IL-10 Is a Major Mediator of Sepsis-Induced Impairment in Lung Antibacterial Host Defense

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IL-10 Is a Major Mediator of Sepsis-Induced Impairment in Lung Antibacterial Host Defense

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To explore the mechanism of immunosuppression associated with sepsis, we developed a murine model of sepsis-induced Pseudomonas aeruginosa pneumonia. CD-1 mice underwent either cecal ligation and 26-gauge needle puncture (CLP) or sham surgery, followed by the intratracheal (i.t.) administration of P. aeruginosa or saline. Survival in mice undergoing CLP followed 24 h later by the i.t. administration of saline or P. aeruginosa was 58% and 10%, respectively, whereas 95% of animals undergoing sham surgery followed by P. aeruginosa administration survived. Increased mortality in the CLP/P. aeruginosa group was attributable to markedly impaired lung bacterial clearance and the early development of P. aeruginosa bacteremia. The i.t. administration of bacteria to CLP-, but not sham-, operated mice resulted in an impressive intrapulmonary accumulation of neutrophils. Furthermore, P. aeruginosa challenge in septic mice resulted in a relative shift toward enhanced lung IL-10 production concomitant with a trend toward decreased IL-12. The i.p., but not i.t., administration of IL-10 Abs given just before P. aeruginosa challenge in septic mice significantly improved both survival and clearance of bacteria from the lungs of septic animals administered P. aeruginosa. Finally, alveolar macrophages isolated from animals undergoing CLP displayed a marked impairment in the ability to ingest and kill P. aeruginosa ex vivo, and this defect was partially reversed by the in vivo neutralization of IL-10. Collectively, these observations indicate that the septic response substantially impairs lung innate immunity to P. aeruginosa, and this effect is mediated primarily by endogenously produced IL-10. The Journal of Immunology, 1999, 162: 392–399.

Sepsis syndrome is an acute systemic response to a variety of noxious insults, particularly bacterial infection, and is manifested clinically by the presence of tachycardia, fever or hypothermia, tachypnea, and multiorgan dysfunction (1). Sepsis is initiated and perpetuated by the overzealous systemic production of inflammatory cytokines, including TNF-α, IL-1β, IFN-γ, and chemokines, resulting in excessive tissue injury and death in approximately 25 to 35% of patients (1, 2). The exaggerated inflammatory response that occurs in sepsis is counterbalanced by the early and sustained expression of potent antiinflammatory cytokines, including IL-10 (3).

There is now compelling evidence to indicate that sepsis syndrome results in a prolonged and profound state of immunosuppression, which is characterized by substantially diminished monocyte/macrophage effector cell function. Specific monocyte/macrophage defects in septic patients include a reduced HLA-DR expression, loss of Ag-presenting capacity, and a striking decrease in the ability to produce important activating and regulatory cytokines, including TNF-α, IL-1β, and IL-6 (4–8). Monocyte/macrophage deactivation appears to be of considerable functional significance, as higher rates of infection and increased mortality have been observed in postoperative or septic patients who display evidence of monocyte deactivation, either in the form of decreased monocyte HLA-DR expression or impaired delayed-type hypersensitivity responses (9–11). Septic patients are especially susceptible to nosocomial infections of the lung, particularly pneumonia secondary to P. aeruginosa (12–14).

Recent studies indicate that IL-10 plays important yet contrasting roles in both the septic response and in the evolution of bacterial pneumonia (15–26), and the expression of IL-10 may contribute to sepsis-induced leukocyte deactivation. In sepsis, IL-10 has been identified as a vital modulator of the often lethal overabundant production of inflammatory cytokines. In several animal models of sepsis, neutralization of IL-10 results in exaggerated proinflammatory cytokine expression and death, while administration of recombinant IL-10 confers significant therapeutic protection (3, 15–17). Conversely, in the setting of bacterial pneumonia, endogenous IL-10 appears to inhibit protective innate immune responses (18–20). For example, IL-10 is a potent suppressor of macrophage production of important activating and/or chemotactic cytokines (21–23). Furthermore, this cytokine directly inhibits neutrophil and macrophage phagocytic and bactericidal activity in vitro (24, 25). In both humans and animal models, the period that follows septic events has been shown to result in the preferential expression of antiinflammatory cytokines, particularly IL-10 (27–29). However, the contribution of IL-10 to the development of infection in the postsepsis period has not been defined either clinically or experimentally.

The purpose of this study was to assess the mechanism(s) by which sepsis syndrome alters subsequent lung innate immune responses, and to determine the specific contribution of IL-10 to sepsis-induced leukocyte deactivation. To do so, we have employed a clinically relevant model of intraabdominal sepsis,
namely, cecal ligation and puncture (CLP)\(^3\) (3, 30), followed by intrapulmonary challenge with \(P. \) aeruginosa to reproduce a common clinical scenario.

