ANIMAL MODEL OF HUMAN DISEASE

Adult Respiratory Distress Syndrome

A Live E coli *Septic Primate Model*

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SINCE its initial description in 1967, the adult respiratory distress syndrome (ARDS) has become a commonly encountered problem in the care of the critically ill patient.[1] Today, ARDS is viewed as a final common pathway in the response of the lungs to a number of insults that eventuate in altered pulmonary capillary permeability, pulmonary edema, and hypoxemia. These insults include direct events such as pulmonary contusion, gastric aspiration, and severe pneumonia as well as indirect events such as sepsis, extensive burns, and massive blood transfusions. The pathophysiologic process is thought to be multifactorial, with both cellular and humoral mediators of injury. Of the many conditions which predispose to the development of ARDS, sepsis is frequently seen. Epidemiologic studies have documented the association of ARDS with sepsis in 10–15% of septic patients. Of the 150,000 patients with ARDS from all causes, at least 50% will die.[1-3]

Despite the diverse primary causes of ARDS, the pathologic findings are similar. During the initial phase interstitial and alveolar edema develops associated with fibrin thrombi in small vessels, aggregates of platelets and neutrophils and extravasation of red blood cells. The pathologic hallmark of the later phase is hyaline membrane formation within the alveolar ducts.[4]

Current investigation emphasizes the central role of polymorphonuclear leukocyte (neutrophil) mediated injury in the pathophysiology of ARDS.[5] Pulmonary leukostasis is commonly noted in this condition and has been demonstrated in animals subjected to acute lung injury.[6] Of note, neutrophil depletion has protected *in vivo* subjects from the deleterious effects of endotoxemia and hyperoxia.[7] Evidence suggests that complement component C5a, which is known to be generated *in vivo* by endotoxemia, serves a chemoattractant function, sequestering neutrophils in the pulmonary capillary bed.[6,8] The neutrophils drawn to the microvasculature are in a metabolically active state and release/generate proteases, oxygen-free radicals, and arachidonic acid metabolites. These toxic effector species presumably damage the pulmonary capillary endothelium in an "innocent bystander" process.[9]

Further evidence suggesting the importance of C5a in lung injury comes from studies utilizing C5-deficient and C5-sufficient twin mouse strains in which the C5-deficient mice develop diminished pulmonary injury in response to septic insult[10] and hyperoxia.[11] Therefore, antiserum directed against C5a might be beneficial in the treatment of ARDS. The model described herein was developed with the goal in mind of trying to prevent ARDS by administering anti-C5a antibodies in a septic primate model.

Primates were chosen because our available anti-C5a antibody was directed against human C5a and

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preliminary *in vitro* work demonstrated cross-reactivity with primate C5a. Primates have previously been shown to have a similarity to humans in response to gram-negative sepsis.[12] As noted below, the pathophysiology of sepsis-induced ARDS in the primates closely mimics the disease state seen in the clinical setting. Additionally, a large animal model allows intensive monitoring of the systemic and organ system changes which accompany sepsis and acute lung injury.

Biologic Features

Adult male cynomolgus monkeys (*Macaca fascicularis*) weighing 4.8–7.2 kg were used. The animals were anesthetized with methohexital intramuscularly (20 mg/kg) and succinylcholine intramuscularly (5 mg/kg) and subsequently maintained on pentobarbital intravenously (5 mg/kg/hr) and pancuronium bromide intravenously (0.1 mg/kg/hr) for the duration of the study. The animals were intubated endotracheally and ventilated with an infant pressure ventilator (Healthdyne Model 121) with a tidal volume of 15 ml/kg, F_IO_2 0.21, and respiratory rate to keep the arterial P_{CO_2} 35 ± 3 mm Hg.

Intravenous access was gained by a cutdown technique involving placing a catheter (SF) in the left femoral vein. Similarly, an arterial line (4F) was placed in the right femoral artery, and a Swan-Ganz catheter (5F) was inserted into the right femoral vein. Flow was directed to its final position in the pulmonary artery.

Sepsis was induced with J96 *Escherichia coli* (04:K6:H+), a human pyelonephritic isolate.[13] Stock cultures were maintained in Luria broth with 10% glycerol at −20 C. Stock broth was inoculated onto Trypticase soy agar plates and harvested into saline after 18 hours at 37 C. The optical density of the saline *E coli* suspension was measured with a spectrophotometer and standardized to an inoculum of 2×10^9 bacteria/ml saline according to previously established curves.

