ANNALS OF SURGERY
Vol. 225, No. 6, 744–756

Poor Outcome from Peritonitis is Caused by Disease Acuity and Organ Failure, Not Recurrent Peritoneal Infection

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Objective
The purpose of the study is to determine whether organ failure develops in patients despite control of peritoneal infection and whether the process is, in part, neutrophil (polymorphonuclear leukocyte [PMN]) mediated.

Summary Background Data
Peritonitis generally responds to prompt surgical intervention and systemic antibiotics; however, some patients continue a septic course and progress to organ failure and death.

Methods
One hundred five consecutive patients with peritonitis between 1988 and 1996 who required operation and a postoperative hospital stay greater than 10 days were studied. Mice were injected with a monoclonal anti-PMN antibody 24 hours before cecal ligation and puncture (CLP) to deplete PMNs.

Results
Thirty-eight patients died, and all but 1 had identified organ failure. Seventy-seven patients had either pulmonary failure alone (25 patients) or as a component of multisystem organ failure (52 patients). All but one of these patients showed resolution of their intraperitoneal infection as evident by clinical course, abdominal computed tomographic scan, second-look laparotomy, or autopsy. Recurrent intra-abdominal infection developed in 15 patients, but only 1 had organ failure, and 2 died. At 18 hours after CLP, lung injury, PMN content, interleukin-1 mRNA expression, and liver injury were significantly reduced by anti-PMN treatment, whereas serum endotoxin levels actually increased.

Conclusions
Disease acuity and organ failure, and not recurrent peritoneal infection, are the major causes of adverse outcome in patients with peritonitis. The authors' experimental data indicate that such organ injury is, in part, PMN mediated but not endotoxin mediated. Attraction of PMNs toward the site of primary infection, and thereby away from remote organs, is a logical future therapeutic approach in such patients who are critically ill with peritonitis.

Secondary bacterial peritonitis generally responds to resuscitation, appropriate systemic antibiotics, and prompt surgical intervention[1]; however, recurrent intra-abdominal infection, complex wound management, and organ failure continue to be challenging clinical problems. Dellinger et al.[2] have shown that disease acuity, as measured by the Acute Physiology Score, and not anatomic site of infection has correlated with mortality in patients with peritonitis. Fry et al.,[3] Norton,[4] and Eiseman et al.[5] reported that the most common cause of multisystem organ failure (MSOF) was intra-abdominal sepsis. The term MSOF was coined initially by Baue[6] and was associated with several clinical disease states and types of physiologic stress. Remote organ failure alone without localizing signs to the abdomen is rarely an indication for operative intervention now but had been a reliable sign of occult intra-abdominal infection in the past.[7] Although overall mortality for peritonitis has changed little in several decades, the increase in remote organ failure probably is the result of more aggressive resuscitation, intensive care support, and operative intervention, which has prolonged survival time and changed the cause of death.

In patients in whom penetrating trauma often preceded the onset of MSOF, uncontrolled infection and sepsis were believed to be a common cause of remote organ injury. Data from numerous models of bacterial translocation, both clinical and experimental, were used to explain the mechanism of organ failure.[8–10] However, MSOF in patients after blunt trauma has been described without bacteremia or persistent abdominal infection.[11–13] Anderson et al.[14] have suggested that the neutrophil is central to remote organ failure, and an increase in the numbers of circulating neutrophils is common in these clinical settings.[15] Organ injury, however, has been postulated to occur after the CD11b/CD18 (integrin) complex on neutrophils has bound to the ICAM-1 receptor on endothelium, allowing neutrophils to migrate across the vascular barrier into tissue parenchyma.[16,17] The beta-2 integrin complex CD11b/CD18 is one of several facilitators of neutrophil sequestration. Botha et al.[16] have shown an upregulation of CD11b on circulating neutrophils from patients who progress to MSOF when compared with patients with similar injury who do not progress to MSOF. Furthermore, the CD11b/CD18–ICAM-1 bound complex appears to have a role in neutrophil oxygen-dependent cytotoxicity,[18] whereas blockade of CD11b attenuates lung injury in models of ischemia–reperfusion and endotoxemia.[19]

To define strategies for treatment of organ failure, determination of the cause of organ injury in the setting of peritonitis and its relation to the systemic inflammatory response[20–22] is a worthwhile goal. The aims of the current clinical studies were to define reasons for poor clinical outcome in patients with severe intra-abdominal infection and to determine if there was a relation between recurrent infection and organ failure. In addition, we examined the role of the neutrophil in the production of acute liver and lung injury by using an antineutrophil (polymorphonuclear leukocyte [PMN]) antibody to deplete the PMN population in a clinically relevant experimental model of peritonitis produced by cecal ligation and puncture (CLP).