### Materials and Methods

#### Reagents

Polyclonal anti-murine TNF-\(\alpha\), IL-12, and IL-10 Abs used in the ELISAs or in neutralization studies were produced by immunization of rabbits with murine recombinant cytokines in multiple intradermal sites with CFA. Carrier-free murine recombinant TNF-\(\alpha\), IL-12, and IL-10 were purchased from R&D Systems (Minneapolis, MN). In IL-10 neutralization experiments, 0.5 ml of control rabbit serum or anti-murine IL-10 was administered i.p. 2 h before \(P. \) aeruginosa administration. This antisera contained an anti-IL-10 Ab titer of 10\(^6\) and has been shown to be neutralizing both in vitro and in vivo (3, 24). Purified Abs for ELISA and i.t. administration were obtained by purification over an endotoxin-free protein A column.

#### Animals

Specific pathogen-free CD-1 mice (6-12 wk females, Charles River Breeding Laboratories, Wilmington, MA) were used in all experiments. All mice were housed in specific pathogen-free conditions within the animal care facility at the University of Michigan (ULAM) until the day of sacrifice.

#### Animal model

The cecal ligation and 26-gauge puncture model (CLP) was used as a model of systemic sepsis syndrome as previously described (3). In distinct contrast to CLP models using larger gauge cecal punctures (19-gauge and larger), in which most animals rapidly develop bacteremia due to enteric organisms and death occurs as a result of polymicrobial sepsis (30), cecal ligation and puncture using a 26-gauge needle results in the development of bacteremia in only 10-15% of animals (data not shown). However, this insult induces a marked septic response, with death occurring in approximately 30-50% of animals. To perform this procedure, pathogen-free female CD-1 mice were anesthetized with ketamine HCl (Ketaset; Fort Dodge Laboratories, Fort Dodge, IA) 50 mg/kg i.p., followed by inhaled methoxyflurane (Metafane; Pitman-Moore, Mundelein, IL) as needed. In these mice, a 1- to 2-cm longitudinal incision to the lower right quadrant of the abdomen was performed, and the cecum was exposed. The distal one-third was ligated with 3-0 silk suture and punctured through and with a 26-gauge needle. The cecum was then replaced into the peritoneal cavity, and the incision was closed with surgical staples. In sham surgery, the cecum was exposed, but not ligated or punctured, and the distal one-third was ligated with 3-0 silk suture and punctured through and with a 26-gauge needle. The skin incision was closed with surgical staples.

#### Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed to obtain AM in pure culture for ex vivo studies. The trachea was exposed and intubated using a 1.7-mm OD polyethylene catheter. BAL was performed by instilling PBS containing 5 mM EDTA in 1 ml aliquots. Fifteen ml of PBS was instilled per mouse, and approximately 10 ml of lavage fluid was retrieved. Lavaged cells from each group of animals were pooled and counted after hypotonic lysis; then cytoarrays for determination of BAL differentials were prepared. Lavaged cells consisted of greater than 95% AM for each of the groups examined (data not shown).

### Phagocytic assay

Alveolar macrophage phagocytic assay was performed as a modification of a previously described method (24). Briefly, murine AM (1 \(\times\) 10\(^5\) cells) were incubated with 2.5% murine \(P. \) aeruginosa immune serum (as source of opsonin) for 5 min at 37°C in eight-well labtaks (Nunc, Naperville, IL). \(P. \) aeruginosa (1 \(\times\) 10\(^5\) bacteria) were added and incubated for 30 min at 37°C on a shaker plate. The supernatants were removed, and the cells were washed three times with sterile HBSS. The gasket was removed, and slides were placed in air dry. Diff-Quick (Baxter Scientific Products, Miami, FL) stain was performed, and 200 cells per well were counted to determine number of intracellular \(P. \) aeruginosa per AM.

### Bactericidal assay

Alveolar macrophages (1 \(\times\) 10\(^5\) cells) were incubated with 2.5% murine \(P. \) aeruginosa immune serum for 5 min at 37°C in 35-mm plastic culture dishes. \(P. \) aeruginosa (1 \(\times\) 10\(^5\) bacteria) were added and incubated for an additional 1 h at 37°C on a rocker plate. The supernatants were removed, and the cells were washed three times with sterile HBSS. The cells were then lysed by adding 1 ml 0°C sterile H\(_2\)O, disrupting the cells with a cell lifter, and incubating them on ice for 10 min. One milliliter 2\% HBSS was added per well, then serially diluted on blood agar plates. Plates were incubated for 18 h at 37°C, and colony counts were performed. Percent survival of intracellular bacteria was calculated by the following formula: percent killing = 100 - (number of \(P. \) aeruginosa CFU/ml AM lysate)/ (total number of intracellular \(P. \) aeruginosa) \(\times\) 100. Total intracellular \(P. \) aeruginosa is the product of total number of AM \(\times\) mean number of intracellular \(P. \) aeruginosa per AM.