The experiment was initiated after baseline measurements were obtained and the animal was hydrated to a pulmonary capillary wedge pressure (PCWP) of 5 mm Hg. Experimental animals were given 1×10^{10} *E coli*/kg over 30 minutes intravenously. Control animals were given an equal volume of saline. Fluid administration was guided by the PCWP to maintain left ventricular filling pressures. This method was chosen, rather than a predetermined rate, in order to give fluid in a more clinically relevant manner. The PCWP was measured at 10-minute intervals, and 20–40-ml boluses of normal saline were given until a PCWP of 5 mm Hg was reached. Adherence to a strict fluid protocol is imperative in a model of ARDS because the volume of fluid given directly affects the development of pulmonary edema. Measurements included systemic and pulmonary hemodynamic pressures, heart rates, cardiac outputs, arterial blood gases, peripheral blood neutrophil counts, complement levels, and quantitative blood cultures.

The experiment was continued for 4 hours after finishing the infusion of *E coli,* and at that time any surviving animals were sacrificed by hyperkalemic cardiac arrest with 10 mEq of KCl given intravenously. The lungs were removed through a thoracotomy for biopsy and gravimetric analysis of extravascular lung water as previously described.[14] Representative samples were taken from the heart, adrenal, kidney, liver, stomach, jejunum, and transverse colon for gross pathologic analysis.

In the development of our primate model the objective was to provide a septic insult sufficient to create an acute lung injury comparable with ARDS. The following parameters were examined in the septic (n=8) and control groups (n=4). For the septic group, the dose of *E coli* given was lethal in 5 of 8 animals (38% survival), compared with the control group (100% survival).

Pulmonary Function

A marked deterioration occurred in the septic group, as manifested by a decline in mean arterial P_{O_2} to 63±18 mm Hg at 2 hours. The control animals maintained an average P_{O_2} of 93 ± 9 mm Hg. Alveolar–arterial O_2 differences determined from arterial blood gases at time of near death or sacrifice increased to 48.5±27 mm Hg over control levels of 22.9±6.5 mm Hg. Fulminant pulmonary edema fluid was noted in the endotracheal tube of septic animals. The extravascular lung water (EVLW) rose to 5.3±1.6 ml/kg over controls, which had an EVLW of 1.7±0.3 ml/kg. The pulmonary edema which accompanied this insult was noncardiogenic in origin as the PCWP values remained below 5 mm Hg for the duration of the experiment. The "capillary leak phenomenon" which accompanies sepsis in the primate affects all vascular beds, as demonstrated by the large fluid requirements in these animals to maintain a PCWP of 5 mm Hg. The amount of saline required to maintain this filling pressure was 57.4±26.8 ml/kg/hr in the septic group, compared with 8.7±7.7 ml/kg/hr over controls.

Hematologic Findings

With the development of sepsis a peripheral neutropenia developed rapidly with absolute neutrophil counts falling to 3100±3500 cells/μmm at 1 hour,

compared with the baseline values of 9800±4100 cells/μmm. The neutropenia persisted throughout the study. Presumably the peripheral depletion of neutrophils represents margination and sequestration of neutrophils in the lungs.

Histology

Histopathologic specimens of lungs from primates infused with live *E coli,* compared with controls, demonstrated moderate amounts of pulmonary edema within scattered alveoli and increased acute cellularity, mainly consisting of neutrophils within the capillaries, in the interstitium and around vascular structures (Figures 1 and 2). Varying amounts of extravasation of red blood cells were also noted. All animals showed some chronic changes, including interstitial fibrosis and clubbing of alveoli.

Abnormalities noted elsewhere in the septic group included mild adrenal cortical and medullary hemorrhages and increased polymorphonuclear cells in liver sinusoids with scattered necrotic hepatocytes. Renal abnormalities were not consistent, but some animals showed fibrin thrombi in the glomeruli and/or scattered necrotic cells in the renal tubules consistent with acute tubular necrosis.

Hemodynamics

Septic animals developed marked hypotension with mean pressures falling to 60 mm Hg at 1 hour from a baseline of 112 mm Hg. Tachycardia was also pronounced in these animals with mean heart rates rising to 202±10.6 bpm over control values of 170±13 bpm at 1 hour. Consistent with septic shock, systemic vascular resistance declined to 3600±1300 dyne · sec · cm^{-5} from mean control values of 7660±2220 dyne · sec · cm^{-5} at 2 hours.

Bacteriology

A pure bacteremia of *E coli* J96 was demonstrated from quantitative blood cultures obtained at 2.5 hours. Bacterial counts ranged from 1.0 to 4.5×10^4 CFU/ml in the septic animals. All control animal blood samples tested were sterile.

