Circulating Polymorphonuclear Leukocyte Activity in Patients with the Adult Respiratory Distress Syndrome*  

Implications for Pulmonary Vascular Injury  

G. A. Zimmerman, M.D.; † A. D. Renzetti, M.D., F.C.C.P.;‡ and H. R. Hill, M.D.§  

The adult respiratory distress syndrome (ARDS) is a common and frequently lethal cause of acute respiratory failure whose major initial pathophysiologic feature appears to be diffuse alveolar capillary membrane injury resulting in increased microvascular permeability. Pulmonary hypertension is a prominent feature in progressive ARDS, and occlusion and destruction of pulmonary capillaries has been reported in autopsy studies of patients dying with this syndrome. Although the specific cellular and biochemical mechanisms contributing to pulmonary vascular injury in ARDS are unknown, circulating humoral and/or cellular factors may cause microvascular damage in patients with this disorder. The polymorphonuclear leukocyte (PMN, granulocyte) may be such a factor. The speculation concerning the role of the PMN as an effector of pulmonary vascular injury in ARDS is primarily based on morphologic observations which indicate accumulation, or "sequestration," of PMNs in the lung microvasculature of animals with acute lung injury (ALI), and on observations which indicate increased pulmonary capillary permeability which is granulocyte-dependent in animal models of ALI. Few observations in humans are available to support this thesis. These are limited to studies which indicate that bronchoalveolar lavage fluid from patients with ARDS contains granulocyte-derived proteolytic activity and increased numbers of PMNs, and that plasma from such patients contains an activity (thought to be C5a) which aggregates control granulocytes in vitro. We studied PMNs from pulmonary artery blood samples from patients with ARDS in order to define the functional and metabolic characteristics of circulating granulocytes early in the course of established ARDS.

METHODS AND RESULTS  

After informed consent, we collected samples of pulmonary artery blood from 24 patients with ARDS a mean of 21 ± 3 SEM hours after satisfying strict criteria for diagnosis of the syndrome. Assays of PMN function and metabolism were performed according to published methods. We found that the mean chemotactic index (CI) of PMNs from patients with ARDS was 172 ± 22 compared to a mean CI of 79 ± 8 of PMNs from normal subjects (p = 0.0001), a 227 ± 24% increase over control (Fig 1). PMN respiratory burst activity, as assessed by the generation of chemiluminescence (CL), was 151 ± 12% of control (p = 0.04), indicating that PMNs from ARDS patients produce increased quantities of active O2 metabolites when stimulated (Fig 1). In addition, we found that superoxide anion (O2−) release was increased over that of control by PMNs from 4 of 8 ARDS patients in response to opsonized zymosan (mean 205 ± 71% of control) and by PMNs from 2 of 4 ARDS patients in response to phorbol myristate acetate. The enhanced CI and CL responses of granulocytes from ARDS patients were not explained by concurrent bacterial infection, did not appear to be influenced by glucocorticoid administration, and were greater than those of critically ill patients without ARDS. PMNs from ARDS patients had significantly increased (p = 0.0002) ratios of intracellular cyclic GMP to cyclic AMP when compared to normal subjects.

DISCUSSION  

The results of this study indicate that the blood perfusing the pulmonary vascular bed of patients with early ARDS

![Figure 1. Chemotactic activity and chemiluminescence response of PMNs from pulmonary artery blood samples of patients with ARDS. The left panel indicates the chemotactic indices of PMNs from ARDS patients, expressed as a percentage of the simultaneously measured normal control. Chemotactic activity was measured with a modified Boyden chamber technique, using a chemotactic factor prepared from E coli culture filtrates as the chemoattractant. The mean value for PMNs from ARDS patients was 227 ± 24% of control and was significantly (p = 0.0001) above that of normal subjects. The right panel indicates the peak chemiluminescence (CL) response generated by PMNs from ARDS patients, also expressed as a percentage of the simultaneously measured normal control. Points indicated by asterisks (*) indicate the CL response to phorbol myristate acetate, all other points indicate the CI response to opsonized zymosan. The mean peak CL of PMNs from ARDS patients was 151 ± 12% of control and was significantly greater than that of normal (p = 0.04).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21359/ on 06/27/2017)
contains PMNs which are in a functionally and metabolically activated state and may respond in an augmented fashion to chemoattractants and stimuli of respiratory burst activity. The alterations in PMN chemotactic activity and CL response which we observed may be in part due to exposure of granulocytes to circulating chemotactic factors or other mediators; exposure of PMNs to chemotactic factors has been reported to cause each of the alterations in granulocyte metabolism and activity which we observed.2,3,4

Our findings of increased chemotactic activity of granulocytes from individuals with ARDS suggests that PMNs from these patients may respond in an enhanced fashion to locally-elaborated or circulating chemotactic factors,2,3 resulting in granulocyte accumulation in the lung microvessels (Fig 2). It is reported that pulmonary capillaries contain increased numbers of PMNs early in the pathogenesis of human ARDS.4

