, we selected *E. coli* as the sole infecting pathogen because it is the most frequent Gram-negative organism isolated from septic patients and because it is generally held most responsible for the high mortality and morbidity observed both clinically and experimentally in peritonitis (11, 16).

E. coli were grown in brain-heart infusion broth for 18 hrs at 37°C (98.6°F) in a shaking water bath to obtain a log-phase growth culture. The bacteria were then harvested after centrifugation, resuspended in sterile saline, divided into aliquots as 10% glycerin stock, and frozen at −70°C (−94°F). Two days before the experiment, an aliquot of stock *E. coli* was thawed and grown as described previously to obtain a log-phase growth culture. The culture was then washed twice in sterile phosphate-buffered saline and resuspended in sterile saline. A sample of the bacterial suspension was cultured aerobically at 37°C (98.6°F) for 24 hrs onto tryptic soy agar to estimate the number of viable *E. coli*. The bacterial suspension was kept at 4°C (39.2°F) until its use. On the experimental day, a volume containing an appropriate number of *E. coli* was added to a mixture consisting of 1.5 mL of sterile saline and 0.5 mL of a sterile rat fecal suspension prepared as described previously (9). The final concentration of the bacterial inoculum was approximately 6.5×10^8 colony-forming units (cfu)/mL. The control inoculum was prepared similarly, substituting the same volume of sterile saline for the *E. coli* suspension in the saline/fecal suspension mixture.

Induction of Intra-Abdominal Infection. The experimental procedures for the induction of intra-abdominal infection in our model have been described in detail elsewhere (9). Briefly, all surgical procedures were performed under aseptic conditions with the animals under general anesthesia (1.5% halothane, 1:1 oxygen:nitrous oxide). A sterile, self-made intraperitoneal cannula (Silastic tubing; 0.76 mm inner diameter, 1.65 mm outer diameter, Dow Corning Medical, Mississauga, ON, Canada) was inserted into the abdominal cavity. The distal end of the cannula was then tunneled subcutaneously along the spine to emerge through a 1-cm skin incision at the nape of the neck and led through a stainless steel tether. All wounds were closed with nonabsorbable 3.0 silk sutures (Johnson & Johnson, Mississauga, ON, Canada), and topical antibiotic (Hibitane, Ayerst Laboratories, Montréal, PQ, Canada) was applied. The cannulated animals received a subcutaneous injection of sterile saline (30 mL/kg of body weight) and were given analgesics (buprenorphine 0.05 μg/g im). The animals were housed individually, and each tether was connected to a temperature-controlled (3–5°C [37.4–41°F]) fecal inoculum infusion system via a swivel to which the intraperitoneal cannula was attached (9). The infusion of 2 mL of either a bacterial inoculum or a control inoculum was initiated 8–10 hrs later.

Experimental Protocol. Intra-abdominal infection was induced in 56 rats (INF), whereas 29 animals received the control inoculum (CON). At the end of the 12-hr infusion period, each cannulated rat (i.e., CON or INF) was anesthetized, a midline laparotomy was performed, and 5 mL of warm sterile saline was injected into the abdominal cavity and thoroughly mixed with the peritoneal fluid, which was then aspirated. Blood and peritoneal fluid were serially diluted in sterile phosphate-buffered saline, plated onto tryptic soy agar enriched with 5% sheep blood, and incubated overnight at 37°C (98.6°F) to determine bacterial content. Small aliquots of peritoneal fluid were frozen at −70°C (−94°F) until further analysis of cytokine concentrations. The abdominal cavity was then lavaged with 70 mL of warm saline in 10- to 15-mL aliquots. Care was taken to ensure that the lavage fluid was entirely aspirated and discarded before closure of the peritoneal cavity in two layers with nonabsorbable silk sutures, this minimized the possibility of a dilutional effect when subsequently measuring bacterial concentrations in the peritoneal cavity. We have observed previously (our unpublished data) that the residual peritoneal bacterial counts were markedly reduced after these peritoneal lavage procedures were completed. All animals received a subcutaneous injection of sterile saline (30 mL/kg), and topical antibiotic was applied to all wounds before housing the rats individually. Fluid replacement was provided every 12 hrs for 48 hrs after the onset of infusion. Analgesia was provided twice daily to all surgical animals until they were randomly killed.

At 12 hrs, 36 hrs, and 7 days after the onset of infusion, animals were randomly killed by exsanguination under general anesthesia. Blood was collected into EDTA, samples were centrifuged immediately, and the plasma was frozen at −70°C (−94°F) until further assay. Peritoneal fluid was collected and processed as described above. The recovered volume of peritoneal fluid varied from 5.5 to 6.5 mL at 12 hrs and from 4.5 to 5.0 mL at 36 hrs and 7 days. Individual volumes were used in calculating the appropriate dilution factor required to correct the concentrations of different cytokines and challenge bacteria in the peritoneal fluid.