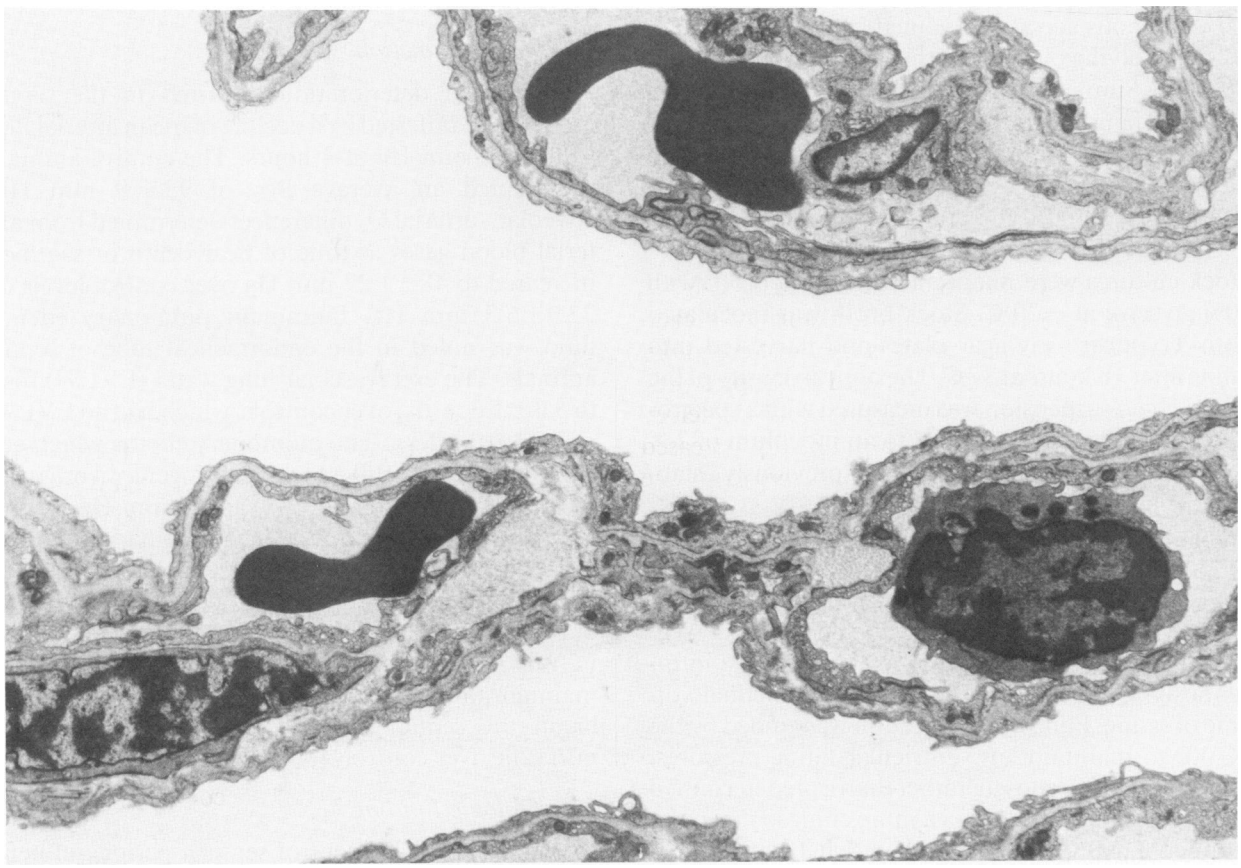

Figure 1 — Electron-microscopic section from a control primate showing normal architecture of interstitium, capillaries, and alveoli. The alveoli are clear from edema fluid, and neutrophils in capillaries are sparse. (×5000)