MATERIALS AND METHODS

Clinical

The hospital charts of patients with peritonitis at two major teaching hospitals at the University of Louisville (University Hospital and Veterans Affairs Medical Center) were reviewed. The general surgical services are similar at both hospitals, with a chief resident under close attending supervision. A retrospective analysis was performed from 1988 to 1995 at the University of Louisville Hospital and from 1992 to 1995 at the Veterans Affairs Medical Center. The inclusion criteria included patients with secondary bacterial peritonitis who underwent surgical intervention (laparotomy) and who had a hospital length of stay >10 days. The excluded patients were those with trauma or burns, those treated solely by the medicine or gynecologic services, those who remained in the hospital <10 days, patients with metastatic cancer, or those patients who were terminal and not operated on. Also excluded were patients whose initial operation involved vascular reconstruction or coronary artery bypass grafting and in whom clinical peritonitis subsequently developed. Finally, patients who remained hospitalized for persistent, well-drained fistulas and those who remained hospitalized for reasons not related to the episode of peritonitis (*e.g.*, psychiatric disorders) also were excluded.

The review included patient demographics, cause of peritonitis, and operative interventions. Also recorded were history of pulmonary disease, Acute Physiology Score and Chronic Health Index (Apache III) scores,[23] length of hospital and intensive care unit (ICU) stays, duration of ventilatory assistance, development of remote organ failure, development of adult respiratory distress syndrome (ARDS), and death. Pulmonary failure was defined (Table 1) as fraction of inspired oxygen >0.6, positive end-expiratory pressure >5-cm water, persistent need

Presented at the 108th Annual Meeting of the Southern Surgical Association, December 1–4, 1996, Palm Beach, Florida.
Supported by VA Merit Review grant and funds from the Mason and Mary Rudd Foundation.
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Accepted for publication December 13, 1996.

Table 1. DEFINITION OF ORGAN FAILURE*

Pulmonary failure (must meet one of the following)
Positive end-expiratory pressure > 5 cmH_2O for more than 24 hr
Persistent need for mechanical ventilation > 48 hr after operation
Chest x-ray consistent with adult respiratory distress syndrome
Renal failure (must meet one of the following)
Need for dialysis
Blood urea nitrogen > 75 mg/dL
Urine output < 10 mL/hr with central venous pressure > 18 mmHg, or pulmonary capillary wedge pressure > 20 mmHg
No response to 120 mg of Lasix
Gastrointestinal failure
Stress ulcers
Gross blood loss via nasogastric tube
Hepatic failure (must meet two of the following)
Bilirubin > 6 mg/dL
Alkaline phosphatase twice normal
Aspartate aminotransferase and alanine aminotransferase twice normal
Albumin < 2.5 g/dL
Serum ammonia level twice normal
Coagulation failure (must meet two of the following)
Platlet count < 60,000/mm^3
Prothrombin time > 18 or requirement for 4 units fresh frozen plasma
Fibrinogen < 150 mg/dL
Fibrin split products > 16 dilutions
Central nervous system failure (must meet the following)
Glasgow Coma Score < 8 in the absence of an anesthetic agent

* Modified with permission from Jordan DA, Miller CF, Kubos KL, Rogers MC. Evaluation of sepsis in a critically ill surgical population. Crit Care Med 1987; 15:897.

for mechanical ventilation >48 hours, and diffuse alveolar pattern on chest x-ray.[22] Patients who were classified as having pulmonary failure possessed at least two of the aforementioned criteria. Individual organ failure also was defined as listed in Table 1.[22] Resolution of intra-abdominal infection was determined by either clinical course, repeat laparotomy, postoperative computed tomographic scan, or autopsy. Persistent or recurrent intraperitoneal infection was determined by using the above criteria as well. Other clinical factors that led to inclusion in the study, such as prolonged ileus, need for pulmonary care, postoperative pneumonia, superficial wound infections, and ostomy complications, also were recorded.

Statistical Analysis

The entire database was analyzed by using Quattro Pro Computer Software (Version 5.00 for Windows, Borland International, Scotts Valley, CA). Differences among groups were analyzed by two-tailed Student's t test for the individual predictors and measure of outcome; differences in gender, mortality rates, and the development of ARDS among groups were determined by chi square analysis with Yates' correction. A one-way analysis of covariance then was performed on several of the predictive criteria to establish whether variable dependence existed.

Experimental

Cecal Ligation and Puncture Protocol

All animal studies were conducted according to the guidelines of the Institutional Animal Care and Use Committee. Animals were housed in an American Association for Accreditation of Laboratory Animal Care-approved facility and provided food and water *ad libitum*. Adult male Swiss Webster mice (Taconic, Germantown, NY) weighing 25 to 30 g received intramuscular injections of a ketamine/xylazine (80/16 mg/kg) anesthetic mixture. The cecum was isolated through a small incision in the left lower abdominal quadrant and then ligated with a 2–O silk ligature near the ileocecal valve. An 18-gauge needle was used to puncture the cecum twice through-and-through, and a small amount of feces was extruded. The cecum was returned to the peritoneal cavity, and the abdominal incision was closed in two layers.