Determination of Cytokine Concentrations. Plasma IL-1β, IL-2, IL-4, IL-6, and TNF-α concentrations were measured using commercially available ELISA kits (MediCorp, Montréal, PQ, Canada) specific for rat cytokines. The sensitivities of the assays were 3 pg/mL (IL-1β), 4 pg/mL (TNF-α), 10 pg/mL (IL-2), 15 pg/mL (IL-4), and 31 pg/mL (IL-6). The within-assay variation was <5% (coefficient of variation).

Statistics. Within each group, data were analyzed using the Kruskal-Wallis test. When the probability ratio proved significant ($p < .05$), Mann-Whitney tests were used to compare the individual time points (12 hrs, 36 hrs, and 7 days) to baseline values for each of the variables measured (i.e., cytokines, bacterial counts in peritoneal fluid). Mann-Whitney tests were also used to compare intergroup differences (e.g., INF vs. CON) for each of those variables. All data throughout the text and figures are expressed as mean ± SEM.

RESULTS

Gross Effect of Surgical Procedures and Peritonitis. There were no apparent signs of stress or illness in any of the CON after the 12-hr infusion period. In contrast, INF showed piloerection, diarrhea, and nasal and/or ocular discharge. Postmortem examination confirmed ongoing peritonitis in the INF, as suggested by the presence of apparent Peyer's patches on the intestines and slightly edematous abdominal walls with increased capillarization and enlarged mesenteric lymph node. There were no intra-abdominal abscesses or pus in the abdominal cavity. We have shown previously that peritonitis in this model was associated with mild to moderate morphologic alterations in the spleen, lung, and liver at 7 days (9).

As reported previously in our model (9, 13), the intraperitoneal cannulae of INF were sometimes loosely wrapped in loops of omentum or epididymal fat infiltrated with inflammatory cells. However, this phenomenon was severe enough to completely occlude the tip of the intraperitoneal cannula in four of the 56 animals that received the bacterial challenge; these animals were humanely killed at 12 hrs, and their data were excluded from the analysis. Data from all other infected animals, whether they were bacteremic or not at the end of the 12-hr infusion period, were included in the data analysis.

Lethality of Peritonitis Model. All CON ($n = 29$) survived the 7-day study period. Most deaths attributable to the sequelae of infection (i.e., nonsurvivors. animals that were not randomly killed; 15 of the 52 infected rats) occurred within 24–48 hrs after the onset of bacterial infusion. Because the remaining infected animals ($n = 37$) were killed randomly at different times (i.e., 12 hrs, 36 hrs, and 7 days) after the onset of bacterial infusion, the overall 7-day mortality rate cannot be calculated from our data. However, we have shown that under similar experimental conditions, the dose of *E. coli* used in the present study corresponded to the LD_{50} in our model (9, 13). It is also noteworthy that the infected rats that were randomly killed at 12 and 36 hrs likely included animals that might have survived for 7 days as well as others that might have died of the sequelae of infection; thus, the cytokine patterns of INF described below are likely to represent those of a "mixed" population of infected animals.

Bacteriology. Bacteremia was observed in all but one of the INF killed at 12 and 36 hrs; the systemic bacterial counts averaged $2.9 \pm 0.7 \times 10^4$ cfu/mL and $7.6 \pm 4.2 \times 10^4$ cfu/mL, respectively. None of the infected rats was bacteremic at 7 days.

Only the challenge strain of *E. coli* was recovered in the peritoneal fluid of the INF, suggesting that there was minor gut bacterial translocation into the inflamed peritoneal cavity. Bacterial counts in the peritoneal fluid collected at the end of the 12-hr infusion period were approximately 78% lower ($p < .05$) in the infected rats that survived the 7-day study period (SURV; $5.5 \pm 1.0 \times 10^7$ cfu/mL) than in those that died before the end of the experiment (NONSURV; $25.5 \pm 3.9 \times 10^7$ cfu/mL), even though all animals had received comparable doses of *E. coli* during the infusion period ($6.5 \pm 0.4 \times 10^8$ cfu/mL). Bacterial counts in peritoneal fluid collected 24 hrs after performing the peritoneal lavage ($14.2 \pm 5.5 \times 10^7$ cfu/mL) were comparable to those measured immediately at the end of the bacterial infusion period ($19.1 \pm 3.7 \times 10^7$ cfu/mL). However, bacteria remained detectable in the peritoneal fluid of only 45% of INF at 7 days ($2.5 \pm 1.6 \times 10^4$ cfu/mL). There were no detectable bacteria in body fluids of the CON throughout the study.

Peritoneal Cytokine Concentrations. It is noteworthy that, as a result of technical difficulties and insufficient biological specimens, not all cytokine determinations were performed on the same peritoneal (or plasma) samples, which explains the differences in the sample size for a given group of samples (see Figs. 1–3).