### P. aeruginosa inoculation

\(P. \) aeruginosa was administered i.t. to sham or CLP animals 24 h post CLP or sham surgery. We chose to use \(P. \) aeruginosa (strain UI-18, Parke-Davis, Ann Arbor, MI) in our studies, since this organism is a common respiratory pathogen in patients with sepsis and immunocompetent mice are generally resistant to infection with this particular strain when administered by the intrapulmonary route (18). \(P. \) aeruginosa was grown in tryptic soy broth (Difco, Detroit, MI) for 18 h at 37°C. The concentration of bacteria in broth was determined by measuring the amount of absorbance at 600 nm. A standard of absorbencies based on known CFU was used to calculate inoculum concentration. Bacteria were pelleted by centrifugation at 3000 rpm for 15 min, washed twice in saline, and resuspended at the desired concentration. Animals were anesthetized with approximately 2.5-3.2 mg ketamine per animal i.p. The trachea was exposed, and 30 \(\mu\)l of inoculum or saline was administered via a sterile 26-gauge needle. The inoculum contained either 4 or 8 \(\times\) 10\(^5\) \(P. \) aeruginosa. The skin incision was closed with surgical staples.

#### Lung harvesting for cytokine analysis and histologic examination

At designated time points, the mice were then anesthetized with inhled methoxyflurane, blood was collected by orbital bleeding, and the animals were sacrificed. Whole lungs were then harvested for assessment of IL-12, IL-10, and TNF-\(\alpha\) protein expression. Before lung removal, the pulmonary vasculature was perfused with 1 ml of PBS containing 5 mM EDTA, via the right ventricle. After removal, whole lungs were homogenized in 2 ml lysis buffer containing 0.5% Triton X-100, 150 mM NaCl, 15 mM Tris, 1 mM CaCl\(_2\), and 1 mM MgCl\(_2\) (pH 7.4) using a tissue homogenizer (Biospec Products, Racin, WI). Homogenates were incubated on ice for 30 min, then centrifuged at 2500 rpm for 10 min. Supernatants were collected, passed through a 0.45-micron filter (Gelman Sciences, Ann Arbor, MI), then stored at -20°C for assessment of cytokine levels. Lungs for histologic examination were excised en bloc without perfusion and inflated with 1 ml 4% paraformaldehyde in PBS to improve resolution of anatomic relationships.

#### Determination of lung and plasma \(P. \) aeruginosa CFU

At the time of sacrifice, plasma was collected, the right ventricle was perfused with 1 ml PBS, then lungs were removed asepically and placed in 2 ml sterile saline. The tissues were then homogenized with a tissue homogenizer under a vented hood. The lung homogenates were placed on ice. Serial 1:10 dilutions of both lung homogenates and plasma were made. Ten microliters of each dilution was plated on soy base blood agar plates (Difeo). Plates were incubated for 18 h at 37°C, after which colonies were counted.

#### Lung myeloperoxidase (MPO) assay

Lung MPO activity (as assessment of neutrophil influx) was quantitated by a method described previously (31). Briefly, whole lungs were homogenized in 2 ml of a solution containing 50 mM potassium phosphate, pH 6.0, with 5% hexadecyltrimethylammonium bromide and 5 mM EDTA. The resulting homogenate was sonicated and centrifuged at 12,000 \(\times\) g for 15 min. The supernatant was mixed 1:15 with assay buffer and read at 490 nm. MPO units were calculated as the change in absorbance over time.

#### Murine cytokine ELISA

Murine TNF-\(\alpha\), IL-12, and IL-10 were quantitated using a modification of a double ligand method as previously described (3). Briefly, flat-bottom 96-well microtiter plates (Nunc Immuno-Plate I 96-F, Denmark) were
coated with 50 μl/well of rabbit Ab against the various cytokines (1 μg/ml in 0.6 M NaCl, 0.26 M H₂BO₃, and 0.08 M NaOH, pH 9.6) for 16 h at 4°C and then washed with PBS, pH 7.5, 0.05% Tween 20 (wash buffer). Microtiter plate nonspecific binding sites were blocked with 2% BSA in PBS and incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer, and diluted (neat and 1:10) cell-free supernatants (50 μl) in duplicate were added, followed by incubation for 1 h at 37°C. Plates were washed four times, followed by the addition of 50 μl/well biotinylated rabbit Abs against the specific cytokines (3.5 μg/ml/PBS, pH 7.5, 0.05% Tween 20, and 2% FCS), and plates were incubated for 30 min at 37°C. Plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. Plates were washed again four times, and chromogen substrate (Bio-Rad) was added. The plates were incubated at room temperature to the desired extinction, and the reaction was terminated with 50 μl/well of 3 M H₂SO₄ solution. Plates were read at 490 nm in an ELISA reader. Standards were 1/2 log dilutions of recombinant murine cytokines from 1 pg/ml to 100 ng/ml. This ELISA method consistently detected murine cytokine concentrations above 25 pg/ml. The ELISA did not cross-react with IL-1, IL-2, IL-4, or IL-6. In addition, the ELISA did not cross-react with members of the murine chemokine family, including murine JE/monocyte chemotactic protein (MCP)-1, RANTES, KC, macrophage inflammatory protein (MIP)-2, growth-related gene-α (GRα), or epithelial cell dermid neutrophil-activating protein-78 (ENA-78).