Antipolymorphonuclear Leukocyte Antibody

Twenty-four hours before CLP, animals were injected intraperitoneally with 1.44-mg rat antimouse neutrophil monoclonal antibody RB6-8C5 diluted in 0.2 mL normal saline (generously supplied by Charles Czuprymski, Department of Pathological Sciences, University of Wisconsin). Two control groups received either an equal amount of saline injected intraperitoneally before CLP (saline control subjects) or had saline injection but no CLP performed (normal subjects). At 6 and 18 hours after CLP, the mice were killed and blood obtained by cardiocentesis for leukocyte count and differential, serum endotoxin, liver transaminase levels, and lung myeloperoxidase (MPO) assay. Mice killed at 18 hours had lungs harvested for mRNA isolation, and lung Evans blue quantification.

Cell Count and Differential

Fifty microliters of blood was stained with Turk's solution, and total leukocyte count was performed manually by hemocytometer. Thin smears of whole blood were prepared and stained with Wright's and Giemsa stains. A differential count then was performed manually.

Evans Blue Protocol

Evans blue dye was used to measure capillary leak (acute lung injury) of dye-bound albumin into lung tissue

homogenates. Evans blue dye diluted in normal saline was injected into the lateral tail vein of anesthetized mice at a concentration of 20 mg/kg mouse weight (approximately 0.2 mL for a 25-g mouse). Thirty minutes after dye injection, mice underwent midline laparotomy and thoracotomy. The trachea was cannulated with an 18-gauge angiocatheter, and bilateral hemidiaphragms then were transected. The inferior vena cava was ligated with a 2–O silk ligature, whereas the superior vena cava was ligated with a medium/short titanium ligaclip (Ethicon, Cincinnati, OH). After transection of the descending thoracic aorta, a 23-gauge butterfly needle was inserted into the right ventricle. Five milliliters of phosphate-buffered saline with 6-mmol ethylenediaminetetraacetic acid (EDTA) then was infused at 20-cm water pressure while ventilating the lungs through the tracheal cannula. Ventilation was performed under direct inspection until the lungs were inflated at a rate of 40 breaths per minute. Care was taken not to overinflate the lungs because this could cause endothelial damage and increased Evans blue extravasation. After the infusion of phosphate-buffered saline, both lungs were removed. The right lung was weighed and placed into 2-mL formamide, whereas the left lung was weighed and placed in a dry container. The specimen in formamide was homogenized and then incubated at 36 C for 18 hours, whereas the specimen in the dry container was incubated at 60 C until no further reduction in weight occurred. After the 18-hour incubation in formamide, specimens were centrifuged at 3000 × g to remove the particulate matter. Supernatant Evans blue was determined by comparing the optical density at 620 nm against a standard curve and expressed as micrograms of dye per gram of dry weight.

Myeloperoxidase Assay

Lung MPO is a direct reflection of the quantity of neutrophils in the lungs.[24] The resected lobe was weighed and placed into 2 mL of 20-mM phosphate homogenization buffer. The tissues then were homogenized at high speed using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Samples were centrifuged at 10,000 rpm for 20 minutes, after which the supernatant was discarded. Myeloperoxidase was extracted using a 50-mM phosphate buffer with 10-mM EDTA and 0.5% hexadecyltrimethylammonium bromide and quantitated against a standard curve of human MPO using tetramethylbenzidine as the color reagent.

mRNA Extraction

The left lobe of the lung was removed and RNA extraction performed as described previously by Chomczynski and Sacchi.[25]

Semiquantitative Polymerase Chain Reaction of Interleukin-1 Beta

This is a previously described semiquantitative competitive differential polymerase chain reaction (PCR)-based protocol adapted to quantitate murine interleukin-1β (IL-1β) mRNA.[26–28] Total cellular RNA (400 ng) underwent reverse transcription according to the Geneamp RNA PCR kit (Perkin–Elmer, Norwalk, CT) protocol using random hexamers to prime the reverse transcriptase. The primers for murine GAPDH (Clontech, Palo Alto, CA) and IL-1β (Stratagene, La Jolla, CA) were added in the same tube at a final concentration of 0.5 mM each. The ratio of IL-1β to GAPDH was calculated from densitometric analysis of negative films for each individual specimen after electrophoresis and ethidium bromide staining of the PCR products. The size of the fragments was confirmed with molecular weight markers. Data are presented as ratio to GAPDH, and nonparametric statistics (*i.e.*, Mann–Whitney U test) were used for the semiquantitative analysis.

Liver Transaminase Quantitation

Serum was assayed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using a colorimetric assay (Sigma Chemical Company, St. Louis, MO) according to the manufacturer's instructions. Serum AST and ALT are expressed as Sigma–Frankel units/mL.

Serum Endotoxin Measurement

Endotoxin levels were determined in 1:10 dilutions of serum by the chromogenic limulus amoebocyte assay (QCL-1000; BioWhittaker, Walkersville, MD) according to the manufacturer's protocol.

Statistical Analysis

For comparison of IL-1β mRNA levels, Mann–Whitney U tests were used. All other measurements in the various groups were compared using two-tailed Student's t tests for unpaired data.