Figure 1 depicts the changes in cytokine concentrations in peritoneal fluid collected 12 hrs after the onset of infusion (i.e., at the time of laparotomy) for the CON and INF. Data for INF are separated into two groups: animals that survived for 7 d (SURV) and animals that died before 7 d of the sequelae of infection (NONSURV). Although the peritoneal concentration of IL-1β tended to be higher in NONSURV compared with SURV, this trend did not achieve statistical significance (Fig. 1, *top left*; $p = .07$). In contrast, peritoneal concentrations of TNF-α and IL-6 at the time of laparotomy were approximately three and two times greater ($p < .05$), respectively, in NONSURV than in SURV (Fig. 1, *middle* and *bottom left*). There were no significant differences in IL-2 and IL-4 peritoneal concentrations between SURV and NONSURV (Fig. 1, *right*). Although the peritoneal concentrations of IL-1β, TNF-α, and IL-6 at 12 hrs were significantly higher ($p < .05$) in INF compared with CON, peritoneal IL-2 and IL-4 concentrations were decreased ($p < .05$) in the septic rats compared with CON by 85% and 68%, respectively.

Figure 2 depicts the changes in cytokine concentrations in peritoneal fluid collected at different times after the onset of infusion. Although the concentrations of IL-1β and TNF-α in the peritoneal fluid of INF gradually decreased during the study, these concentrations remained significantly higher ($p < .05$) than those of CON at 7 days (Fig. 2, *top* and *middle left*). Peritoneal IL-6 concentrations in INF were markedly increased at 12 hrs ($p < .05$), but these concentrations were comparable to those of CON for the remainder of the study (Fig. 2, *bottom left*). In contrast, peritoneal concentrations of IL-2 remained lower ($p < .05$) in INF than in CON for most of the experiment (Fig. 2, *top right*). Although IL-4 concentrations in peritoneal fluid in INF at 12 hrs were approximately half of those of CON ($p < .05$), these concentrations were 71% greater at 36 hrs ($p < .05$) and returned to baseline values at 7 days. There were minimal or no changes in peritoneal cytokine concentrations throughout the study period for CON (Fig. 2).

Circulating Cytokine Concentrations. Fig. 3 depicts the changes in circulating cytokine concentrations in INF and CON at different times after the onset of infusion. Plasma IL-1 concentrations were undetectable in any of the animals throughout the experiment. Plasma TNF-α concentrations increased ($p < .05$) up to five-fold in INF compared with CON during the first 36 hrs after the onset of infusion, with values returning to baseline by 7 days (Fig. 3, *top left*). Plasma IL-2 concentrations were unaltered for 36 hrs in INF and CON, but