Statistical analysis

Ratio scale data were evaluated by ANOVA with Newman-Keuls follow-up, while survival curves were analyzed by the log-rank test. All calculations were performed on a Power Macintosh 7200 computer using the programs Statview 4.5 (Brain Power, Calabasas, CA) and Primer of Biostatistics 3.01 (McGraw Hill) for ratio scale data, and Prism 2.0 (Graphpad Software, San Diego, CA) for survival curves.

Results

Effect of P. aeruginosa administration on mortality in sham-operated and CLP mice

Initial studies were performed to assess the effects of intraabdominal sepsis syndrome on mortality after i.t. challenge with P. aeruginosa. Mice were anesthetized with ketamine and then underwent CLP or sham surgeries. Twenty-four hours later, animals were administered 30 μl of saline or saline containing 8 × 10⁵ CFU of P. aeruginosa i.t. As shown in Fig. 1, minimal lethality or evidence of systemic toxicity was noted in sham-operated animals after P. aeruginosa administration. In contrast, mice undergoing CLP followed by i.t. P. aeruginosa displayed substantial systemic toxicity and early mortality, with only 10% of mice surviving long term, as compared with the 58% survival in CLP animals receiving saline i.t. (p < 0.001). In all experimental groups, no lethality was observed after 72 h post bacterial challenge.

Effect of CLP on bacterial clearance in mice challenged with P. aeruginosa i.t.

To determine whether the increased lethality observed in CLP mice challenged with P. aeruginosa was due to impaired intrapulmonary bacterial clearance, plasma and lungs from CLP and sham mice were harvested 24 h after the i.t. administration of P. aeruginosa (8 × 10⁵ CFU). The 24 h time point was chosen, since appreciable differences in survival between the sham and CLP groups was emerging by this time point, and preliminary studies indicated marked differences in bacterial clearance at 24 h post P. aeruginosa challenge. As shown in Fig. 2, 8 × 10⁵ CFU P. aeruginosa was recovered from the lungs of CLP mice 24 h after Pseudomonas administration, whereas no organisms were isolated from the lungs of sham-treated animals challenged with either saline or P. aeruginosa i.t., or CLP mice administered saline i.t. (p < 0.001; Fig. 2). Furthermore, 7 of 11 CLP mice inoculated with P. aeruginosa were bacteremic at 24 h, while none of 14 sham-operated animals were bacteremic (p < 0.01). In addition, no P. aeruginosa was isolated from the plasma of CLP mice 24 h after the administration of saline i.t. These observations indicate that abdominal sepsis syndrome results in substantial impairment in the ability to clear P. aeruginosa from the lung after intrapulmonary administration.

Effect of intraabdominal sepsis syndrome on lung PMN influx in mice challenged with P. aeruginosa i.t.

To determine whether the sepsis-induced impairment in lung bacterial clearance was due to inadequate inflammatory cell recruitment, histologic examination of the lung was performed 24 h post i.t. administration of P. aeruginosa 8 × 10⁵ CFU in both sham and CLP mice. As shown in Fig. 3A, modest intraalveolar inflammatory cell influx was present in sham-operated animals 24 h after bacterial administration, as compared with sham-operated animals administered saline. In addition, the appearance of lungs from CLP mice administered saline i.t. was not appreciably different from that observed in sham-operated mice after i.t. administration of saline (data not shown). In contrast, a substantial influx of airspace leukocytes, predominantly PMN, was observed in CLP mice administered P. aeruginosa i.t. (Fig. 3B), resulting in dense patchy areas of consolidation (Fig. 3C). To better quantitate the number of

FIGURE 1. Effect of P. aeruginosa administration on survival in CLP and sham-operated mice. P. aeruginosa at a dose of 8 × 10⁵ CFU was administered i.t. Control CLP mice were administered sterile saline i.t. Time zero was assigned as the time of Pseudomonas inoculation (24 h post CLP or sham surgery). Survival in CLP/P. aeruginosa group was significantly different from that in CLP/saline group (p < 0.01) and sham/P. aeruginosa (p < 0.01). Experimental n = 20–24 per group.