RESULTS

Clinical Study

One hundred five patients met the inclusion criteria and were reviewed. There were 48 men and 57 women with a mean age of 59 years (range, 20 to 87 years). Thirty-eight of the 105 patients died and organ failure developed in 77. All but 1 of the 38 patients died with organ failure. Recurrent or persistent intraperitoneal infection occurred in 15 patients, but interestingly, organ failure developed in only 1 of these patients and 2 died. Fourteen patients

Table 2. SOURCES OF ABDOMINAL SEPTIC FOCI

Source	No. of Patients Affected
Ruptured gastric or duodenal ulcers	22
Small bowel obstruction with perforation	19
Pancreatitis	12
Perforative diverticulitis	10
Infarcted bowel secondary to a hypotensive episode	8
Perforated cecum or large intestine	8
Appendicitis	6
Cholecystitis	5
Retroperitoneal abscess	4
Perforated colon cancer (without evidence of distant metastasis)	4
Iatrogenic injury	3
Perforated small bowel tumors	2
Tubo-ovarian abscess	1
Idiopathic sepsis	1

recovered from their bout of peritonitis and organ failure did not develop in them. The sources of peritonitis are listed in Table 2, and perforated hollow viscus was the most common.

The 105 patients were placed into 1 of 4 clinical outcome groups for comparison. Fifteen patients had a clinical outcome of persistent or recurrent intraperitoneal infection. Fifty-one had MSOF and another 25 had pulmonary failure alone; none of these 76 patients had recurrent or persistent intra-abdominal infection. Fourteen patients had no organ failure and recovered from their abdominal infection, but stay was prolonged for other reasons such as wound complication and nutritional management. Thirty-one deaths (82%) occurred in the MSOF group compared with 7 deaths (18%) in the remaining groups ($p < 0.001$). Only five patients with pulmonary failure, and not MSOF, died; this was not significantly different from the population without remote organ injury that had two deaths. All patients with MSOF met previously defined criteria for sepsis (Table 3)[29], whereas 24 of 25 patients with pulmonary failure alone, and 15 of 28 patients with no remote organ failure, met these criteria. There was little difference in the mean number of operations per patient in the four groups (1.9 in the recurrent infection group, 1.6 in the MSOF group, 1.4 in the pulmonary failure group, and 1.1 in the group without organ failure or infection). In the survivors who had organ failure, the average number of days from operation to resolution of organ failure was 8 in those with pulmonary failure alone and 23 in those with MSOF. However, it took another 28 days between resolution of failure and discharge from the hospital in the pulmonary failure group and 40 days in the MSOF group.

A significantly larger proportion of patients (72%) were able to recover from their abdominal infection but suffered from either pulmonary failure alone or MSOF as compared to those (28%) who suffered no remote organ failure ($p < 0.05$). Peritoneal infection in all but one of the patients with pulmonary failure or MSOF resolved, as evident from the defined clinical course (15 patients), postoperative abdominal computed tomographic scan (19 patients), second-look laparotomy (35 patients), or autopsy (7 patients). All patients in whom MSOF developed had pulmonary failure. In fact, the lungs were the first organ to fail (either alone or in conjunction with the coagulation system) in every case of MSOF. The second most common remote organ system involved in MSOF was the coagulation system (28 patients), followed by the renal and hepatic systems (23 patients), and then the central nervous system and gastrointestinal system with 5 and 4 patients, respectively.

APACHE III scores were significantly higher in those patients who died, with the mean 64 *versus* 40 ($p < 0.0001$). APACHE III scores for patients in whom pulmonary failure developed without other remote organ system failure averaged 43, whereas those patients who experienced no remote organ injury averaged 28 ($p < 0.001$). APACHE III scores were significantly higher in patients in whom MSOF developed than in patients in whom pulmonary failure alone developed, with the mean 63 *versus* 43 ($p < 0.0001$). The patient's age, gender, and a history of pulmonary disease (*e.g.*, chronic obstructive pulmonary disease, emphysema, history of active tuberculosis) were not significantly different among patients who died *versus* those who survived, patients in whom MSOF developed *versus* those in whom MSOF did not develop, or patients in whom MSOF developed *versus* those in whom pulmonary failure alone developed.

Remote organ injury after peritonitis, either pulmonary failure alone or in conjunction with MSOF, resulted in an increased number of days in the ICU (mean, 26 days compared to 6 days, $p < 0.0001$), an increased number

Table 3. SEPSIS CRITERIA*

Temperature > 38° C
Heart rate > 100 beats/min
Respiratory rate > 20 breaths/minute or partial pressure O_2 < 32 mm Hg
White blood cell count > 12,000 cells/mm^3 or <4000 cells/mm^3

* Patient must meet two of the four criteria.