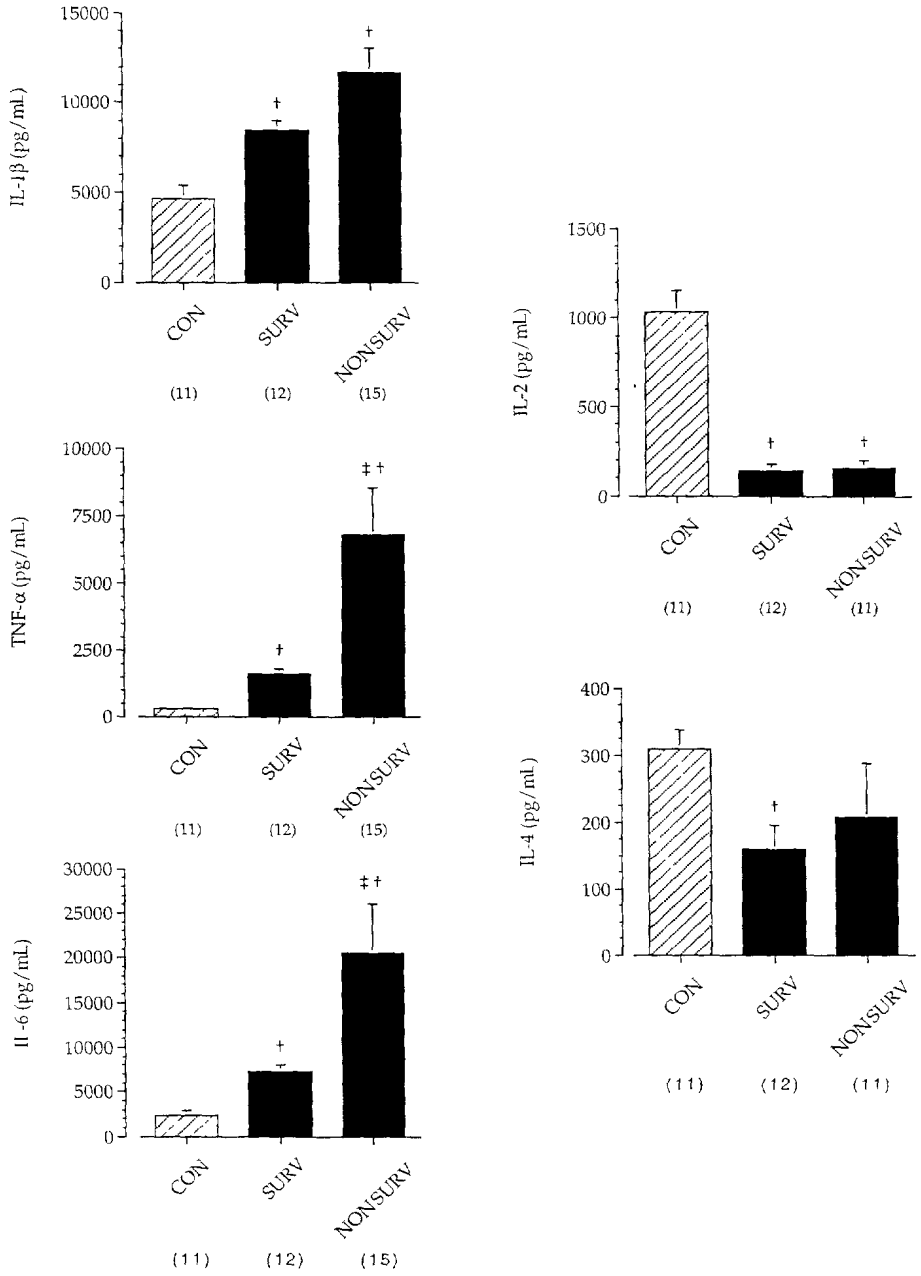

Figure 1. Cytokine concentrations in peritoneal fluid taken 12 hrs after the onset of infusion for the control animals (*CON*), for the infected rats that survived the 7-day study period (*SURV*), and for the animals that died before 7 days (*NONSURV*) The number of experimental animals in each group is indicated in *parentheses* Results are expressed as mean ± SEM. †Significantly different from CON ($p <$ .05), ‡significantly different from SURV ($p <$ 05) *IL*, interleukin, *TNF*, tumor necrosis factor

these values had almost doubled ($p <$ .05) in both experimental groups at 7 days (Fig. 3, *top right*). The circulating concentrations of IL-4 and IL-6 were comparable in INF and CON throughout the study, being unaltered by any of the experimental procedures. It is noteworthy that the plasma cytokine concentrations measured in INF were far below those measured simultaneously in peritoneal fluid during the experiment (Figs. 2 and 3; $p <$ .05).

DISCUSSION

We have described the alterations in circulating and peritoneal concentrations of cytokines in a recently developed rat model of Gram-negative peritonitis. This model allows a continuous intraperitoneal infusion of a standardized *E. coli* inoculum over 12 hrs, simulating a trauma-induced, slow bacterial release from the gut. Under these experimental conditions, we observed that high peritoneal concentrations of TNF-α and IL-6 at the end of the bacterial infusion period correlated with adverse outcome. In contrast, Zanetti et al. (7) observed that peritoneal TNF-α concentrations in rats injected with an intraperitoneal bolus *E. coli* challenge were similar in both survivors and nonsurvivors, whereas concentrations of IL-6 were more closely associated with survival. Furthermore, the magnitude of the TNF-α response in rats receiving an intraperitoneal bolus of polymicrobial fecal inoculum was similar to that observed when a nonlethal sterile inoculum was injected, suggesting that TNF-α concentrations were also not related to mortality in that model of bacterial peritonitis (6). Such discrepancies with our data may be attributable in part to differences in the mode of infection (i.e., bolus vs. infusion). Indeed, the magnitude of the peritoneal concentrations of TNF-α after murine cecal ligation and puncture, a model of peritonitis simulating persistent leakage of bacteria from a focus on infection, correlated adversely with outcome (17). However, Evans et al. (18) could not detect TNF-α concentrations in the peritoneal fluid at varying time intervals after murine cecal ligation and puncture. Nevertheless, our findings are in agreement with those from clinical studies in patients with secondary bacterial peritonitis (19) and severe intra-abdominal infections (20).

Although this and other experimental studies have identified individual proinflammatory cytokines as potential predictors of sepsis, Walley et al. (8) recently showed that the severity and mortality in murine peritonitis was better related to the balance of proinflammatory and anti-inflammatory mediators. In the present study, we measured IL-4, an important anti-inflammatory cytokine thought to contribute to immune modulation in sepsis by its inhibition of the production of IL-1, IL-6, and TNF-α (1). The lack of a relationship between the peritoneal concentrations of IL-4 and survival outcome in our infected rats appears to suggest that this cytokine may not play an important role in the early anti-inflammatory response to peritonitis. This suggestion is supported by results from a recent study showing that administration of IL-4 had

Our data would suggest the use of peritoneal rather than systemic concentrations of cytokines to evaluate the extent of the inflammatory response in intra-abdominal infections.

no effect on survival in a murine model of chronic peritonitis (21). Alternatively, IL-4 has been shown either to play a detrimental role in the immune response to *Staphylococcus aureus* infection (22) or to improve survival in acute lethal endotoxemia (23). Nevertheless, the increased production of IL-4 in peritoneal fluid after 36 hrs in our infected rats was likely an important aspect of the cytokine balance contributing to the resolution of the inflammation process.