The observation of enhanced respiratory burst activity of PMNs from ARDS patients, indicated by the augmented CL response, may have important relevance to the pathogenesis of pulmonary vascular injury in ARDS. Active oxygen metabolites (O2•, hydrogen peroxide, and hydroxyl radical) which are produced during the PMN respiratory burst have been reported to directly or indirectly injure human endothelial cells,9,10 and to cause increased vascular permeability and pulmonary vasoconstriction in animal lungs.4,5,6 In addition, active O2• metabolites degrade protease inhibitor activity8 and thus may cause endothelial injury by promoting local concentrations of unopposed neutrophil proteases.7,8 Superoxide anion and hydroxyl radical degrade the biologic activity of prostacyclin, a potent local vasodilator and inhibitor of platelet aggregation.9 Thus, PMN-derived O2• metabolites have the potential to contribute to the increased microvascular permeability and to the occlusion and destruction of lung capillaries which occurs in ARDS.1-4,6 Granulocytes in the pulmonary artery blood of patients with ARDS, with the functional characteristics which we have defined, may be primed to participate in pulmonary vascular injury; further, they may also contribute to alveolar cell injury if they are recruited to the alveolar space.

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Pneumococcal Killing in the
Alveolus*
Evidence for a Nonphagocytic Defense
Mechanism for Early Clearance

J. D. Coonrod, M.D.; S. R. Rehm, M.D.; and K. Yoneda, M.D.

It is frequently stated that inhaled bacteria are killed by
alveolar macrophages. This view is based principally on studies of the fate of aerosolized Staphylococcus aureus. With nonstaphylococcal bacteria, the exclusive role of macro-
phages in early clearance is not proven and an extracellular
bactericidal system has been postulated. We have been interested in the mechanism of clearance of encapsulated pneumoccci. In the first series of experiments, 3 pneu-
mooccal serotypes which exhibited widely degrees of opsonization by complement in vitro were aerosolized into normal and decomponented rats. The pneumococcal serotypes employed were type 1 (no detectable opsonization by complement); type 3 (opsonized partly by the classic and partly by the alternative complement pathway); and type 25 (readily opsonized via the alternative pathway). The rats
employed had no detectable type-specific antibodies, as
judged by an indirect fluorescent antibody test. The rats
were decomponented by an iv injection of 50 units of cobra
venom factor (CoVF) (Naja naja, Cordis Laboratories) 18-22
hr before aerosolization of bacteria. The efficacy of C3
depletion in serum and alveolar lining material was estab-
lished by monitoring levels of C3 protein by immunodiffusion
and by evaluation of heat-labile opsonic activity and lytic
activity for rabbit red blood cells. Log phase pneumococci
were aerosolized into normal or decomponented, adult
Sprague-Dawley rats for 30 min, followed by a 10 min cloud
decay period before removal of the rats from the aerosoliza-
tion chamber. Particle size in the aerosol was checked with an
Andersen air sampler. The rats were sacrificed and their
lungs homogenized with a tissue homogenizer. Viable bacteria were
determined by culturing the lung homogenates on 5% sheep
blood agar overnight. The results of these experiments, now
published, are summarized in Figure 1. Quantitative cul-
tures at 30 min revealed about 50% clearance of each
pneumococcal type, with no difference between normal and
component-depleted rats. The similarity of clearance rates
for the different serotypes and the lack of effect of comple-
ment depletion on clearance indicated a negligible role for comple-
ment in pulmonary clearance in this model. This conclusion appeared even more likely when it was demon-
strated that Sprague-Dawley resident alveolar macrophages
lack detectable complement receptors, as measured by
studies with complexes bearing C3b, C3a, or C5a. Since
unopsonized encapsulated pneumococci are known to be
highly resistant to phagocytosis in vitro, our findings ap-
peared to indicate the presence of an unusual (noncom-
plement, nonimmunoglobulin) opsonin for pneumococci in rat
lungs, or possibly an extracellular bactericidal system.

The latter possibility was examined directly by collecting
alveolar lining material in PBS from rats by bronchoalveolar
lavage, removing the leukocytes from the fluid by centrifuga-
tion at 160 g, and testing the fluid for anti-pneumococcal
activity. The cell-free fluid was concentrated 20-fold by
Amicon filtration (10,000 mol wt exclusion level) and 0.2 ml of

*From the VA Medical Center and the University of Kentucky
School of Medicine, Lexington.
Reprint requests: Dr. Coonrod, University of Kentucky Medical
Center, Lexington 40536

Figure 1 Pulmonary clearance of aerosolized pneumococci in
normal and decomponented Sprague-Dawley rats.