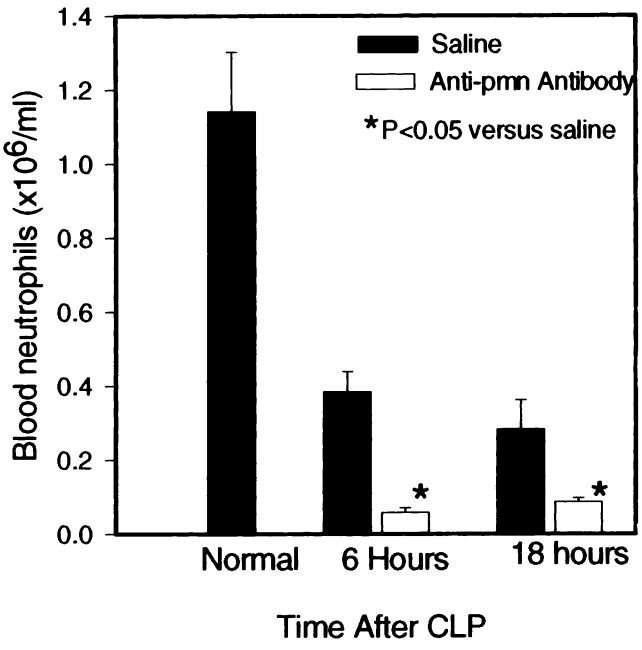

Figure 1. The number of peripheral blood neutrophils from normal subjects (unmanipulated control subjects), saline control subjects, and mice that received antipolymorphonuclear leukocyte antibody at 6 and 18 hours after cecal ligation and puncture.

of days of mechanical ventilation (mean, 18 days *versus* 2 days, $p < 0.0001$), and a longer hospital stay (mean, 42 days *versus* 26 days, $p < 0.01$). When compared to patients with lung failure alone after peritonitis, patients with MSOF required a longer stay in the ICU (mean, 31 days *vs.* 15 days, $p < 0.01$) and longer mechanical ventilation (mean, 23 days *vs.* 8 days, $p < 0.001$), but not a longer hospital stay. The use of total parenteral nutrition increased steadily from 2.6 ± 1.3 days/patient in the group of 14 without organ failure or recurrent infection, 5.9 ± 2.7 days/patient in the recurrent infection group, and 7.6 ± 1.8 days/patient in the pulmonary failure alone group to 13.2 ± 2.1 days/patient in those with MSOF ($p < 0.05$ compared to other groups). The analysis of covariance showed that in those patients without organ failure, there was a positive correlation between age and both ICU stay and duration on the ventilator, APACHE III score and duration on the ventilator, ICU stay and duration on the ventilator, and duration on the ventilator and length of hospital stay. In those patients with organ failure, there was a positive correlation between the APACHE III score and duration on the ventilator, and the ICU stay and duration on the ventilator. Thus, APACHE III score and organ failure were independent predictors of mortality.

Twenty-two patients met the definition for ARDS, 18 of whom died. Twenty of the 22 progressed to MSOF, whereas only 2 had isolated pulmonary failure after peritonitis. Seventeen of the 20 patients with both ARDS and MSOF eventually died, whereas 1 of the 2 patients with ARDS and isolated pulmonary failure died. Pneumonia, as defined by positive sputum culture, fever, leukocytosis, and infiltrate on chest x-ray, occurred in 71 patients. Common organisms recovered from sputum included *Pseudomonas sp.* from 25 patients, *Staphylococcus sp.* from 18 patients, *Enterobacter* from 12 patients, *Klebsiella* from 8 patients, and other organisms recovered from 8 patients. Pneumonia was most common in those with MSOF (44/51), followed by those with pulmonary failure alone (17/25), persistent infection (5/14), and those without organ failure or recurrent peritoneal infection (5/15).

Experimental Study

In previous studies, mice injected intraperitoneally with the anti-PMN antibody RB6-8C5 showed a profound decrease in circulating neutrophils for up to 5 days after injection, without markedly altering other circulating leukocytes.[30] After injecting five mice with the antibody intravenously and four littermates with an equal volume of saline (control subjects), CLP was performed. At 6 and 18 hours after CLP, the number of circulating blood neutrophils was significantly reduced in the antibody-treated group when compared with that of saline control subjects (Fig. 1).

In this same experiment, the lungs were harvested for mRNA quantitation. After CLP, lung IL-1β mRNA ex-

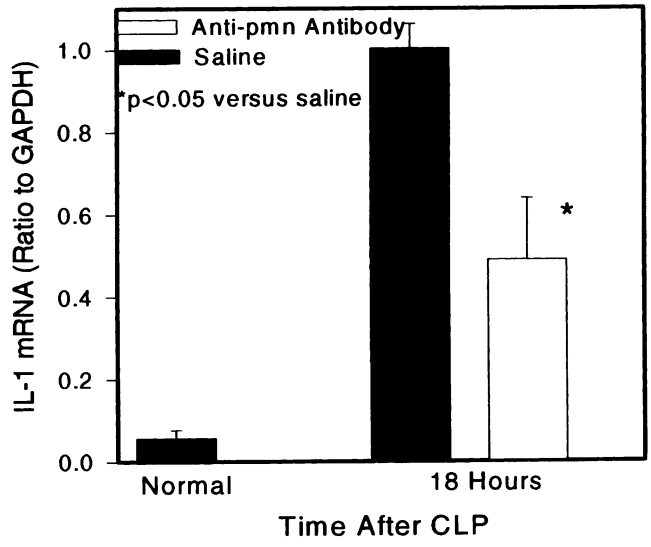

Figure 2. The ratio of lung interleukin-1 mRNA to GAPDH RNA (polymerase chain reaction product) from normal subjects, saline control subjects, and antipolymorphonuclear leukocyte-treated mice 18 hours after cecal ligation and puncture.