We chose not to measure simultaneously the cytokine concentrations in peritoneal fluid and plasma of our infected animals at the time of laparotomy, not only for technical reasons but also because the recent literature suggests that local concentrations are more important biologically and, therefore, better reflect the severity of an initially local insult such as peritonitis (24, 25). In fact, our observation of minimal or no alterations in plasma concentrations of proinflammatory and anti-inflammatory cytokines in infected animals of this study would suggest that systemic concentrations were not a good indication of an ongoing inflammatory process in our model of peritonitis.

The magnitude of the changes in peritoneal concentrations of TNF-α, IL-1, and IL-6 during the first 12 hrs in infected relative to control animals was comparable to that reported in other models of Gram-negative bacterial infection with similar mortality (7, 8). However, the prolonged inflammatory response (i.e., 7 days) in our infected rats is in contrast to reports showing peritoneal concentrations returning to normal within 6–48 hrs (6–8). The sustained production of cytokines may have been attributable to endotoxin-related mechanisms. Indeed,

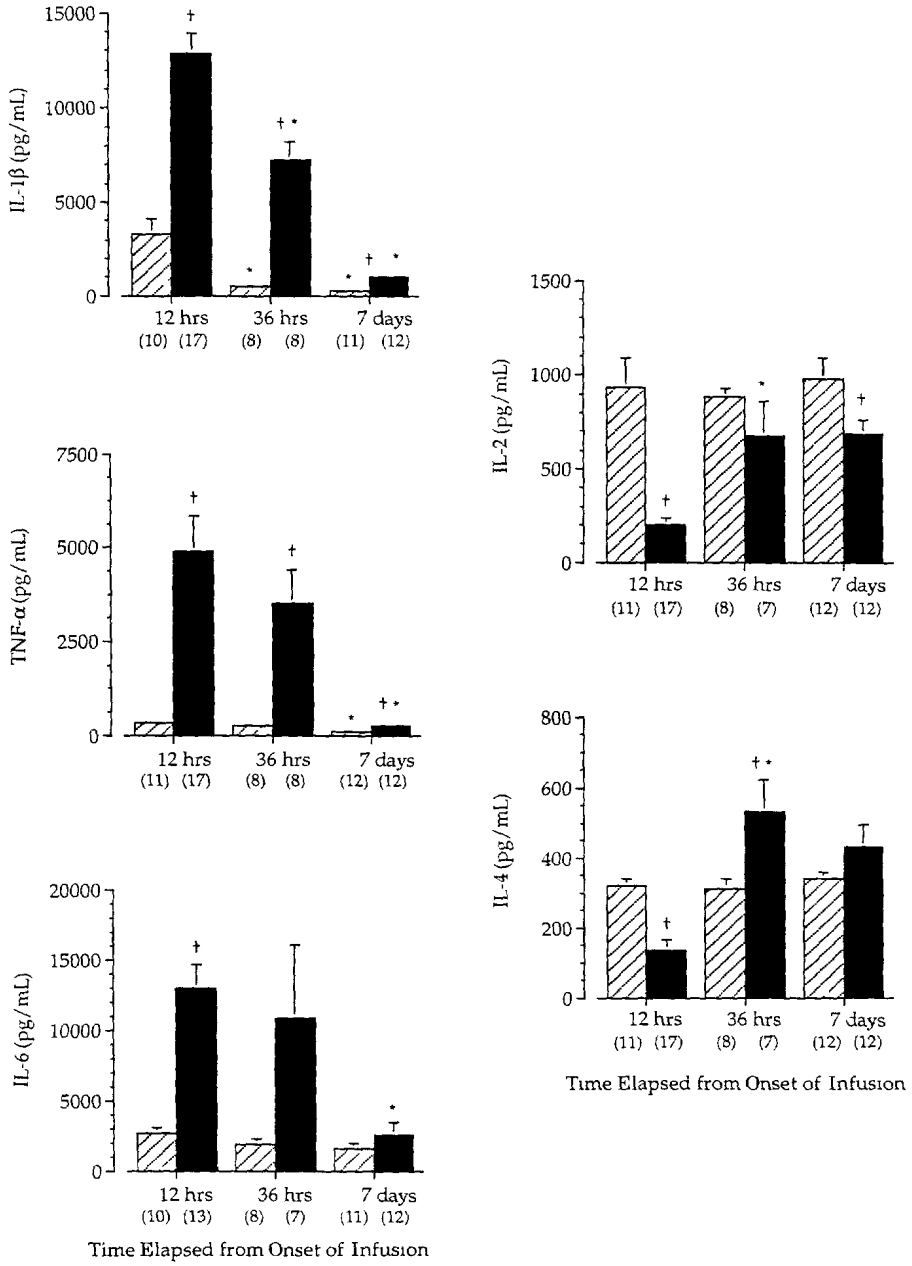

Figure 2. Cytokine concentrations in peritoneal fluid taken at the time of necropsy in control animals (*hatched bars*) and infected rats (*closed bars*). The number of experimental animals randomly killed at a given time period is indicated in *parentheses*. Results are expressed as mean ± SEM †Significantly different from control animals ($p < .05$); *significantly different from the preceding time period ($p < .05$) *IL*, interleukin; *TNF*, tumor necrosis factor.