FIGURE 2. The effect of CLP on P. aeruginosa CFU in lung homogenates 24 h postinoculation. Log₁₀ P. aeruginosa CFU are expressed on the vertical axis. * p < 0.001 as compared with all other groups. Experimental n = 11–14 per group.
infiltrating PMN, total lung myeloperoxidase (MPO) activity was determined in CLP and sham-operated animals given *Pseudomonas* or saline i.t. (Fig. 4). Despite relatively normal-appearing lung histologic examination, lung MPO activity was approximately 2.5-fold greater in CLP animals administered saline i.t. as compared with animals undergoing sham surgery ($p < 0.01$). In addition, an approximately 2.7-fold increase in lung MPO activity was observed in sham-operated animals challenged with *P. aeruginosa* i.t., as compared with sham animals administered saline i.t. ($p < 0.01$). Importantly, lung MPO activity observed in CLP animals administered *P. aeruginosa* i.t. was further increased as compared with that observed in similarly challenged sham animals ($p < 0.05$).

**Effect of intraabdominal sepsis syndrome on lung cytokine expression in mice challenged with *P. aeruginosa***

To determine whether intraabdominal sepsis resulted in alterations in the expression of important pro- and antiinflammatory cytokines during the evolution of *Pseudomonas* pneumonia, lungs were harvested from sham-operated and CLP mice 24 h after inoculation with *P. aeruginosa*. TNF-$\alpha$, IL-12, and IL-10 levels were then determined by specific ELISAs (Fig. 5). These cytokines were examined because we and others have previously shown them to be important both in the septic response and in lung innate immunity against bacterial pathogens. Interestingly, an increase in lung TNF levels was observed in CLP animals with pneumonia, as compared with sham-operated animals given *P. aeruginosa* i.t. ($p < 0.01$). However, IL-12 levels tended to be decreased in CLP/Pseudomonas animals, as compared with sham/Pseudomonas-treated mice ($p = 0.058$). In addition, CLP followed by i.t. *P. aeruginosa* resulted in marked increases in the expression of the antiinflammatory cytokine IL-10, whereas little induction of this cytokine was observed in sham-operated animals challenged with *P. aeruginosa*. These studies suggest that a second insult (i.e., intrapulmonary bacterial challenge) in septic mice results in a shift in the balance of pro- and antiinflammatory cytokines, favoring an IL-10 predominant response.

**Effect of neutralization of IL-10 on survival in septic mice challenged with *P. aeruginosa***

Given that the antiinflammatory properties of IL-10 have been shown to be detrimental to the host in the setting of Gram-negative pneumonia, and that IL-10 is expressed in increased amounts in the postseptic period (3, 18, 27–29), we next determined whether the sepsis-induced suppression of lung antibacterial host defense was mediated, in part, by the enhanced and/or persistent expression of IL-10. In these studies, CLP animals were treated with rabbit IL-10-specific antisera i.p. or purified rabbit IL-10 Abs i.t. 2 h before the i.t. administration of *P. aeruginosa*, rather than at the time of CLP. The administration of anti-IL-10 serum was delayed until 24 h post CLP, because systemic inhibition of IL-10 at the time of CLP has been shown to enhance proinflammatory cytokine production and sepsis-induced lethality (3). To best assess for either a protective or detrimental effect of anti-IL-10 therapy, an approximately LD$_{50}$ inoculum of *P. aeruginosa* in septic mice was employed ($4 \times 10^5$ CFU). Interestingly, the i.p. administration of IL-10-specific antiserum 2 h before *P. aeruginosa* administration resulted in significantly improved survival as compared with CLP animals with *Pseudomonas* pneumonia given preimmune serum.
i.p. (Fig. 6). However, survival of CLP animals given anti-IL-10 Abs (100 μg) i.t. at the time of P. aeruginosa administration was not different from that observed in CLP animals with Pseudomonas pneumonia given control IgG (data not shown).