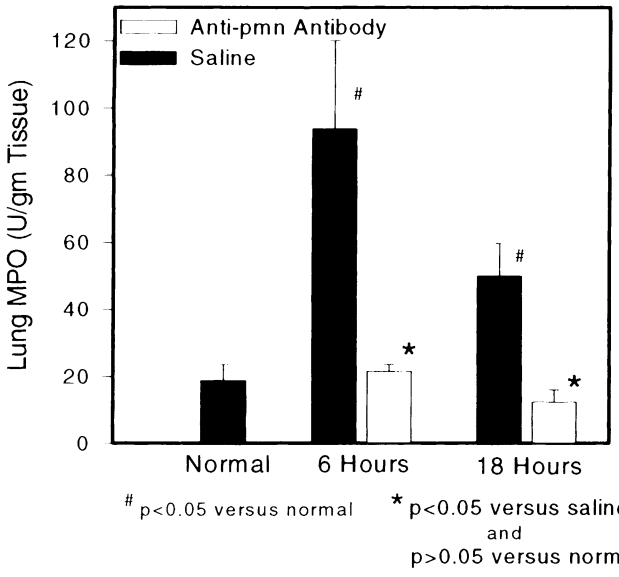

Figure 3. Lung myeloperoxidase levels from normal subjects, saline control subjects, and antipolymorphonuclear leukocyte-treated mice 6 and 18 hours after cecal ligation and puncture.

pression compared to GAPDH was markedly increased in both groups after CLP when compared with that of normal subjects. However, the group treated with the antibody had significantly lower expression of IL-1β mRNA compared to GAPDH than the mice treated with saline before CLP (Fig. 2).

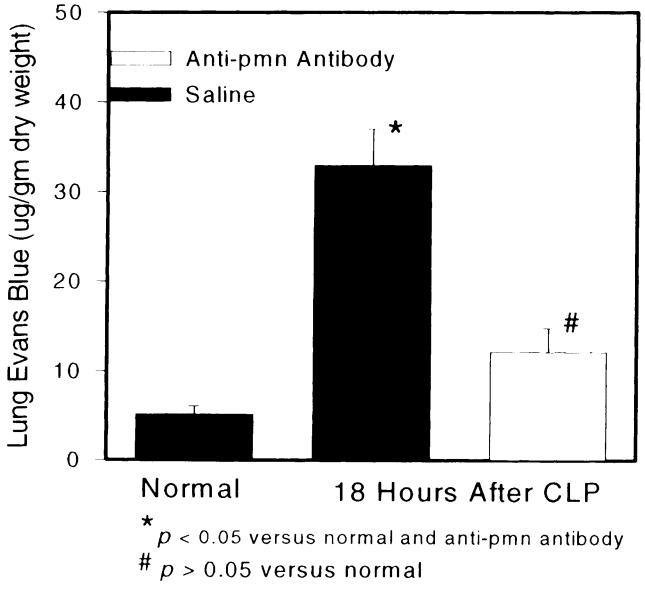

Figure 4. Evans blue content of lungs from normal subjects, saline control subjects, and antipolymorphonuclear leukocyte-treated mice 18 hours after cecal ligation and puncture.

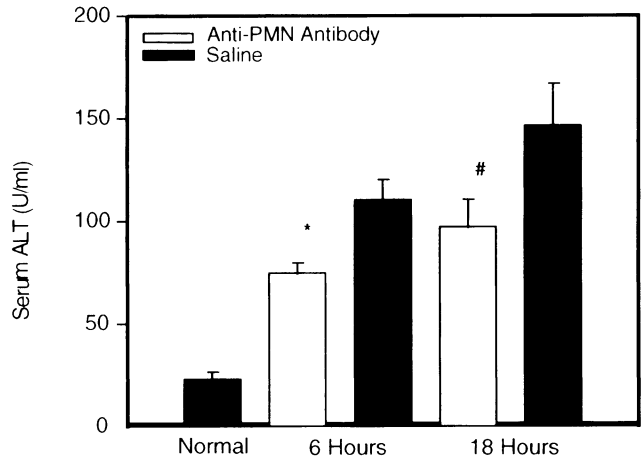

Figure 5. Serum alanine aminotransferase levels from normal subjects, saline control subjects, and antipolymorphonuclear leukocyte-treated mice 6 and 18 hours after cecal ligation and puncture. *,# = $p < 0.05$ compared to saline control subjects.

In a separate experiment, six mice were treated with the antibody, whereas seven were injected with an equal volume of saline. After CLP, lung MPO was significantly increased in the saline-treated animals when compared with that of normal subjects. Those animals treated with the anti-PMN antibody showed a complete return to baseline MPO levels equivalent to those levels measured in animals not subjected to CLP (Fig. 3).