the septic conditions associated with intra-abdominal infections have been shown to increase intestinal permeability (26), thus releasing endotoxin from the gut microflora. The persistence of bacteria in the peritoneal cavity for 7 days may also have caused the sustained release of cytokines. However, some of the infected animals showed increased peritoneal IL-1β and TNF-α concentrations at 7 days in the absence of bacteria, suggesting that other mechanisms may also have contributed to this prolonged response. Alternatively, our finding of comparable peritoneal concentrations of IL-6 in infected and control animals at 7 days concomitantly with significantly different concentrations of IL-1β and TNF-α between these groups would suggest that IL-6 might no longer dampen the inflammatory response in the late phase of peritonitis.

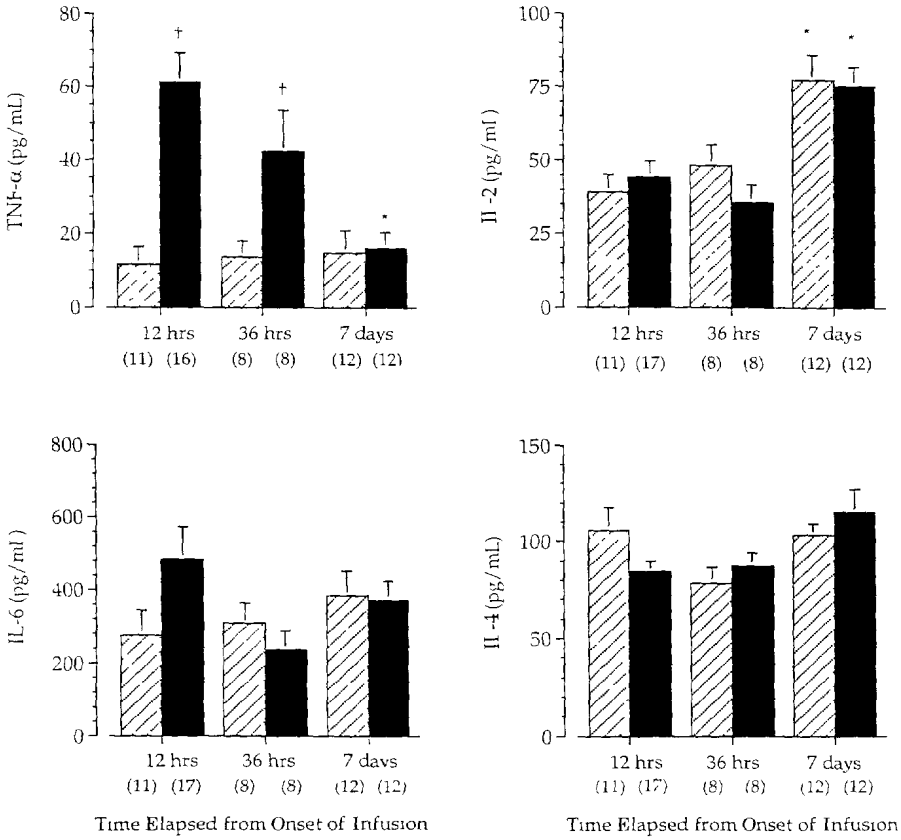

Figure 3. Cytokine concentrations in plasma obtained at the time of necropsy in control animals (*hatched bars*) and infected rats (*closed bars*). The number of experimental animals randomly killed at a given time period is indicated in *parentheses* Results are expressed as mean ± SEM †Significantly different from control animals ($p < 05$), *significantly different from the preceding time period ($p < .05$) *TNF*, tumor necrosis factor, *IL*, interleukin

Our finding of peritoneal concentrations of cytokines several times higher than those measured in the circulation is in agreement with results from several clinical studies of peritonitis (19, 20, 27) and experimental models using bolus intraperitoneal bacterial challenges (6, 7, 28). Our observation of an elevated cytokine response in the peritoneum also supports the hypothesis of a compartmentalized inflammatory process in intra-abdominal infections (25). However, in contrast to other experimental studies, there was no parallel between the plasma and peritoneal profiles of inflammatory cytokines. Whether this discrepancy indicates that in our model of peritonitis the cytokines produced locally could not diffuse easily into the systemic circulation or that there were differences in the clearance rates of the cytokines between these two compartments cannot be assessed from our data. Nevertheless, the similarity between the plasma cytokine concentrations for the control and infected animals (except for TNF-α), together with our observation that these concentrations are significantly different from those of normal rats (data not shown), may suggest that the systemic cytokines in the infected rats were produced in response to the surgical stress rather than resulting from the bacterial infection.