**Effect of neutralization of IL-10 on bacterial clearance in septic mice challenged with P. aeruginosa**

Having shown that the delayed treatment of mice undergoing CLP followed by intrapulmonary bacterial challenge with anti-IL-10 serum resulted in improved survival as compared with similarly treated animals receiving preimmune serum, we next determined whether the improved survival observed after anti-IL-10 therapy was attributable to improved pulmonary clearance of P. aeruginosa. Twenty-four hours following CLP, mice were treated with rabbit anti-IL-10 antiserum or control preimmune serum i.p., then 2 h later administered P. aeruginosa 4 x 10⁵ i.t. Importantly, septic mice passively immunized with anti-IL-10 antiserum 2 h before P. aeruginosa administration resulted in significant reductions in P. aeruginosa CFU recovered from lungs or plasma 24 h post bacterial administration, as compared with CLP animals with Pseudomonas pneumonia given preimmune serum (Fig. 7). Specifically, mean P. aeruginosa CFU recovered from plasma and lungs of anti-IL-10 treated animals were 18.6- and 25.7-fold less than that observed in animals receiving preimmune serum, respectively. These studies indicate that the delayed administration of anti-IL-10 serum significantly enhanced bacterial clearance and survival in septic animals challenged with P. aeruginosa.

**Effect of neutralization of IL-10 in vivo on AM phagocytic and bactericidal activity ex vivo**

To identify specific mechanisms by which abdominal sepsis syndrome altered lung antipseudomonal host defense, and how these effects were substantially reversed by neutralization of IL-10 in vivo, AM were isolated from CLP and sham-operated mice for assessment of phagocytic and bactericidal activity ex vivo. In these studies, CD-1 mice underwent sham surgery or CLP, then 24 h later were administered either rabbit anti-IL-10 serum or control serum i.p. Two hours following Ab administration, bronchoalveolar lavage was performed, AM were adherence-purified, and then the ability of these cells to ingest and kill P. aeruginosa was assessed. As shown in Table I, AM recovered from CLP animals treated in vivo with control serum displayed a marked impairment in the ability to both ingest and kill P. aeruginosa ex vivo, as compared with that observed in AM isolated from sham-operated animals (p < 0.001 for numbers of cells ingested, p < 0.01 for percentage of ingested bacteria killed). Treatment of sham-operated animals with anti-IL-10 Ab did not significantly improve AM phagocytic or bactericidal activity. However, treatment of CLP mice with anti-IL-10 Ab in vivo before AM isolation resulted in a significant improvement in both numbers of P. aeruginosa ingested (p < 0.01) and percentage of ingested organisms killed (p < 0.05).

**Discussion**

Sepsis syndrome predisposes the host to the development of a variety of nosocomial infections, particularly bacterial infection of the lung. (12–14) The prevalence of nosocomial lung infections is especially high in septic patients complicated by the adult respiratory distress syndrome (ARDS), with as many as 70% of these patients developing nosocomial pneumonia during the course of their disease. (32, 33) The outcome of bacterial pneumonia in ARDS patients is grim, with observed mortality as high as 90% (13, 33–35). The high mortality observed is due primarily to the inability of the compromised host to eradicate pathogenic organisms from the airspace (34). Interestingly, while P. aeruginosa is essentially nonpathogenic in the immunocompetent host, this organism represents the most common respiratory pathogen in critically ill patients, including those with sepsis and/or ARDS (33–35). In our studies, we attempted to recreate this all too common
In initial studies, we established a higher mortality rate in septic mice challenged with P. aeruginosa as compared with sham-operated mice administered P. aeruginosa or CLP mice given saline i.t. The higher mortality in the CLP/P. aeruginosa group was attributed to the development of pneumonia, since this group displayed an inability to clear bacterial organisms from the airspace, whereas nonseptic mice rapidly and completely cleared P. aeruginosa from the lung. Furthermore, mice undergoing CLP were less able to contain the spread of infection, since 64% of the CLP/P. aeruginosa mice were bacteremic by 24 h post P. aeruginosa administration, while none of the sham-operated mice were bacteremic at this time point. Taken together, these data suggest that, like critically ill humans, septic mice displayed a marked predisposition to the development of Pseudomonas pneumonia.

Mechanisms that account for enhanced susceptibility to nosocomial infection in septic patients have not been clearly defined. Inadequate PMN recruitment represents one possible explanation for the sepsis-induced impairment in lung bacterial clearance. Impaired PMN influx could occur as a result of several events, including insufficient generation of chemotaxins locally, impaired PMN responsiveness to chemotactic stimuli, and/or sequestration of PMN at other sites of noxious insult (i.e., the abdominal cavity). However, MPO activity in the lungs of septic mice with Pseudomonas pneumonia was increased, rather than decreased, by nearly twofold as compared with sham-operated mice after P. aeruginosa challenge, indicating a brisk influx of PMN. Furthermore, lung histologic sections from CLP mice challenged with P. aeruginosa also revealed a substantial PMN influx. In sum, these data suggest that the defect in innate lung immunity in septic animals cannot be attributed to impaired PMN recruitment to the lung, although effects of sepsis syndrome on PMN phagocytic or bactericidal activity cannot be excluded.