Six mice were given the antibody and five littermates were injected with an equal volume of saline followed by CLP. Eighteen hours later, these mice were administered Evans blue dye, and 30 minutes later, these mice were killed. Lung Evans blue dye was significantly increased in the saline control group that underwent CLP when compared with that of normal subjects and three times higher than the group treated with anti-PMN antibody before CLP ($p < 0.05$), whereas there was no difference between the normal subjects and those in the antibody-treated groups (Fig. 4).

To assess liver injury, serum transaminases were measured after CLP. Four mice were injected with the antibody, whereas four littermates were injected with an equal volume of saline followed by CLP. When compared with normal subjects, CLP significantly elevated both serum AST and ALT. Serum ALT, which is more specific for liver injury, was significantly increased after CLP in the saline-treated group when compared with that of the antibody-treated animals at both 6 hours and 18 hours (Fig. 5). Serum AST was significantly elevated at 6 hours in the saline control group. At 18 hours, serum AST was greater in the animals of the saline control group than the antibody-treated animals, but the difference did not achieve statistical significance (Fig. 6).

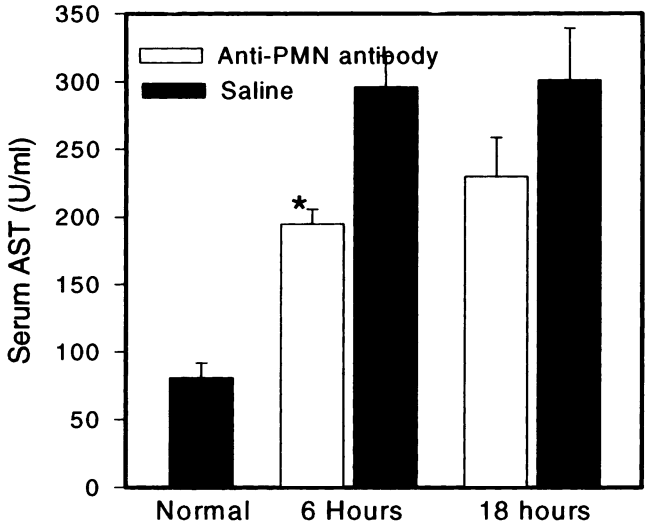

Figure 6. Serum aspartate aminotransferase levels from normal subjects, saline control subjects, and antipolymorphonuclear leukocyte-treated mice 6 and 18 hours after cecal ligation and puncture. * = $p < 0.05$ compared to saline control subjects.

In the same experiment that was performed to measure total circulating neutrophils and lung IL-1β mRNA, serum was obtained for endotoxin measurement. Serum endotoxin was 40-fold greater than for normal subjects in the antibody-treated animals and 4-fold greater than for saline control subjects (Fig. 7) at the 6-hour timepoint ($p < 0.05$). At the 18-hour timepoint, serum endotoxin in the antibody-treated CLP animals was 60-fold greater than for normal subjects and 2.5-fold greater than for saline CLP control subjects ($p < 0.05$).

DISCUSSION

Fry et al.[3,31] have reported that MSOF is the most lethal determinant of death in patients with intra-abdominal infection and that secondary bacterial peritonitis is the primary cause of MSOF.[3,5,31,32] Progression to MSOF after peritonitis remains a grave situation, and mortality rates after sepsis-induced MSOF have ranged between 45% and 90%.[3,31,33,34] Nearly 61% of all patients in our study with MSOF eventually died, despite adequate treatment of the intra-abdominal process in all cases. In a multicenter trial conducted by members of the Surgical Infection Society, 80% of deaths were found to occur with a persistent abdominal source of infection.[2] However, only 2 of the 38 patients who died in our study were unable to recover from their intraperitoneal infections. Similar results were seen by Norton,[4] where only 3 of 16 patients died with persistent intra-abdominal infection.

Populations of patients in whom remote organ failure might develop for reasons in addition to intra-abdominal infection were excluded from this study. Patients with disseminated cancer were eliminated because the immune system is generally suppressed, and trauma victims were excluded because remote organ failure might be attributed to hemorrhagic shock or direct organ injury.[35–38] For similar reasons, patients who recently had undergone coronary artery bypass grafting or major vascular reconstruction[39,40] also were excluded. Despite the exclusion of patients who might have organ failure from causes other than peritonitis, nearly 73% of the study patients who remained hospitalized for >10 days after surgery did so because of remote organ injury.

Pulmonary failure during sepsis is generally attributed to lung microvascular injury, and delayed bacterial clearance from the lung has been reported in patients with peritonitis.[41] The injury may occur by several pathways, including the direct effects of endotoxin on the vascular endothelium, endotoxin-induced neutrophil activation (including recruitment and release of preformed proteases and elastases), and activation of the complement and coagulation cascades as well as the contact activation system.[42–44] In our study, the lungs were the remote organ system most commonly affected and also were either the first organ to fail or were one of several organ systems to fail simultaneously. Although lung failure alone without concomitant MSOF did not result in a significantly higher mortality, it did predispose to prolonged ICU and total hospital lengths of stay. Age, gender, and history of