It is noteworthy that the cytokine kinetics data presented reflect the natural course of peritonitis but perhaps not the pattern resulting from the combined effects of infection and supportive therapy as provided clinically. Thus, one might argue that continuous infusion of fluids to the infected animals may be more relevant to the clinical setting. However, Wilson et al. (29) have shown that providing even a single volume of saline attenuates the systemic cytokine gene expression after murine cecal ligation and puncture compared with untreated animals. Whether or not repeated administration of saline, as provided to our infected rats for 48 hrs, had a persistent effect on cytokine expression cannot be determined from our data. Rosman et al. (30) have also shown that a single dose of intraperitoneal antibiotics transiently decreased the concentration of TNF-α in plasma and abdominal fluid in rats with generalized peritonitis. Similarly, we recently observed that twice-daily intravenous administration of cefoxitin to our infected rats reduced the plasma TNF-α concentrations after 24 hrs to values comparable to those of time-matched controls (our unpublished data); unfortunately, TNF-α concentrations in peritoneal fluid were not measured simultaneously in that study. Interestingly, ascitic fluid concentrations of TNF-α and IL-6 were reduced within the first 48 hrs of repeated systemic antibiotic therapy in cirrhotic patients with spontaneous bacterial peritonitis (27). However, it should be stressed that hypercytokinemia sometimes persists in clinical peritonitis despite the provision of effective antimicrobial agents and maximal supportive therapy (31).

The advantage of mechanically removing bacteria as part of the therapeutic regimen in generalized peritonitis is supported by both experimental and clinical data, which have shown that it significantly reduces the mortality associated with this condition (6, 32). However, one may argue that the lavage procedures also remove essential components of the local host response, such as macrophages and cytokines. Although cytokines were undetectable immediately after completion of the lavage procedures in the infected rats (data not shown), these concentrations had either returned to prelavage values or become elevated 24 hrs later. Because midline laparotomy *per se* is known to reduce the capacity of peritoneal macrophages to release cytokines for at least 24 hrs (33), the peritoneal cytokines that we measured were likely produced by other cells Several studies have shown that insults to the peritoneum induce the migration of polymorphonuclear neutrophils (PMN) from the blood vessels into the peritoneal cavity (34). Although PMN counts in the peritoneal fluid were not measured in the present study, we have observed previously (35) a ten-fold decrease in circulating PMN concentrations at 36 hrs in infected rats. Peritoneal mesothelial cells, mast cells, fibroblasts, and intestinal enterocytes have also all been shown to contribute to the peritoneal cytokine network in peritonitis (34, 36).

In conclusion, the septic conditions in our rat model of intra-abdominal infection are associated with a significant and prolonged peritoneal inflammatory response, which is adversely correlated with survival outcome. We can only speculate about the clinical significance of the results of this study. We acknowledge that it is technically difficult to ensure appropriate timing and specificity of intraperitoneal cytokine therapy in a clinical situation, even with rapid quantification of cytokine concentrations in individual patients. Nevertheless, our data would suggest the use of peritoneal rather than systemic concentrations of cytokines to evaluate the extent of the inflammatory response in intra-abdominal infections, and they should be considered when designing cytokine-specific therapies.

ACKNOWLEDGMENTS

The authors are indebted to D. Saunders and C. Bogard for expert technical assistance.