An alternative explanation for the sepsis-induced impairment in innate lung immunity is that sepsis results in impaired resident AM function. We have previously demonstrated the importance of AM in host defense against Klebsiella pneumoniae (36). Furthermore, sepsis has been shown to induce a state of monocyte/macrophage “deactivation” analogous to that observed in LPS desensitization, whereby prior exposure to endotoxin results in impaired cytokine production by monocytes rechallenged with LPS (4). Specific defects noted in peripheral blood monocytes isolated from septic patients include decreased phagocytic capabilities, markedly lower HLA-DR expression, a loss of Ag-presenting ability, and a significantly decreased ability to produce inflammatory cytokines like IL-6, TNF-α, and IL-1β (4–11). Our ex vivo studies indicate that AM isolated from CLP mice displayed a marked impairment in the ability to ingest and kill P. aeruginosa, as compared with AM isolated from animals undergoing sham surgery. Similarly, it has been shown that AM isolated from septic dogs and rats undergoing CLP have increased susceptibility to Escherichia coli or P. aeruginosa colonization of the lung at 4 h postintratracheal challenge, and the host’s inability to effectively clear the bacteria was linked to a decreased AM bactericidal activity (37, 38). These findings indicate that sepsis not only inhibits peripheral blood monocyte

Table I. Effect of in vivo neutralization of IL-10 on phagocytic and bactericidal activity of AM cultured ex vivo

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P. aeruginosa/AM</th>
<th>% Killing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham/IgG</td>
<td>0.70 ± 0.1</td>
<td>74.3 ± 3</td>
</tr>
<tr>
<td>Sham/α-IL-10</td>
<td>0.86 ± 0.2</td>
<td>76.3 ± 5</td>
</tr>
<tr>
<td>CLP/IgG</td>
<td>0.29 ± 0.1*</td>
<td>44.0 ± 3*</td>
</tr>
<tr>
<td>CLP/α-IL-10</td>
<td>0.58 ± 0.1</td>
<td>61.2 ± 4*</td>
</tr>
</tbody>
</table>

* P. aeruginosa/AM represents mean ± SEM number of intracellular bacteria per AM, while % Killing represents mean ± SEM percentage of ingested bacteria that were killed. AM from six to eight animals per group were pooled.

*p < 0.05 as compared with AM from all other groups.

†, p < 0.05 as compared with AM recovered from sham-operated animals. Representative of two separate experiments.
IL-10 has recently been identified as a crucial modulator of the inflammatory response in sepsis and is therefore a likely candidate for mediating sepsis-induced impairment in lung host defense. We have shown that IL-10 levels in plasma are increased by as much as 50-fold over baseline in mice undergoing 26-gauge CLP, and the expression of IL-10 is prolonged relative to the expression of proinflammatory cytokines, including TNF-α, chemokines, and Th1-phenotype cytokines IL-12 and IFN-γ (Ref. 3, and data not shown). In addition, we observed a fourfold increase in the levels of IL-10 within the lungs of septic animals 24 h after a second inflammatory insult (i.e., P. aeruginosa), as compared with sham-operated mice administered P. aeruginosa. Conversely, there was a trend toward lower levels of IL-12 in the CLP/P. aeruginosa mice vs the sham/P. aeruginosa group. Collectively, these data suggest that, as a result of sepsis, the host becomes primed for an antiinflammatory, rather than a proinflammatory response, favoring the production of IL-10 while attenuating the production of the inflammatory response in sepsis and is therefore a likely candidate for mediating sepsis-induced impairment in lung host defense. We have shown that IL-10 levels in plasma are increased by as much as 50-fold over baseline in mice undergoing 26-gauge CLP, and the expression of IL-10 is prolonged relative to the expression of proinflammatory cytokines, including TNF-α, chemokines, and Th1-phenotype cytokines IL-12 and IFN-γ (Ref. 3, and data not shown). In addition, we observed a fourfold increase in the levels of IL-10 within the lungs of septic animals 24 h after a second inflammatory insult (i.e., P. aeruginosa), as compared with sham-operated mice administered P. aeruginosa. Conversely, there was a trend toward lower levels of IL-12 in the CLP/P. aeruginosa mice vs the sham/P. aeruginosa group. Collectively, these data suggest that, as a result of sepsis, the host becomes primed for an antiinflammatory, rather than a proinflammatory response, favoring the production of IL-10 while attenuating the production of the inflammatory response. Specifically, PGE2 has been identified as a primary inducer of IL-10 production from monocytes, while inhibiting the expression of proinflammatory cytokines, including IL-12 and IFN-γ (40, 41). Furthermore, indomethacin, an inhibitor of PGE2 synthesis, has been shown in vitro to partially reverse the inhibition of TNF-α production from endotoxin-desensitized monocytes (39). Collectively, sepsis syndrome-induced changes in PG profiles favoring increases in PGE2 could account for most, but not all, of the findings observed. However, we observed increased production of TNF-α within the lungs of CLP animals after P. aeruginosa challenge, which is contrary to what would be expected given the presence of increased amounts of IL-10 (and possibly PGE2). A possible explanation for this discrepancy is that the persistence of bacterial organisms within the airspace may continue to drive the expression of TNF-α despite the presence of down-regulatory signals. Alternatively, PMN synthesize significant quantities of TNF-α, and the presence of increased numbers of PMN within the lung airspace of CLP mice challenged with P. aeruginosa may account for these differences.