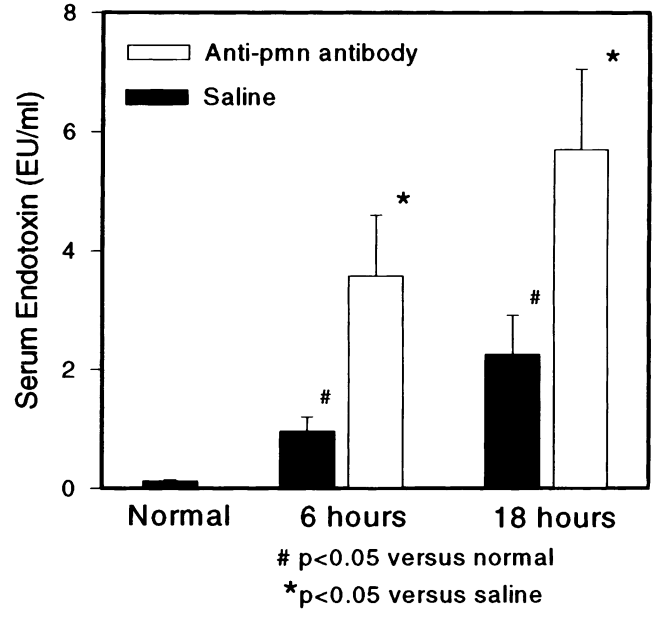

Figure 7. Serum endotoxin levels from normal subjects, saline control subjects, and antipolymorphonuclear leukocyte-treated mice 6 and 18 hours after cecal ligation and puncture.

chronic obstructive pulmonary disease were not predictive of pulmonary failure. Adult respiratory distress syndrome, by definition, is a more severe type of acute lung injury, defined as bilateral infiltrates on chest x-ray with an alveolar-arterial gradient of >200. In our study, 22 of the 24 patients who met the strict criteria for ARDS progressed to have MSOF develop. In that group, 18 of the 22 eventually died, a higher mortality rate than has been reported previously.[29,45] In concordance with results reported by Spec–Marn et al.,[46] the majority of patients (15 of 18) in our study with ARDS who died did so because of progressive organ failure rather than inadequate oxygenation.

In most patients, peritonitis will induce a host systemic inflammatory response but not any substantial degree of organ failure. However, as in our series, all those who developed organ failure manifested the systemic inflammatory response syndrome at some point in their clinical course. This may be related to a specific infection, but the majority of our patients with organ failure had recovered from their intraperitoneal infection. It has long been hypothesized that remote organ injury in the setting of intra-abdominal infection is mediated primarily by neutrophils.[47–49] To test this hypothesis, the CLP model of peritonitis was used, because it reproduces the same peritonitis as that observed in humans, with a necrotic tissue focus and gram-negative enteric bacteria. The antigranulocyte antibody RB6-8C5 virtually eliminated PMNs in the peripheral circulation. Lung MPO in anti-PMN-treated mice returned to a level equivalent to that observed in mice that had not undergone CLP. Acute lung injury and liver injury were significantly reduced in concordance with the decrease in both Evans blue extravasation and liver function test results. These data indicate that the PMN, at least in part, mediates such remote organ injury, as observed in this experimental model of human peritonitis after CLP.

In previous work,[27,50] we have shown a strong correlation between lung tissue levels of IL-1β mRNA and lung MPO after CLP-induced peritonitis. However, this increase in lung MPO was not abrogated by treatment with anti-IL-1α and anti-IL-1β before CLP (unpublished data, 1994). After CLP, there was a significant reduction in lung IL-1β mRNA in the anti-PMN antibody-treated animals, suggesting that neutrophil sequestration in the lung is mediated by other cytokines. Indeed, the source of IL-1β in the lung may be the neutrophil itself. This would account for our previous observation that IL-1β blockade did not reduce lung MPO after CLP. Several studies have shown that endothelial damage may be induced by endotoxin alone, in the absence of granulocytes,[51,52] whereas others have shown a requirement for PMNs.[53,54] Our results show significantly elevated serum endotoxin levels in the anti-PMN group yet a simultaneous reduction in both lung and liver injury after CLP. Clearly other factors are involved because abrogation of tissue injury with anti-PMN treatment was incomplete.

In summary, the primary cause of prolonged hospital stay after operation in patients with secondary bacterial peritonitis is pulmonary and MSOF, despite the technical success of the initial operation. Peritonitis may induce a systemic inflammatory response in which the lungs succumb more often and earlier than other remote organs. Although pulmonary failure alone did not predispose to death, ARDS and MSOF were both associated with increased mortality. A primary mediator of remote organ injury after peritonitis is the neutrophil, although such injury is multifactorial and complex in nature. Future therapeutic approaches should involve strategies to block neutrophil adherence to the endothelium of remote organs while simultaneously preserving or enhancing their ability to enter the peritoneal cavity.

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Discussion

DR. JOHN A. MANNICK (Boston, Massachusetts): Mr. President, Mr. Secretary, Ladies, and Gentlemen. I certainly enjoyed this paper, which brings daylight into what has been a pretty cloudy clinical area. Dr. Cheadle has shown, I think, convincingly in the animal model, and I believe the clinical data support what he has found in the animal model, what is going on in patients who are recovering from peritonitis really is not, as far