REFERENCES

1. Dinarello CA: Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. *Chest* 1997; 112:S321–S329
2. van der Poll T, Keogh CV, Helfgott D: Effects of anti-interleukin-6 on inflammatory responses during murine septic peritonitis. *J Endotox Res* 1996; 3:463–470
3. Deitch EA: Animal models of sepsis and shock: A review and lessons learned. *Shock* 1998; 9:1–11
4. Cross AS, Opal SM, Sadoff JC, et al. Choice of bacteria in animal models of sepsis. *Infect Immun* 1993; 61:2741–2747
5. Wichterman KA, Baue AE, Chaudry IH: Sepsis and septic shock: A review of laboratory models and a proposal. *J Surg Res* 1980; 29:189–201
6. Bagby GJ, Plessala KJ, Wilson LA, et al. Divergent efficacy of antibody to tumor necrosis factor-α in intravascular and peritonitis models of sepsis *J Infect Dis* 1991; 163: 83–88
7. Zanetti G, Heumann D, Gérain J, et al: Cytokine production after intravenous or peritoneal Gram-negative bacterial challenge in mice. *J Immunol* 1992, 148:1890–1897
8. Walley KR, Lukacs NW, Standiford TJ, et al: Balance of inflammatory cytokines related to severity and mortality of murine sepsis. *Infect Immun* 1996, 64:4733–4738
9. Martineau L, Shek PN: A sustained release bacterial inoculum infusion model of intra-abdominal infection in conscious rats: Bacteriology, metabolism, and histopathology. *Shock* 1996; 5:446–454
10. Holzheimer RG, Cavaillon JM, Mosea F, et al: Clinical and basic science aspects of immune pathogenesis of sepsis and peritonitis. *Med Microbiol Lett* 1996; 5 386–393
11. Bartlett JG: Intra-abdominal sepsis. *Med Clin North Am* 1995; 79:599–617
12. Canadian Council on Animal Care: Laboratory rats. *In:* Guide to the Use and Care of Experimental Animals. Vol. 2. Ottawa, National Library of Canada, 1984, pp 175–189
13. Martineau L, Shek PN: Efficacy of liposomal cefoxitin therapy in a fecal infusion model of bacterial peritonitis in the rat. *Shock* 1997, 7(Suppl) 137
14. Kresta A, Shek PN, Odumeru J, et al: Distribution of free and liposome-encapsulated cefoxitin in experimental intra-abdominal sepsis in rats. *J Pharm Pharmacol* 1993; 45: 779–783
15. Finegold SM, Martin WJ: Diagnostic Microbiology. St. Louis, CV Mosby, 1982, p 651
16. Bartlett JG, Onderdonk AB, Louie T, et al: Lessons from an animal model of intra-abdominal sepsis. *Arch Surg* 1978; 113: 853–857
17. Astiz ME, Saha DA, Carpati CM, et al: Induction of endotoxin tolerance with monophosphoryl lipid A in peritonitis: Importance of localized therapy. *J Lab Clin Med* 1994; 123 89–93
18. Evans GF, Snyder YM, Butler LD, et al: Differential expression of interleukin-1 and tumor necrosis factor in murine septic shock models *Circ Shock* 1989; 29:279–290
19. Propst T, Propst A, Herold M, et al: Spontaneous bacterial peritonitis is associated with high levels of interleukin-6 and its secondary mediators in ascitic fluid. *Eur J Clin Invest* 1993; 23:832–836
20. Holzheimer RG, Schein M, Wittmann DH: Inflammatory response in peritoneal exudate and plasma of patients undergoing planned relaparotomy for severe secondary peritonitis. *Arch Surg* 1995; 130:1314–1320
21. Sawyer RG, Rosenlof LK, Pruett TL: Interleukin-4 prevents mortality from acute but not chronic murine peritonitis and induces an accelerated tumor necrosis factor-α response. *Eur Surg Res* 1996; 28:119–123
22. Hultgren O, Kopf M, Tarkowski A: *Staphylococcus aureus*-induced septic arthritis and septic death is decreased in IL-4 deficient mice: Role of IL-4 as promoter for bacterial growth. *J Immunol* 1998; 160:5082–5087
23. Baumhofer JM, Beinhauer BG, Wang JE, et al. Gene transfer with IL-4 and IL-13 improves survival in lethal endotoxemia in the mouse and ameliorates peritoneal macrophages immune competence. *Eur J Immunol* 1998; 28:610–615
24. Cavaillon JM, Munoz C, Fitting C, et al: Circulating cytokines: The tip of the iceberg? *Circ Shock* 1992; 38:145–152
25. Schein M, Wittmann DH, Holzheimer R, et al: Hypothesis: Compartmentalization of cytokines in intraabdominal infection *Surgery* 1996; 119:694–700
26. Fink M, Antonson J, Wang H, et al: Increased intestinal permeability in endotoxic pigs: Mesenteric hypoperfusion as an etiologic factor. *Arch Surg* 1991; 126:211–218
27. Zeni F, Tardy B, Vindiman M, et al: High levels of tumor necrosis factor-α and interleukin-6 in the ascitic fluid of cirrhotic patients with spontaneous bacterial peritonitis *Clin Infect Dis* 1993; 17:218–223
28. Montravers P, Mohler J, Saint Julien L, et al: Evidence of the proinflammatory role of *Enterococcus faecalis* in polymicrobial peritonitis in rats. *Infect Immun* 1997; 65:144–149
29. Wilson MA, Chou MC, Spain DA, et al: Fluid resuscitation attenuates early cytokine mRNA expression after peritonitis. *J Trauma* 1996; 41:622–627
30. Rosman C, Westerveld GJ, van Oeveren W, et al: Effect of intraperitoneal antimicrobials on the concentration of bacteria, endotoxin, and tumor necrosis factor in abdominal fluid and plasma in rats. *Eur Surg Res* 1996; 28: 351–360
31. Rotstein OD, Meakins JL. Diagnostic and therapeutic challenges of intraabdominal infections. *World J Surg* 1990; 14:159–166
32. Baker CC, Chaudry IH, Gaines HO, et al Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. *Surgery* 1983; 94:331–335
33. Zellweger R, Ayala A, Zhu XL, et al: Effect of surgical trauma on splenocyte and peritoneal macrophage immune function *J Trauma* 1995; 39:645–650
34. Topley N, Williams JD: Role of the peritoneal membrane in the control of inflammation in the peritoneal cavity *Kidney Int* 1994, 46: S71–S78
35. Martineau L, Shek PN: Characterization of a bacterial infusion model of peritonitis in the rat. II. Circulating levels of leukocytes, lymphocyte subsets and platelets. Abstr *Crit Intensive Care* 1995; 6(Suppl): 21
36. Ogle CK, Guo X, Hasselgren PO, et al. The gut as a source of inflammatory cytokines after stimulation with endotoxin. *Eur J Surg* 1997; 163:45–51

513557