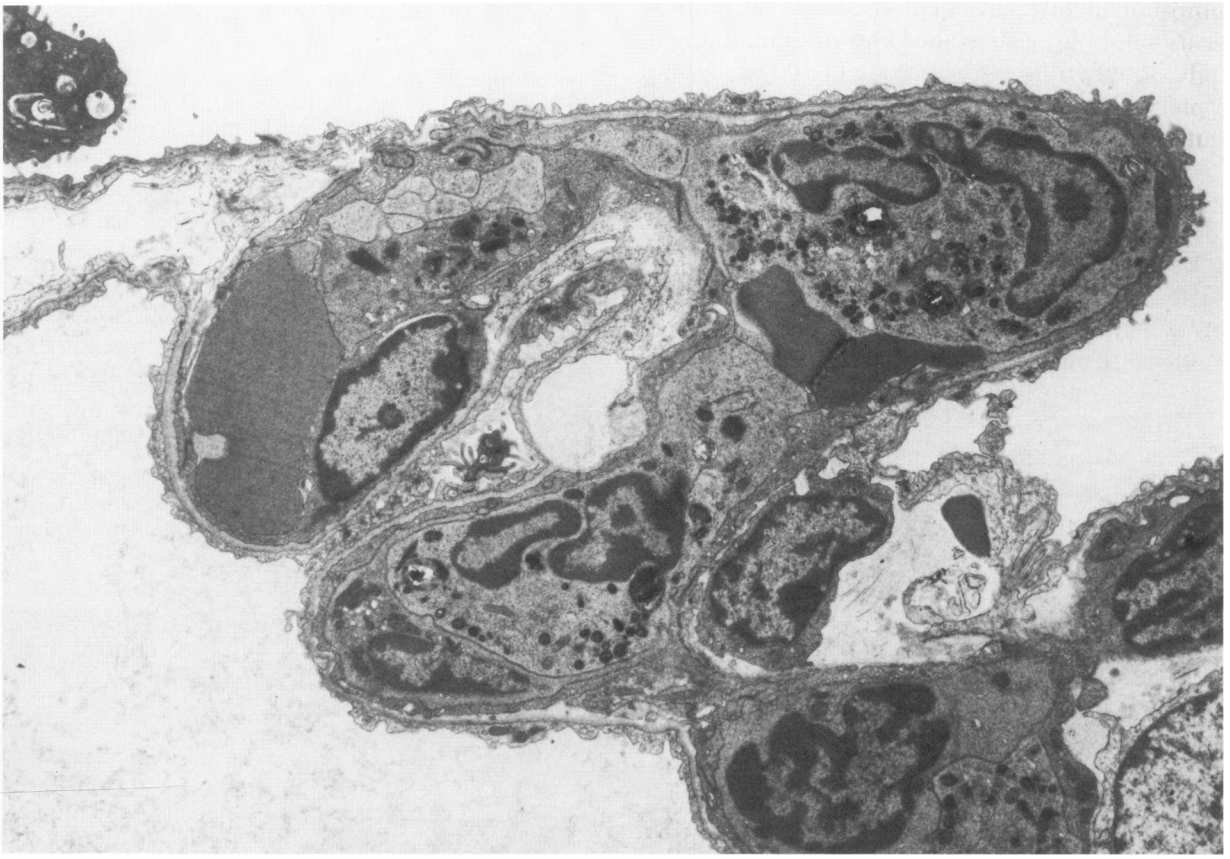

Figure 2— Electron-microscopic section from a septic primate demonstrating edema withn the alveolar space. Several neutrophils within vascular structures are noted, as well as a thickened interstitium due to edema fluid. (×4200)

Comparison With Human Disease

The diagnosis of ARDS in the clinical setting includes respiratory distress with a history of a catastrophic event, of which sepsis is the most common, hypoxemia, which was clearly noted in the primates, and exclusion of cardiogenic edema, which is inferred from a nonelevated PCWP.[15] The markedly increased EVLW in the septic group is consistent with the edema fluid noted by roentgenographic changes in the human patient. Typically in patients severely affected, respiratory compliance is reduced, the shunt fraction is elevated, and an increase in alveolar–arterial oxygen difference is noted. All of these parameters are compatible with the ARDS seen in the primate model.

The histopathologic changes seen in the primates were similar to the early changes described in humans. As noted above, primates infused with *E coli* developed pulmonary edema, with increased numbers of neutrophils within the capillaries. Of interest, there was no desquamation of alveolar lining cells or formation of hyaline membranes. The lack of these changes was probably due to the acute nature (4 hours) of this model.

Usefulness of the Model

The use of a primate septic model allows in-depth investigation into the pathophysiology of ARDS utilizing simultaneous measurements of hemodynamic pressures, pulmonary function, hematologic data, and histopathologic specimens. The model is also ideal for investigating the efficacy of potential therapeutic agents in sepsis-induced lung injury. For example, in our laboratory we have demonstrated that anti-C5a IgG antibodies (1 mg/kg) showed promise in attenuating the manifestations of ARDS in this model. Treated animals did not develop significantly increased extravascular lung waters nor decreased oxygenation, and they also had a recovery in their mean arterial blood pressure.[16] Monkeys given anti-C5a antibodies developed significantly less interstitial edema than septic controls, as shown by electron microscopy.[17]

Additionally, we have demonstrated that infusing prostaglandin E_1, a substance known to inhibit neutrophil function *in vitro,* at a rate of 100 ng/kg/min did not affect the hemodynamic changes or pulmonary injury seen in this model.[18]

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