Our current in vivo observations, taken together with previous in vitro studies, provide direct evidence that IL-10 represents a critical mediator of monocyte/macrophage deactivation in LPS desensitization and sepsis. In vitro, TNF production from endotoxin-desensitized blood monocytes and monocytes isolated from septic patients can be nearly completely restored when treated in culture with anti-IL-10 Abs (39). Importantly, we observed that the systemic (i.p.) administration of IL-10-specific antisera given 24 h after CLP conferred significant protection against mortality associated with the development of Pseudomonas pneumonia. Furthermore, the protective effect associated with anti-IL-10 therapy resulted from improved bacterial clearance since mice receiving this treatment displayed significantly reduced CFU recovered from lungs and plasma. The enhancement of bacterial clearance appeared to be partially mediated by enhanced AM antimicrobial activity, since ex vivo studies indicated that anti-IL-10 treatment in vivo significantly, but not completely, reversed CLP-induced impairment in P. aeruginosa phagocytosis and killing by AM. Given that anti-IL-10 therapy has been shown to be detrimental if given to the host at the time of endotoxin challenge or CLP, our findings indicate that neutralization of IL-10 can be achieved safely if Ab administration is delayed until after the acute phase of the septic response has dissipated. In a similar manner, the proinflammatory cytokine IFN-γ has been administered to a select group of septic patients with evidence of reduced monocyte HLA-DR expression, resulting in restoration of HLA-DR expression and in vitro TNF production, as well as clinical resolution of sepsis (7). Finally, to provide further evidence linking IL-10 expression with sepsis-induced immunoparalysis, we have employed intratracheal adenoviral gene therapy to transiently overexpress IL-10 within the lung, which resulted in an increased susceptibility of CD-1 mice to the development of pneumonia and lethality after intratracheal challenge with P. aeruginosa (our unpublished observations).

Despite the efficacy of systemic administration of anti-IL-10 serum, the direct intrapulmonary administration of purified IL-10 Abs concurrently with P. aeruginosa failed to influence survival, as compared with CLP/P. aeruginosa animals given control IgG. Unfortunately, this observation suggests that compartmentalized anti-IL-10 therapy may not be a therapeutic option in the setting of pneumonia. However, we cannot exclude that this lack of effect may have resulted from inadequate delivery of Ab to the site of IL-10 production, or from a concomitant injurious effect of Ab on lung parenchyma that negated any potential beneficial effects of compartmentalized Ab therapy.

While IL-10 appears to be a major mediator of leukocyte deactivation in sepsis, the cause of this shift toward an IL-10-dominated antiinflammatory response remains unclear. Our laboratory is currently exploring the possibility that elevated levels of PGE2 during sepsis is partially responsible for promoting antiinflammatory responses. Specifically, PGE2 has been identified as a primary inducer of IL-10 production from monocytes, while inhibiting the expression of proinflammatory cytokines, including IL-12 and IFN-γ (40, 41). Furthermore, indomethacin, an inhibitor of PGE2 synthesis, has been shown in vitro to partially reverse the inhibition of TNF-α production from endotoxin-desensitized monocytes (39). Collectively, sepsis syndrome-induced changes in PG profiles favoring increases in PGE2 could account for most, but not all, of the findings observed. However, we observed increased production of TNF-α within the lungs of CLP animals after P. aeruginosa challenge, which is contrary to what would be expected given the presence of increased amounts of IL-10 (and possibly PGE2). A possible explanation for this discrepancy is that the persistence of bacterial organisms within the airspace may continue to drive the expression of TNF-α despite the presence of down-regulatory signals. Alternatively, PMN synthesize significant quantities of TNF-α, and the presence of increased numbers of PMN within the lung airspace of CLP mice challenged with P. aeruginosa may account for these differences.

In conclusion, our findings indicate that IL-10 is an important cytokine mediator of sepsis-induced immunosuppression. Given the emergence of multidrug-resistant bacterial strains, particularly P. aeruginosa (35), it will be vital to develop new treatment strategies aimed at altering the balance of pro- vs antiinflammatory cytokines as adjuvant therapy in the treatment of septic patients with nosocomial pneumonia. The neutralization of IL-10 in this clinical setting may provide an additional approach to therapy in these critically ill patients.

References


